The Role of Oxytocin in Older Adults' Facial Emotion Recognition Difficulties

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Declaration

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other institution and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made.

The ethical principles and procedures specified by the Australian Catholic University's policy document on Human Research and Experimentation have been adhered to in the preparation of this report

Signed: .....

Date: 13/08/2020

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Abstract

The ability to recognise what someone is feeling from non-verbal cues, such as their facial expression, is a core social cognitive skill. Emotion recognition a crucial aspect of interpersonal function and as such, emotion recognition deficits can lead to diminished quality of life. There is substantial evidence that healthy older adults are not as accurate as younger adults at recognising facial expressions of emotions, which may make them vulnerable to impaired interpersonal functioning. However, there is debate about whether this age-related difficulty is due to biological decline or a shift in motivation, or a combination of these two factors. The overarching aims of this thesis were to clarify the pattern and magnitude of older adults' difficulty recognising different facial expressions of emotions, and to advance our understanding of the underlying mechanisms of older adults' difficulty in recognising emotions with a focus on the oxytocin system. This was achieved through three studies. The first was a comprehensive meta-analytic review of the facial emotion recognition and ageing literature, investigating the moderating effects of task characteristics (Chapter 3). The second was a clinical trial of the modulatory effects of oxytocin nasal spray administration on young and older adults' facial emotion recognition accuracy (Chapter 6), and the third was a comparison of young and older adults' peripheral oxytocin levels as measured from saliva samples (Chapter 7).

The meta-analytic review served to clarify the pattern of age-effects across emotion types, but also to identify if there were specific task features that moderated this variance. This was an important avenue of inquiry due to the numerous different task designs used to measure facial emotion recognition in the field of ageing, and the varied outcomes in terms of the magnitude and direction of age-effects reported for each emotion. The meta-analytic review included 102 data sets from 81 studies comparing older and young adults' facial emotion recognition accuracy. The combined variance, accounted for by the number of

response options included in the task, the stimulus format (e.g., videos, photographs of full-intensity expressions), and the image set from which the stimuli were sourced, was significant. Notably, videos produced relatively moderate age-effects across *all* emotions, which challenged the previously reported positivity effects in ageing. For disgust recognition, older adults demonstrated superior accuracy to young adults for the most common image set (Pictures of Facial Affect), but older adults were less accurate than young adults at recognising disgust on all other stimulus formats and image sets. This finding suggests that older adults do no retain disgust recognition. The overall finding that task characteristics impact on older adults' facial emotion recognition performance transforms our understanding of their deficits, but may also have broader implications given these task are used across many different clinical populations.

In terms of biological decline, research has largely investigated neurobiological changes in brain function and structure in relation to age-related emotion recognition ability. However, the potential modulatory effects of neurotransmitters and neuropeptides linked to social cognitive function remain to be thoroughly examined. Specifically, the neuropeptide oxytocin is known to play a role in social processes, such as emotion recognition, and is also thought to decline in healthy ageing. Yet, minimal research has been conducted investigating the role that oxytocin might play in older adults' emotion recognition accuracy. Thus, one aspect of the empirical research component of this thesis was to test the role of oxytocin, when administered intranasally, in modulating young and older adults' facial emotion recognition accuracy. Sixty young adults (18 – 33 years) and 60 older adults (65 – 80 years), with equal proportions of men and women, completed a facial emotion recognition task of six basic emotions (anger, fear, sadness, disgust, happiness and neutral) in a placebo-controlled cross-over randomised trial of 24 IU of oxytocin nasal spray versus placebo nasal spray. Mixed-effects models revealed that oxytocin had no impact on older adults' facial emotion

recognition accuracy, contrary to the expectations. Oxytocin did improve young females' sadness recognition, however, this effect was small. The findings from this study suggest that intranasal oxytocin had no age-related benefit on recognising facial expressions of emotion in older adults, and this thesis, therefore, does not provide evidence in support of oxytocin being a biological mechanism behind these difficulties.

The second aspect of the empirical research component of this thesis was a comparison of young and older adults' baseline peripheral oxytocin concentration levels from saliva samples. Although peripheral oxytocin concentration levels cannot be used to infer oxytocin concentration in the central nervous system (the oxytocin pathway of primary interest to social cognitive research), it has been postulated that intranasal oxytocin exerts effects on the central oxytocin system via indirect peripheral effects. Thus, when investigating the effects of oxytocin on central processes, it is important to have an understanding of the oxytocin system as a whole in the population of interest. Based on animal research, an age-related decline of peripheral oxytocin has been speculated, but there is little research confirming this trajectory. The results of this thesis indicated that older and young adults of both sexes have similar peripheral oxytocin concentration levels, which suggests that parts of the oxytocin system may be maintained in healthy ageing.

Overall, this thesis provides new information to the understanding of the mechanisms behind older adults' facial emotion recognition difficulties. This thesis does not provide direct support for either motivation or biological models of emotion recognition decline in healthy ageing, as the findings have implications for both perspectives. Specifically, the task characteristics that were shown to moderate older adults' performance on measures of facial emotion recognition accuracy vary in terms of the cognitive load required but also the level of motivation engagement. As such, the findings from the meta-analytic review have implications for both motivational and biological models. Intranasal oxytocin not modulating

older adults' facial emotion recognition, and parts of the oxytocin system appearing to be maintained in healthy ageing, may initially seem to contradict biological models and in turn, provide support for motivational models. However, the findings from this thesis are not enough to eliminate the oxytocin system as a biological mechanism behind older adults' facial emotion recognition difficulties, as more direct testing of the central oxytocin system is required. While this thesis does not provide conclusive evidence for or against oxytocin playing a role in older adults' emotion recognition deficits, it makes important contributions to the growing foundation of knowledge, which will serve to inform future research.

Research Outputs

Published Peer-Reviewed Papers as Chapters of the Thesis

Hayes, G. S., McLennan, S. N., Henry, J. D., Phillips, L. H., Terrett, G., Rendell, P. G., Pelly, R. M., Labuschagne, I. (2020). Task characteristics influence facial emotion recognition age-effects: A meta-analytic review. *Psychology and Aging, 35(2)*, 295-315. doi:https://doi.org/10.1037/pag0000441

Conference Presentations and Awards

Hayes G., McLennan S., Henry J., Phillips L., Terrett G., Rendell P., Pelly R., Labuschagne I., *Task Characteristics Influence Facial Emotion Recognition Age-Effects: A Meta-Analytic Review*, Australian Catholic University Psychology Conference, 24th September 2018

Hayes G., Heinrichs M., Rendell P., Terrett G., De Bono N., Labuschagne L., *Acute Intranasal Oxytocin Administration Modulates Social Cognitive Functions in Older and Young Adults,* Aikenhead Centre of Medical Centre Research Week, 5th August 2019

** Awarded best Junior Investigator Oral Presentation on the day.

Oral Presentations and Awards

Hayes G., *The Role of Oxytocin in Older Adults' Emotion Recognition Difficulties*, Australian Catholic University Three Minute Thesis Melbourne Heat, 23rd July 2019.

**Awarded winner of Melbourne heat.

Hayes G., *The Role of Oxytocin in Older Adults' Emotion Recognition Difficulties*, Australian Catholic University Three Minute Thesis Finals, 21st August 2019.

** Awarded winner of national finals.

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List of Abbreviations

ADFES – Amsterdam Dynamic Facial Expression Set

ANOVA – Analysis of variance

ASD – Autism Spectrum Disorder

AQ – Autism Quotient

BFI – Big Five Inventories

CI – Confidence interval

CATS – Comprehensive Affect Testing System

CSF – Cerebrospinal fluid

DANVA-2 – Diagnostic Analysis of Nonverbal Behaviour

DIT – Dynamic Integration Theory

DNA – Deoxyribonucleic acid

ECF – Extracellular fluid

EIA – Enzyme Immunoassay

ER-40 – Penn Emotion Recognition Test

FAN – Florida Affect Naming task

FEEST – Facial Expressions of Emotions Stimuli and Tests

HPA – hypothalamic-pituitary-adrenal

IU – International units

JACFEE – Japanese and Caucasian Facial Expressions of Emotion

KDEF – Karolinska Directed Emotional Faces

MANOVA – Multivariate analysis of variance

MRI – Magnetic resonance imaging

MSFDE – Montreal Set of Facial Displays of Emotion

NART – National Adult Reading Test

NYEB – New York Emotion Battery

PET – Positron emission tomography

POFA – Pictures of Facial Affect

PVN – Paraventricular nucleus

RIA – Radioimmunoassay

SFS – Social Functioning Scale

SON – Supraoptic nucleus

SPSS – Statistical Package for the Social Sciences

SST – Socioemotional Selectivity Theory

STAI – State and Trait Anxiety Inventories

TICS – Telephone Interview for Cognitive Status

CHAPTER 1: THESIS OVERVIEW

1.1 Thesis Rationale

Despite the 'fountain of youth' being merely a legend, life expectancy continues to rise, and older adults today can expect to live longer than any previous generation. In the last 55 years, life expectancy in Australia has risen by a decade, from an average of 71 years to 82 years (Australian Institute Health and Welfare, 2019). However, this increase in the quantity of years may not necessarily be accompanied by an adequate quality of life. For example, while average life expectancy is the highest it has been in the written record, morbidity rates (i.e., the frequency with which a deficit/illness appears within a population) of many age-related physical and cognitive declines have remained relatively unchanged. An increase in life expectancy, without a mirrored shift in morbidity rates, means that older adults are living with age-related loss of functioning for longer (Crimmins, 2011). To allow the growing numbers of old and very-old adults to enjoy a greater quality of life in their later years, research is therefore needed that focuses on delaying the onset of age-related loss of functioning.

Age-related loss of function is not limited to physical and neurocognitive losses, such as becoming less mobile and experiencing memory difficulties. Healthy older adults also demonstrate difficulties with several social skills (Von Hippel & Henry, 2012). It has been suggested that this may reflect age-related declines in social cognition. Social cognition refers to "the human ability and capacity to perceive the intention and disposition of others" (Brothers, 1990, pg. 28) and is essential for positive social interactions and relationships (Adolphs, 2009). Given that social cognitive declines are a critical predictor of mental health and well-being in older adults (Phillips, Scott, Henry, Mowat, & Bell, 2010), social cognitive declines may be more detrimental to the quality of life than declines in other cognitive functions such as memory in this age group (Henry, von Hippel, Molenberghs, Lee, & Sachdev, 2016; Holt-Lunstad, Smith, & Layton, 2010; Steptoe, Shankar, Demakakos, &

Wardle, 2013; Yang et al., 2016). However, to date, research investigating social cognition in healthy ageing is more limited than research focusing on cognitive abilities such as working memory and processing speed. Consequently, despite the need to delay age-related loss of function, the pattern of social cognitive declines with age, and particularly, the mechanisms underpinning these declines are still being determined. These issues are the focus of the current thesis.

One core social cognitive ability is emotion recognition, and age-related difficulties in this ability have been well documented (Sullivan & Ruffman, 2004b; Sullivan, Ruffman, & Hutton, 2007; West et al., 2012; Williams et al., 2009). However, a small but pivotal review of the literature by Ruffman, Henry, Livingstone, and Phillips (2008) indicated that age-related deficits in emotion recognition may to some extent vary depending on the nature of the emotion. More specifically, this review reported that while older adults demonstrated lower facial emotion recognition accuracy than younger adults, typically, older adults found anger, fear, and sadness more difficult to recognise than happiness and surprise, and that they may retain the ability to recognise disgust (Ruffman et al., 2008). Since this review, there have been many adaptations made to the tasks used to measure facial emotion recognition, such as: reducing the intensity of the expressions, using videos instead of photographs, and providing contextual cues. There is considerable evidence that these task features influence the degree to which older adults demonstrate facial emotion recognition difficulty (Richter, Dietzel, & Kunzmann, 2011; Voelkle, Ebner, Lindenberger, & Riediger, 2014; Wieck & Kunzmann, 2017; Zhang, Fung, Stanley, Isaacowitz, & Ho, 2013). It is thought that different task features influence the cognitive load required to perform well on the task, as well as older adults' motivation to engage with the task (Isaacowitz & Stanley, 2011; Zhang et al., 2013). It is now unclear if the pattern and magnitude of age-effects reported by Ruffman et

al. (2008) accurately reflect older adults' abilities or is only true for the traditional task designs on which the review mostly reported.

To improve functioning, however, it would be valuable to identify mechanisms underpinning older adults' compromised ability to recognise emotions, which can then be targeted for intervention. Currently, there is some debate regarding the basis for emotion recognition deficits in older adults, with researchers disagreeing on whether older adults' inferior accuracy, for example, is a function of motivation or biology (Ruffman et al., 2008). Several different motivational theories could account for why older adults are less accurate than young adults on emotion recognition tasks. These include Socioemotional Selectivity Theory (SST, Carstensen, Pasupathi, Mayr, & Nesselroade, 2000), Dynamic Integration Theory (DIT; Labouvie-Vief, 2009), and the selective engagement hypothesis (Hess, Rosenberg, & Waters, 2001). Generally, these theories suggest that older adults retain the ability to recognise emotion but lack the motivation to allocate their limited cognitive resources to emotion processing, particularly when the stimulus is unpleasant such as someone expressing anger. Conversely, biological theories attribute the age-related decline in emotion recognition accuracy to neurological degeneration and an associated loss of ability (Isaacowitz et al., 2007; Suzuki & Akiyama, 2013; Williams et al., 2006). For example, neurodegeneration of the orbitofrontal cortex, amygdala and cingulate cortex has been proposed to contribute to older adults' difficulties recognising anger, fear and sadness (Ruffman et al., 2008).

Although biological theories have predominately focused on the atrophy of neural regions associated with emotion recognition, it has been suggested that key social neurotransmitters and neuropeptides are also likely to play a role. In particular, oxytocin has been shown to play a role in emotion recognition accuracy (Melchers, Montag, Markett, & Reuter, 2013; Shahrestani, Kemp, & Guastella, 2013) though this has been understudied in

healthy ageing (Ebner, Maura, Macdonald, Westberg, & Fischer, 2013; Huffmeijer, van Ijzendoorn, & Bakermans-Kranenburg, 2013). Indeed, despite numerous studies investigating the effects of intranasal oxytocin administration on emotion recognition in clinical populations (e.g., Guastella et al., 2010; Lischke et al., 2012; Schulze et al., 2011), only three studies have examined the effects of intranasal oxytocin administration on older adults' emotion recognition accuracy (Campbell, Ruffman, Murray, & Glue, 2014; Grainger et al., 2018b; Horta et al., 2019). Two of these studies reported no oxytocin effects (Grainger et al., 2018b; Horta et al., 2019), however, Campbell, Ruffman, Murray, and Glue (2014) reported that oxytocin significantly improved emotion recognition accuracy for older men, but not older women or young adults. However, this latter study used independent measures, rather than repeated measures, so the reported oxytocin effects could have been confounded by individual differences between the two groups. Thus, it remains unclear whether oxytocin plays a role in older adults' emotion recognition difficulties.

1.2 Thesis Aims

The two overarching aims of this thesis were to clarify the pattern and magnitude of older adults' difficulty recognising different facial expressions of emotions, and to advance our understanding of the underlying mechanisms of older adults' difficulty in recognising emotions, focusing on the oxytocin system.

The first study was a meta-analytic review of all previous research that had measured older adults' ability to recognise facial expressions of emotion. This study investigated the influence of task design on older adults' recognition performance. The task characteristics selected as variables of interest were the number of response options participants had to choose from, the format of the stimulus (e.g., dynamic videos vs. static photographs), and the image set from which the stimuli were obtained. These task characteristics were selected because they were considered likely to influence the cognitive resources required by older

adults to do well on the task, and to also affect their motivation to engage with the task. This study contributed to the thesis aims by identifying the task conditions under which older adults demonstrate difficulty recognising facial expressions of emotions and by reporting the patterns and magnitudes of the age-effects observed across different facial emotion recognition tasks.

The second and third studies investigated the oxytocin system in healthy ageing. The primary focus of the second study was to identify whether oxytocin within the central nervous system is involved in older adults' facial emotion recognition difficulties. The study involved an acute double-blind placebo-controlled trial of oxytocin administration (via a nasal spray) to artificially inflate oxytocin levels in the brain and test the impact on young and older adults' facial emotion recognition accuracy. Factors, that might influence the strength of any oxytocin effect such as age and sex were also explored. Central oxytocin was the primary focus because peripheral and central oxytocin systems are distinct, and there is evidence that they are not coordinated (Kagerbauer et al., 2013; Valstad et al., 2017). Moreover, peripheral oxytocin is linked to physiological functions such water balance, bone density, metabolism, muscle tissue regeneration, and homeostasis (Colaianni, Sun, Zaidi, & Zallone, 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019), and has not been proposed to play a role in social cognition.

Despite there being no a theoretical basis for correlating peripheral oxytocin with emotion recognition accuracy, there are many examples of peripheral oxytocin levels predicting emotion recognition ability in clinical populations (e.g., Goldman, Marlow-O'Connor, Torres, & Carter, 2008; Rubin et al., 2011; Strauss et al., 2015; Taurines et al., 2014). While peripheral oxytocin concentrations would not be expected to directly impact on emotion recognition, it may therefore be that there is an indirect relationship between oxytocin concentrations in the peripheral and emotion recognition ability. Futhermore, there

is a theory that intranasal oxytocin may modulates social cognitive skills via an indirect peripheral effects (Leng & Ludwig, 2016; Martins et al., 2016). Given the current lack of knowledge about the oxytocin system in healthy ageing (Ebner et al., 2013; Huffmeijer et al., 2013), the focus of the third study was to identify whether older adults demonstrate a decline in peripheral oxytocin concentration. An age-related decline in peripheral oxytocin concentration may suggest that peripheral oxytocin is indirectly connected to age-related social cognitive deficits in some way and warrants further investigation.

1.3 Thesis Structure

This thesis comprises manuscripts that have been published or were prepared with publication in mind. It is presented as a traditional thesis in line with the Australian Catholic University's Guidelines on the Preparation and Presentation of a Research or Doctoral Thesis for Examinations (Australian Catholic University, 2015). It includes a comprehensive multi-level meta-analytic review (published), results from a substantiative double-blind randomised trial investigating the effect of acute intranasal oxytocin on facial emotion recognition accuracy in younger and older adults, and a brief report on peripheral oxytocin age-differences.

The thesis begins with this introductory chapter (Chapter 1). A literature review addressing social cognitive difficulties in healthy ageing is then presented in Chapter 2, with a key focus on emotion recognition and behavioural outcomes. Chapter 2 reviews both neurobiological and behavioural accounts of age-related emotion recognition deficits. Chapter 3 presents the published meta-analytic review of facial emotion recognition age-effects and the influence of task design. The meta-analytic review also outlines the main theoretical accounts of age-related facial emotion recognition difficulties. Chapter 4 is an overview of oxytocin and healthy ageing. It details what is known about the role oxytocin plays in emotion recognition, the oxytocin system in old age, and what is still to be explored.

This chapter includes a review of behavioural studies that have investigated the effect of intranasal oxytocin on facial emotion recognition in both older adults and other populations more broadly. Chapter 5 presents the methodology for the empirical research papers in greater detail than is presented in the manuscripts. Chapter 6 presents the study investigating the effects of oxytocin on older and younger adults' facial emotion recognition accuracy and potential moderating variables. Chapter 7 provides a report contrasting young and older adults' peripheral oxytocin, vasopressin, testosterone, and estrogen concentration levels, as measured in saliva samples provided by participants prior to nasal spray administration. Finally, Chapter 8 involved a general discussion of the findings of the thesis and presents the conclusions, limitations, and contributions of this research.

CHAPTER 2: EMOTION RECOGNITION DIFFICULTIES IN HEALTHY AGEING

2.1 Chapter Guide

The research presented in this thesis explores task-related conditions and biological mechanisms that influence healthy older adults' ability to recognise facial expressions of emotion. The current chapter starts with a definition of healthy ageing followed by an overview of social cognition as a broad construct. Emotion recognition is then defined and highlighted as a core social-cognitive skill that is present across all social cognitive models, for which older adults demonstrate significant difficulty. The evidence of an age-related difficulty recognising emotion from facial expressions is detailed through the presentation of both behavioural and neuroimaging research. The behavioural evidence of an age-related decline in facial emotion recognition accuracy is presented within the context of there being task variations that likely impact on older adults' performance. Lastly, a summary is provided of the different theoretical accounts for older adults' inferior facial emotion recognition accuracy compared to young adults.

2.2 Healthy Ageing

The term healthy ageing seems paradoxical. Ageing is by its very definition not healthy. To age is not just to live longer but to degenerate (Rose, 1994). Healthy ageing may therefore be better understood as typical ageing. Typical ageing can be defined as typical rate and onset of age-related degeneration that is separate to the declines associated with age-related clinical disorders such as Alzheimer's disease, other dementias, and Parkinson's disease.

2.3 Overview of Social Cognition

Social cognition is a broad term for the varying cognitive processes used in social interactions. Given the relative newness of this field, compared to the study of traditional cognitive processes such as memory and processing speed, there is little consensus in terms

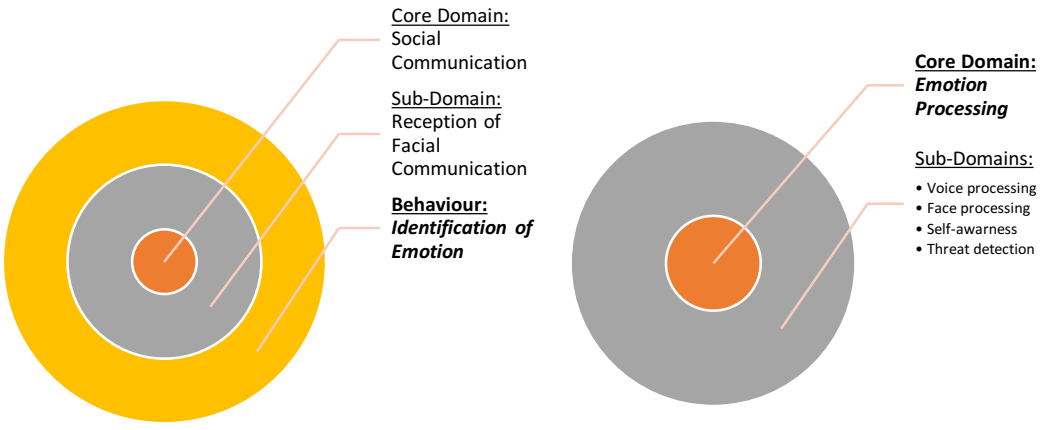
of the structure and components that comprise social cognition as a construct (Happé, Cook, & Bird, 2017). Across the literature, the proposed number of overarching social cognitive domains range from four (e.g., National Institute of Mental Health's research domain criteria; National Institute of Mental Health, 2012) to fourteen (e.g., Fiske and Taylor, 2013) (Happé, et al., 2017; Stevens & Woodruff, 2018). The National Institute of Mental Health (2012) list the core domains of social cognition as; affiliation and attachment; social communication; perception and understanding of self; and perception and understanding of others. In contrast, Happé and Frith (2014) link a multitude of social processes to ten overarching domains; emotion processing, self-processing, mental state attribution, in-group/out-group, social hierarchy mapping, affiliation, agent identification, individuals' information store, social policing, and empathy. Thus, despite some overlap in domains, there is clearly a lack of consensus in terms of the breadth and structure of this construct.

Adding to the complexity, different models of social cognition also assign different labels are assigned to the same processes or domains. For example, 'perception and understanding of others' (National Institute of Mental Health, 2012) is defined as "*the processes and/or representations involved in being aware of, accessing knowledge about, reasoning about, and/or making judgments about other animate entities, including information about cognitive or emotional states, traits or abilities.*" Yet this definition could also be used to describe 'agent identification', 'mental state attribution', and 'emotion processing'; three separate domains from the Happé and Frith (2004) model, which all involve perceiving and making judgements about others. Moreover, it is unclear which social processes are distinct and which overlap. For example, empathy is considered by some to be a unique social cognitive component (Happé et al., 2017), whereas others consider it to be an aspect or moderator of emotion recognition (Balconi & Canavesio, 2016).

Despite some disagreement in terms of the breadth and structure of social cognition as a construct, one process that consistently emerges across the proposed models of social cognition is the ability to observe and interpret what other people are feeling. For example, as depicted in Figure 2.1, the Happé and Frith (2004) model lists 'emotion processing' as a core domain, and the National Institute of Mental Health (2012) lists 'identification of emotion' as a behaviour within their 'reception of facial communication' sub-domain. Therefore, while the field of social cognition is still developing a robust model for encapsulating the relevant social processes, there is consensus that emotion recognition is a key aspect of social cognition.

Figure 2.1

Visual depiction of emotion recognition as a behaviour within National Institute of Mental Health's (2012) research domain criteria matrix, and as a core domain with the Happé and Frith (2014) model of social cognition.



National Institute of Mental Health (2012)

Happé and Frith (2014)

2.4 Emotion Recognition

As noted, the ability to infer emotion from other people's cues has multiple associated terms. These include, but are not limited to emotion recognition, emotion processing,

empathy, emotional intelligence, emotion perception, affect recognition, and emotion detection. Some of these terms, such as emotional intelligence, encompass a broader set of skills (i.e., recognising others' emotions, recognising one's own emotions, and using emotional information to guide behaviour) (Salovey & Sluyter, 1997). Other terms, such as emotion detection, are used to convey a more specific skill (i.e., the action of noticing the presence of emotion, rather than categorising the emotion). As such, one can detect emotion without being able to accurately label it, and one can be impaired in emotion recognition of others' but maintain other aspects of emotional intelligence. **For the purpose of this thesis, emotion recognition is defined as i) the ability to detect the presence of emotion in other people, ii) to accurately interpret the emotion being expressed (i.e., what the other person is feeling), and iii) is considered to only be the mental categorisation of the emotion rather than subsequent behavioural responses.**

A person can be deemed as accurate or inaccurate at emotion recognition, because these interpretations are not considered to be subjective, rather there are prototypical facial expressions of emotion that are universal across cultures. For example, a smile denotes happiness, a frown is a suggestion of anger, and wide eyes are typical of fearful faces. Emotions are likely universal because of their evolutionary origins (Darwin, 1872). For example, fear biologically prepares the body to escape danger. The specific components of facial expressions of emotions may have also originated from biological functions (e.g., the wide eyes expressed for fear may serve to increase the field of vision) (Darwin, 1872). However, the universality of facial expressions has led to them taking on a communicative function (Fridlund, 1997). For example, a fearful expression can communicate to others that they too may be in danger, or that you perceive them as a threat. A disgust expression can alert others to the presence of contamination, or that you perceive that they will contaminate you. Thus, it is the universality of emotional expressions, due to the evolutionary origins of

emotions, that allow facial expression to be an efficient means of non-verbal communication and a skill for which people can be accurate or inaccurate.

There is a general consensus that the basic emotions for which facial expressions are universal are the six outlined by Ekman (2008): anger, sadness, disgust, happiness, fear, and surprise. These emotions are called basic emotions because they are thought to have evolutionary origins and comprise the base level of a hierarchical classification of more nuanced emotional terms (Johnson-laird & Oatley, 1992; Shaver, Schwartz, Kirson, & O'connor, 1987). Although other emotions such as contempt, guilt, love, and contentment have also been proposed to be basic emotions, there is a lack of evidence supporting these emotions as either separate from the original six, or expressed in a prototypical fashion (Levenson, 2011; Ortony, 1987; Widen, Christy, Hewett, & Russell, 2011). It therefore remains unclear if these emotions can be classified as basic emotions, with evolutionary origins and universal expression, or if they are culturally specific expressions (Elliott & Jacobs, 2013) or sub-emotions. For example, contentment may be a form of happiness rather than a unique emotional expression. **As such, the research presented in this thesis focused only on the six basic emotions outlined by Ekman (2008): anger, sadness, disgust, happiness, fear and surprise.**

Since different emotions motivate different behavioural actions and communicate different messages, it is not surprising that different emotions are processed in different neural regions (Vytal & Hamann, 2010). Anger, fear and sadness are known to place particular demands on the orbitofrontal cortex, amygdala and cingulate cortex (Adolphs, 2002; Calder, Lawrence, & Young, 2001; Iidaka et al., 2001; Murphy, Nimmo-Smith, & Lawrence, 2003; Phan, Wager, Taylor, & Liberzon, 2002; Phillips, Drevets, Rauch, & Lane, 2003; Posamentier & Abdi, 2003; Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998; Vuilleumier & Pourtois, 2007). In contrast, the superior temporal gyrus is typically implicated with happiness

recognition (Vytal & Hamann, 2010) and the basal ganglia and insula are implicated in the recognition of disgust (Calder et al., 2001; Vytal & Hamann, 2010). These neurological differences in emotion processing, suggest that it is possible to be impaired at recognising one emotion but not another. As such, identification of each emotion type can be considered an individual process rather than one general process.

Emotion recognition accuracy is typically measured by showing participants photographs of posers demonstrating prototypical facial expressions of emotions, and asking the participants to label the basic emotion being expressed. However, it should be noted that facial expressions are not the only modality through which emotion can be recognised. Emotions are expressed, and thus recognised, through tone of voice and body posture. Therefore, while participants are typically shown images of facial expressions, alternatively, participants might listen to audio clips of statements read with emotional tone (Isaacowitz et al., 2007; Wong, Cronin-Golomb, & Neargarder, 2005), or images of full bodies rather than isolated faces (Ruffman, Halberstadt, & Murray, 2009b).

2.5 Emotion Recognition in Ageing

Though one can never truly observe another person's emotional experience, accurate interpretation of what someone might be feeling, based on their external cues, is vital for appropriate and validating social responding. Misread the cues or fail to notice the presence of emotion in the first place, and you may quickly earn a reputation for being cold, unsympathetic, unkind, or distant. In clinical populations, social cognitive difficulties can be more detrimental to the quality of life than deficits in other cognitive functions (Henry et al., 2016). Unfortunately, there is substantial evidence that older adults demonstrate inferior emotion recognition accuracy to younger adults, which will be detailed in the remainder of this chapter. There is also evidence that social cognitive declines are a critical predictor of

mental health and well-being in older adults (Phillips, Scott, Henry, Mowat, & Bell, 2010; Szanto et al., 2012). Given the links between social isolation and mortality in healthy ageing (Henry et al., 2016; Holt-Lunstad et al. , 2010; Steptoe et al., 2013; Yang et al., 2016), social cognitive declines may also be more detrimental to the quality of life than other cognitive declines in healthy ageing. Though the trajectory of emotion recognition accuracy in ageing differs across emotions, typically there is a sharp decline in emotion recognition accuracy observed between ages 60 and 70 onwards (Mill, Allik, Realo, & Valk, 2009; Williams et al., 2009). This is particularly true for anger, fear and sadness, with disgust recognition potentially retained until the age 80, and happiness recognition observed to remain stable across all ages (Williams et al., 2009).

Across emotion recognition literature, measures of facial emotion recognition are the most commonly assessed emotion modality, and as such, there is more evidence of age-effects for emotion recognition from faces than from auditory or bodily cues. Thus, the behavioural study presented in Chapter 6 of this thesis, which trialled the effects of oxytocin administration on emotion recognition in young and older adults, used a measure of *facial* emotion recognition to ensure age-effects emerged. This chapter and the meta-analytic review that follows (see Chapter 4) also therefore focused predominately on *facial* emotion recognition in healthy ageing.

2.6 Behavioural and Neuroimaging Evidence

2.6.1 Behavioural studies

In a small (n = 28) but a pivotal review of the behavioural studies, Ruffman et al. (2008) reported that older adults consistently demonstrated inferior accuracy on at least some emotions across all modalities; faces, voices, bodies, and face to voice matching. For facial expressions of emotion, the focus of this thesis, an interesting pattern emerged across

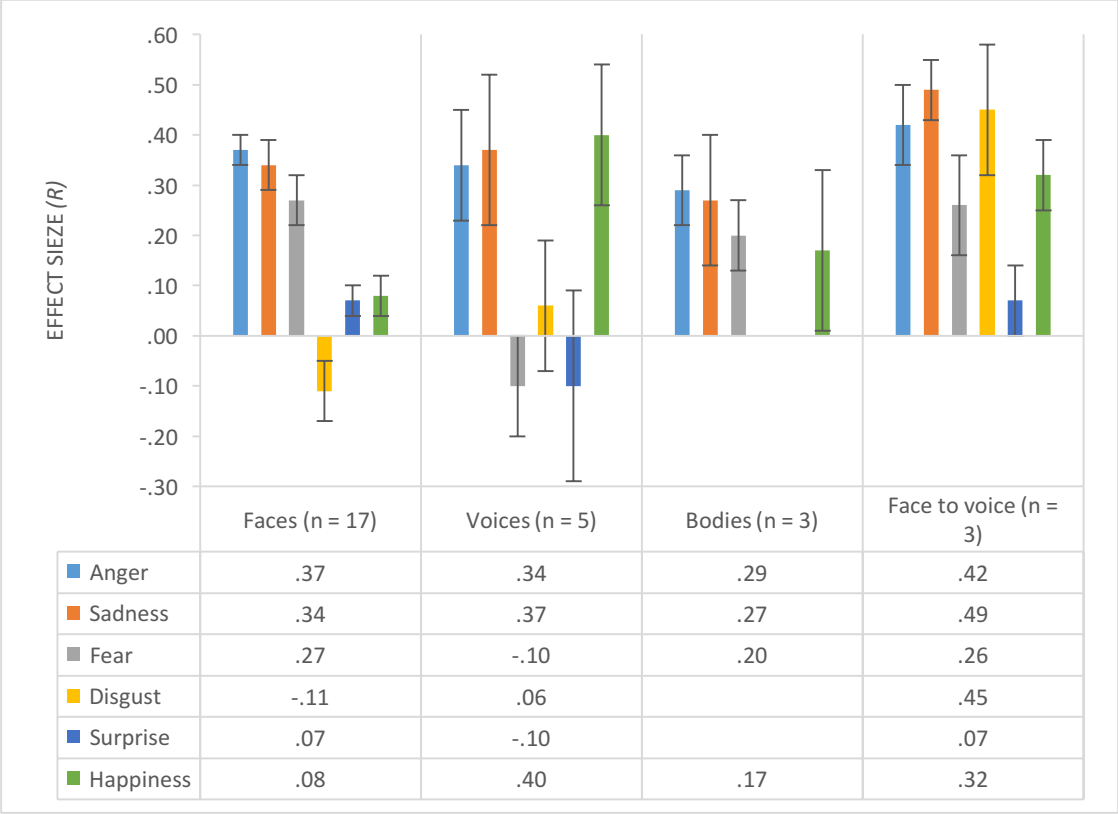
emotions whereby older adults demonstrated the strongest difficulties for sadness, fear, and anger; less difficulty for happiness and surprise; and no age-related difficulty for disgust. In fact, a trend emerged where older adults were more accurate at recognising expressions of disgust than young adults. Figure 2.2 shows the pattern of effects reported by (Ruffman et al., 2008) for each modality. Notably, on tasks that required participants to matching facial expressions to voices of same emotional tone, older adults demonstrated as much difficulty with disgust stimuli as they did anger and sadness (Figure 2.2), which suggests that there may be some age-related difficulty with disgust recognition that was not captured by the single modality facial emotion recognition tasks.

Since Ruffman et al.'s (2008) meta-analysis, the body of research into facial emotion recognition in healthy ageing has increased substantially, with large variations emerging in the reported magnitudes of the age-effects across emotions and studies. Concerning the three emotions identified as most impaired in this earlier review (i.e., anger, fear, sadness), many newer studies have also reported significant age-related difficulties for these emotions (e.g., Hunter, Phillips, & MacPherson, 2010; Kessels, Montagne, Hendriks, Perrett, & de Haan, 2014; Krendl & Ambady, 2010; Murphy & Isaacowitz, 2010), however, others have failed to identify any age-effect for one or more of these emotions (e.g., Circelli, Clark, & Cronin-Golomb, 2013; Demenescu, Mathiak, & Mathiak, 2014; Fölster, Hess, Hühnel, & Werheid, 2015; Horning, 2012; Orgeta & Phillips, 2008; Sze, Goodkind, Gyurak, & Levenson, 2012). For surprise and happiness, the small age-related difficulty identified by Ruffman et al. (2008) has not reliably been replicated, with several more recent studies identifying either a null effect (e.g., Ebner, Johnson, & Fischer, 2012; Hot et al., 2013; Keightley, Chiew, Winocur, & Grady, 2007) or a small effect that is in favour of older adults (e.g., Ruffman, Ng, & Jenkin, 2009b). Moreover, the trend identified by Ruffman et al. (2008) for disgust recognition to be slightly enhanced in late adulthood contrasts with more recent studies,

which have found that older adults performed worse than young adults (e.g., Chaby, Boullay, Chetouani, & Plaza, 2015; Demenescu et al., 2014; Ngo & Isaacowitz, 2015).

Figure 2.2

Mean age-effects (r) from the Ruffman et al. (2008) meta-analysis for each emotion and modality.



	Faces (n = 17)	Voices (n = 5)	Bodies (n = 3)	Face to voice (n = 3)
Anger	.37	.34	.29	.42
Sadness	.34	.37	.27	.49
Fear	.27	-.10	.20	.26
Disgust	-.11	.06		.45
Surprise	.07	-.10		.07
Happiness	.08	.40	.17	.32

Note. Positive values denote older adults' inferior accuracy over young adults, with negative values indicating the reverse. Error bars represent standard error.

A likely contributor to the inconsistencies across studies is the variation in stimuli and task designs used when assessing facial emotion recognition. To date, the most common method of assessing facial emotion recognition has been to present participants with a series of static photographs from the Pictures of Facial Affect database (POFA; Ekman & Friesen, 1976) depicting most, or all, of the six basic facial expressions proposed by Ekman (1992) (i.e., anger, disgust, fear, happiness, sadness, and surprise). Participants are then instructed to select a word label that categorises each expression. While many researchers still use traditional facial emotion recognition task designs of this type, more recently there has been a

shift towards assessing this construct using different emotion-related stimuli and methodological designs.

2.6.1.1 'Either or' tasks

A predicament within the field of facial emotion recognition is to estimate facial emotion processing difficulties separate from other more general cognitive processing. A critique of the traditional facial emotion recognition measure is that providing participants with multiple lexical response options, places demands on working memory and linguistic processing. One way that the traditional task design has therefore been altered is by presenting participants with fewer than six facial expressions to choose from. Many recent studies have reduced the number of response options in any given item to only two. Tasks with two response options include go/no-go tasks (i.e., 'click for happy, do not click for sad'), tasks where the target emotion is present or absent (i.e., 'click left key if expression is happy, click right key if expression in not happy'), facial discrimination (i.e., 'which of these two faces is happy?'), and those including two labels (i.e., 'is this face happy or sad?') (e.g., Ma, Li, Niu, Yu, & Yang, 2013; Roring, Hines, & Charness, 2006). When items only include two responses, participants have an 'either-or' choice that allows for the use of elimination, a cognitive strategy that is less demanding on working memory (Phillips, Channon, Tunstall, Hedenstrom, & Lyons, 2008).

The literature is mixed in terms of whether or not older adults still demonstrate facial emotion recognition difficulties when the task allows the use of elimination; see (Bailey, Henry, & Nangle, 2009; Noh & Isaacowitz, 2013) but see also (Di Domenico, Palumbo, Mammarella, & Fairfield, 2015). A complication with interpreting these mixed results is that the relevant studies have also used different stimuli (i.e., photographs vs. videos), and the stimulus format would also have influenced the cognitive load of the task. Thus, from this data it remains unclear if older adults demonstrate emotion recognition difficulties that are

separate from other cognitive declines such as working memory. However, Suzuki and Akiyama (2013) controlled for general cognitive ability using four measures of processing speed and fluid intelligence, and reported that age-effects for recognition of anger and disgust were not accounted for by cognitive ability. To some degree, or at least for some emotions, older adults' facial emotion recognition difficulties are separate to decline of general cognitive ability.

2.6.1.2 Other-race and other-age

The POFA (Ekman & Friesen, 1976) is the original and most commonly used set of photographs and depicts black-and-white photographs of young to middle-aged adult Caucasian actors expressing the six basic emotions. The POFA therefore does not test older adults' ability to recognise facial expressions presented in colour, expressions of posers their own age, or expressions of posers of a different race. However, alternative image sets have been developed that use colour photographs to represent a wider variety of ages and racial backgrounds, and thus more accurately depict the diversity of social partners typically encountered in everyday life. That is, some image sets include both older and young to middle-aged posers, such as the 'FACES' image set (Ebner, Riediger, & Lindenberger, 2010), and posers with different racial backgrounds, such as the Japanese and Caucasian Facial Expressions of Emotion (JACFEE) (Matsumoto, 1998).

It appears that older adults' difficulty recognising emotion is consistent across own race and other race. Ruffman et al. (2008) reported that for anger, fear and sadness the four studies that had used the JACFEE stimuli produced the same pattern of results as the 12 studies that had used the POFA stimuli. Furthermore, older adults also retained disgust recognition on the JACFEE stimuli. However, unlike the POFA stimuli, studies using the JACFEE stimuli failed to identify an age-effects for surprise. The authors were unable to examine age-effects for happiness due to at least one age group performing at ceiling in every

study (Ruffman et al., 2008). Thus, older adults' difficulty recognising facial expressions of emotion, appears to be consistent across race and is not own-race or other-race dependent.

For age of poser, it appears that the age of older adults demonstrates even greater difficulty recognising facial expressions of emotion on age-congruent posers than young or middle-aged posers. One might expect an own-age bias to provide older adults with an advantage over young adults when older adult posers are presented. Indeed, it has previously been argued that older adults may also be more motivated to allocate cognitive resources to recognising emotion on age congruent posers (Hess et al., 2001). Yet, the creators of the FACES stimuli report that both young and older adults found older adult posers' expressions of emotions more difficult to identify than those of young adult posers' (Ebner, He, & Johnson, 2011; Ebner & Johnson, 2009). Thus, it would appear that facial expressions on aged faces are generally harder to interpret than those of younger faces, and do not give older adults an advantage.

2.6.1.3 Subtle facial expressions

Although these newer image sets address some shortcomings of traditional stimuli, they are still susceptible to ceiling effects. Studies that incorporate photographs depicting full-blown, very intense or exaggerated expressions frequently report that most young adults, and many older adults, can correctly identify the emotion in more than 95% of trials for at least one of the emotions displayed; usually happiness (Campbell, Murray, Atkinson, & Ruffman, 2015; Ebner et al., 2011; Halberstadt, Ruffman, Murray, Taumoepeau, & Ryan, 2011). Half of the studies of happiness recognition reviewed by Ruffman et al. (2008) report no age-effects, with these studies frequently reporting that one or both age groups performed at, or close to, 100% accuracy for happiness recognition. Thus, full-intensity photographs may be insensitive to detect subtle differences in ability, which is problematic for any studies focusing on emotion-specific difficulties.

In an attempt to increase the sensitivity of these tools, some researchers have digitally altered the static photographs to reduce the intensity of the emotion displayed, such as the emotion mega-mixes in the Facial Expressions of Emotion: Stimuli and Test (FEEST; Young, Perrett, Calder, Sprengelmeyer, & Ekman, 2002). Specifically, the full-intensity photographs are morphed with images depicting either neutral or non-target emotional expressions. The emotions portrayed in the resulting morphed images are presumably more ambiguous, and harder to identify, and therefore are expected to be more sensitive to facial emotion recognition differences such as age-effects. Grainger, Henry, Phillips, Vanman, and Allen (2015) explored the impact of emotion intensity on facial emotion recognition age-effects and reported that subtle expressions disadvantaged older adults, but not younger adults, but only for dynamic stimuli. However, results from studies using morphed images have not been compared systematically to those using the original full-intensity photographs. Consequently, it remains to be established whether the magnitude of age-effects reported in Ruffman et al.'s (2008) review, which primarily comprised full-intensity facial emotion recognition tasks, might be an underestimation of older adults' difficulties.

2.6.1.4 Dynamic stimuli

While the use of expressions depicting subtler emotions, as well as varying the race and age of posers, has arguably made the stimulus sets more reflective of real life, other researchers motivated by concerns about the ecological validity of using static images (Isaacowitz & Stanley, 2011) have developed emotion recognition tasks that use dynamic (moving) stimuli. For example, video clips have been created in which actors' faces naturally transition from a neutral state into a display of a target emotion (e.g., Fölster, Hess, Huhnel, & Werheid, 2015; Grainger et al., 2015; Richter et al., 2011). A second form of dynamic stimuli, referred to as morphed animations, has also been developed which emulates the moving nature of videos, but uses pre-existing static stimuli and digital morphing software to

create a dynamic stimulus. For the latter, photographs displaying target emotions are digitally morphed from either photographs of neutral faces (e.g., Altamura et al., 2016; Di Domenico et al., 2015; Grainger et al., 2015) or from photographs of other, non-target emotions (e.g., Sullivan & Ruffman, 2004a). These animations serve to create a morphing sequence in which the target expression is initially absent but then slowly emerges.

On the one hand, because dynamic stimuli more closely emulate real-world expressions of emotion relative to static photographs, it has been suggested that dynamic stimuli may benefit older adults by allowing them to use well-practiced, crystallised strategies based on their more extensive real-world experiences (Isaacowitz & Stanley, 2011). In contrast, dynamic expressions likely involve a more rapid presentation of maximum intensity expressions and require quicker processing. Dynamic stimuli may therefore impose greater demands on processing speed, which could disadvantage older adults due to the well-documented age-related declines in this cognitive capacity (Hedden & Gabrieli, 2004). If so, variation in the speed of motion of the dynamic stimuli would then be expected to moderate the extent to which older adults are disadvantaged.

Given these competing influences of greater life experience versus processing demands, and the limited research directly comparing stimulus formats, it is unclear whether the provision of dynamic cues would be beneficial or detrimental to older adults. Sullivan, Ruffman, and Hutton (2007) co-varied for processing speed and fluid intelligence in their analyses. They found that older adults demonstrated difficulties accurately recognising emotion in morphed animations that were independent of these cognitive capacities. In two separate experiments, Grainger et al. (2015) compared facial emotion recognition age-effects on a static photograph task and a video-based task. Overall, Grainger et al. (2015) found no significant interaction between age and stimulus format, although they detected a complex interaction involving stimulus intensity whereby only dynamic stimuli presented at 50%

intensity provided older adults, but not young adults, with an advantage over static stimuli. In a recent meta-analysis, Goncalves et al. (2018) considered stimulus features (dynamic versus static) as a moderator of facial emotion recognition age-effects, and did not observe any significant differences across emotions. However, they only included one study in their review that had used dynamic stimuli. Given these competing influences of greater life experience versus speed, and the limited research directly comparing stimulus formats, it is unclear whether the provision of dynamic cues would be beneficial or detrimental to older adults.

2.6.1.5 Virtual agents

There are also images used that do not involve human posers, but rather are digital productions of virtual agents. Creation of digital avatars has a number of important strengths, in particular greater experimental control with respect to the features of the expressions. However, use of this stimulus format may disadvantage older adults who, due to generational differences, are not familiar with, or as motivated by, clearly artificial computer-based images. In a recent meta-analysis, Goncalves et al. (2018) found that this stimulus feature (i.e., virtual versus human faces) did not moderate facial emotion recognition age-effects. Thus, older adults demonstrate equal facial emotion recognition difficulties on human and virtual faces. However, Goncalves et al. (2018) categorised the stimuli used in Hunter, Phillips, and MacPherson (2010) as virtual agents, when the stimuli used in that study (images from the FEEST) involved digitally manipulated human faces. Furthermore, this small meta-analysis (n = 24) was not comprehensive, their findings may therefore need to be interpreted with caution.

2.6.1.6 Contextual cues

It has previously been argued that when older adults are given the opportunity to draw from their life experience and to process emotional information in context, they may not

exhibit the same level of difficulty as they do when completing lab-based tasks, which typically fail to closely represent facial emotion recognition as it occurs in daily life (Isaacowitz & Stanley, 2011). Furthermore, it has also been argued that tasks that more closely emulate real-world experiences tend to be more engaging and meaningful for older adults, relative to tasks that are lower in ecological validity (Gesn & Ickes, 1999; Hall & Schmid Mast, 2007; Zaki, Bolger, & Ochsner, 2008). Richter, Dietzel, and Kunzmann (2011) reported no age effects for a test of facial emotion recognition that involved videos of autobiographical accounts involving age-relevant contexts. Additionally, Wieck and Kunzmann (2017) found that older women demonstrated equal facial emotion recognition accuracy to young women when stimuli involved age-relevant context. It may therefore be that in everyday life, where recognition of facial expressions occurs in a contextual setting, age-related difficulties may be smaller than that observed in lab-based task void of context. This does not necessarily suggest that older adults do not have trouble recognising facial expressions of emotion in the real world, rather it may mean that in certain circumstances context helps them to compensate for their difficulties by relying on crystallised intelligence.

2.6.2 Neuroimaging studies

Not only do young and older adults differ in their emotion recognition behavioural performance, they also demonstrate different patterns of neural activation when viewing and/or labelling facial expressions of emotion. The first study to investigate neural activation in older adults' compared to young adults when viewing facial expressions of emotion reported that both age groups activated visual cortices and prefrontal gyri, but the young adults showed greater activation of the visual cortices and the older adults showed greater activations of the prefrontal regions (Gunning-Dixon et al., 2003). Additionally, the younger adults activated limbic regions, in particular the amygdala, and the older adults did not (Gunning-Dixon et al., 2003). The finding that older adults show reduced amygdala

activation and increased prefrontal activation compared to young adults when viewing facial expressions of emotions has since been replicated (Fischer, Nyberg, & Backman, 2010; Fischer et al., 2005; Iidaka et al., 2002; Tessitore et al., 2005). Collectively, the evidence suggest that older adults experience a dynamic neural shift towards processing emotional stimuli in prefrontal regions, which are areas known to be involved in language and complex cognitive processes, rather than the limbic regions, which are areas more typically involved in emotion processing (Tessitore et al., 2005).

There are two dominant perspectives on why older adults demonstrate a dynamic neural shift from activating the limbic regions in response to facial expressions of emotion to activating prefrontal regions. The first perspective is that the shift is compensatory. Specifically, older adults utilise prefrontal regions to recognise emotions to compensate for dampened amygdala functioning. This perspective may account for why older adults are not only less accurate at recognising emotions than young adults but slower than younger adults at recognising emotions, as rerouting the visual information through the cognitive/linguistic systems comes at the cost of efficiency (Tessitore et al., 2005). The second perspective is that older adults maintain amygdala functioning but demonstrate a change in the type of emotional stimuli for which amygdala activation occurs (Mather et al., 2004; St Jacques, Bessette-Symons, & Cabeza, 2009). Specifically, that older adults demonstrate similar amygdala activation to young adults when viewing emotional expressions that they perceive to have negative valence (Leclerc & Kensinger, 2008a; Mather et al., 2004; St Jacques, Dolcos, & Cabeza, 2010; Wright, Wedig, Williams, Rauch, & Albert, 2006), but demonstrate dampened amygdala activation when they perceive the stimuli to be neutral (St Jacques et al., 2009), as was the case or the emotional faces in the studies by Gunning-Dixon et al. (2003), Iidaka et al. (2002), and Tessitore et al. (2005). St Jacques, Bessette-Symons, and Cabeza (2009) highlight that this perspective is further supported by evidence that older adults

demonstrate dampened amygdala activation compared to young adults when viewing negative emotions but not positive emotions (Gutchess, Kensinger, & Schacter, 2007; Leclerc & Kensinger, 2008b; Mather et al., 2004); but see also (Williams et al., 2006).

In summary, while there is substantial evidence that older adults to demonstrate reduced amygdala activation in response to facial expressions of emotions and instead activate prefrontal regions, it appears that this finding is somewhat task-dependent. In particular, the valance attributed to the stimuli by the older adults seems to influence the areas of activation identified. Further work is required to clearly map how the different emotions and task variations map onto changes in the ageing brain.

2.7 Age-Related Emotion Recognition Decline Theories

The field of healthy ageing research is divided in terms of why older adults demonstrate inferior accuracy to young adults on facial emotion recognition tasks. Sociomotivational theories propose that older adults retain the ability to recognise emotions but are not motivated to engage in this process. Either because they are goal orientated to improve current experience and find recognition or negative emotions unpleasant, or because they want to conserve cognitive resources for more meaningful or relevant tasks. In contrast, neuropsychological theories suggest that there are tangible age-related declines in facial emotion recognition ability that have underlying biological mechanisms.

2.7.1 Neuropsychological models of age differences in emotion perception

Neuropsychological models link age-related declines in facial emotion recognition accuracy to structural and functional changes in key brain regions that underlie emotion recognition processing (Phillips, MacLean, & Allen, 2002). Such models therefore predict that there should be a direct correspondence between the degree of age-related brain change and subsequent functionality in the emotion recognition processes that are specifically

supported by these brain regions. The dominant neuropsychological theory focuses on structural degeneration of neural regions. However, a second neuropsychological theory, which will be discussed in Chapter 4 of this thesis, is that the production of key neurotransmitters involved in social cognitive processes diminishes with ageing.

In their earlier review, Ruffman et al. (2008) argued that the pattern of age-related change observed in facial emotion recognition was most consistent with a neuropsychological model, reflecting volumetric changes in frontal and temporal neural regions and/or changes in neurotransmitters. In particular, Ruffman et al. (2008) noted that anger, fear, and sadness are known to place particular demands on the orbitofrontal cortex, amygdala and cingulate cortex (Adolphs, 2002; Calder et al., 2001; Iidaka et al., 2001; Murphy et al., 2003; Phan et al., 2002; Phillips et al., 2003; Sprengelmeyer et al., 1998; Vuilleumier & Pourtois, 2007), and thus larger age-effects recognising these three emotions might be explained by known age-related volumetric reductions in these brain regions (Lamar & Resnick, 2004). The age-effect for recognition of happiness, however, might be smaller because the orbitofrontal and temporal regions are less involved during happiness recognition than during the recognition of negative emotions (Adolphs & Tranel, 2004; Iidaka et al., 2001). Note, however, that a recent meta-analysis revealed the temporal regions to be implicated in happiness recognition (Vytal & Hamann, 2010). Given that the basal ganglia are implicated in the recognition of disgust (Calder et al., 2001), the finding that older adults' disgust recognition was equal to, or even slightly better than, young adults', was proposed to be explained by the relative sparing of this neural region in the ageing process (Calder et al., 2003; Williams et al., 2006). This neuropsychological model predicts that the nature and magnitude of any age differences observed should vary across different specific emotions. However, as previously discussed in this chapter, since Ruffman et al.'s (2008) review many studies have reported different patterns of age-effects across emotions.

Ruffman et al. (2008), link the retention of disgust recognition to the relative sparing of the basal ganglia, yet this explanation is somewhat tenuous, given that instances of age-related reductions in volume have been documented for both the basal ganglia and insula (Casse-Perrot, Fakra, Jouve, & Blin, 2007; Raz & Rodrigue, 2006). In their review of affective neuroscience and ageing, Mather (2016) suggest that a more plausible explanation for older adults' superior disgust recognition is that different face-processing strategies are used by young and older adults. Specifically, evidence from eye-tracking research consistently indicates that older adults attend more to the lower half of the face, whereas young adults attend relatively more to the upper half (Circelli et al., 2013; Firestone, Turk-Browne, & Ryan, 2007; Grainger et al., 2015; Heisz & Ryan, 2011; Murphy & Isaacowitz, 2010; Sullivan et al., 2007; Wong et al., 2005). In an early paper on this topic, Wong et al. (2005) proposed that these differences might occur due to age effects in the area of the frontal lobes that control visual attention. Differences in the areas of the face that each age group attends to are potentially important, because Calder, Young, Keane, and Dean (2000) showed that fear, sadness, and anger were more recognisable from the eyes, whereas happiness and disgust were more recognisable from the mouth, thereby potentially giving older adults an advantage over young adults for disgust recognition. However, even this explanation lacks strong empirical support. Eye-tracking studies of older adults consistently show this visual attention bias towards the mouth region, and away from the eyes, but studies have failed to consistently show that these patterns of visual attention are related to emotion recognition accuracy (see Grainger et al., 2015).

2.7.2 Motivational theories of age differences in emotion perception

Alternative theoretical perspectives such as Socioemotional Selectivity Theory (SST, Carstensen, Isaacowitz, & Charles, 1999) and the selective engagement hypothesis (Hess, 2006) instead particularly emphasise older adults' motivation to engage with facial emotion

recognition tasks. In these models, motivation is argued to play a key role in determining the allocation of resources, with older adults argued to be more selective in their resource allocation relative to their young counterparts. In this context, selection represents an adaptive motivational process in response to age-related changes. Selection is influenced by capabilities (such as reduced cognitive resources; Hess et al., 2001) and developmental goals (such as increased prioritisation of emotion-related goals; Carstensen et al. , 2000).

Motivational models are also often argued to explain the information processing bias, called the positivity effect, whereby older adults prioritise positive stimuli over negative stimuli (Reed, Chan, and Mikels, 2014). Motivational models therefore also predict that older adults should be more motivated to attend to positive (relative to negative) expressions in facial emotion recognition tasks. Age-related preference for positive emotions is thought to be a reason for why older adults demonstrate a shift in preference for amygdala activation when viewing negative emotions, with the processing of emotions in the prefrontal cortex thought to provide older adults with greater control over balancing positive and negative emotional experience (Williams et al., 2006). The positivity bias is also thought to explain why older adults demonstrate difficulty recognising anger, fear, and sadness but not happiness. Yet, the selective retention of disgust recognition in healthy ageing is incongruent with a positivity bias.

2.7.3 Models including cognitive and motivational components

The Dynamic Integration Theory (DIT; Labouvie-Vief, 2009) is a different model that additionally emphasises the role of cognitive variables and also predicts age-related positivity preferences. A central tenet of this model is that older adults engage in two integrated processes to maintain emotional well-being (Labouvie-Vief, 2016). The first is *affect optimisation*, an almost automatic tendency to engage in processes that maintain well-being. The second is *affective complexity*, which refers to the ability to coordinate positive and

negative feelings into cognitively complex representations. Labouvie-Vief, Diehl, Jain, and Zhang (2007) propose that as older adults' cognitive resources decrease, their affective complexity declines, but they maintain emotional well-being by favouring affective optimisation. The DIT also highlights the protective function of crystallised intelligence and maintains that older adults should be able to rely on pre-existing, well-practiced strategies (Labouvie-Vief, 2009).

2.8 Chapter Summary

Facial expressions of emotion are universally a key form of non-verbal communication. There is substantial behavioural evidence that older adults demonstrate inferior accuracy to younger adults on facial emotion recognition tasks. There is also evidence that when viewing facial expressions of emotion, that older adults show dampened amygdala activation compared to younger adults, and increased prefrontal cortex activation. However, there are competing theories in terms of why older adults demonstrate a shift in neural activation and reduced task accuracy. Generally, there are two overarching theories. That is, older adults experience a neurological functional decline, and utilise their prefrontal cortex to compensate, but are therefore less accurate at recognising emotions. Alternatively, that older adults retain the ability to recognise emotions and the amygdala remains responsive in old age, but due to age-related motivational changes (e.g., a preference to maintain emotional well-being), older adults demonstrate a shift in terms of which stimuli they will attend to and process, and under what condition their amygdala is activated. The substantial variation in facial emotion recognition task design, makes it difficult to draw conclusions in terms of the specific pattern and magnitude of age-related facial emotion recognition difficulties. Thus, the following meta-analytic review explores the impact that task characteristics have on the pattern and magnitude of age-effects observed and discusses the findings within the context of cognitive load and motivational appeal.

CHAPTER 3: STUDY 1 – TASK CHARACTERISTICS INFLUENCE FACIAL EMOTION RECOGNITION AGE-EFFECTS: A META-ANALYTIC REVIEW

3.1 Chapter Guide

The following chapter comprises a comprehensive meta-analytic review of 81 studies comparing young and older adults' facial emotion recognition accuracy. The studies used multiple different task types, and as such, several task characteristics were included as potential moderators in the multi-level models; the number of response options included in the task, the stimulus format (e.g., video, photographs of full-intensity expressions, schematics, etc.), and the image set from which the stimuli were sourced. The review statistically tested the moderating effect of these features (both individually and combined), statistically compared the magnitude of effects produced by different task types, and qualitatively compared the patterns of results produced across emotion types by each task type. The review showed that these task features did account for a significant proportion of the variance in age-effects reported across the field of facial emotion recognition and ageing. Further, trends were uncovered for specific task types (e.g., tasks involving videos and the POFA image set), that are discussed in relation to their theoretical implications. This review has been published in *Psychology and Aging* (see 'Research Outputs', pp. 15). To maintain a publishable manuscript length, task features for which the data was limited (i.e., were represented by 2-3 data sets) were presented as supplementary materials, which are presented directly following the manuscript; see '3.7 Supplementary Material'. Preliminary findings from this review were presented at the Australian Catholic University Psychology Conference, 24th September 2018 (see 'Research Outputs', pp. 15).

3.2 Abstract

Relative to their young counterparts, older adults are poorer at recognising facial expressions. A 2008 meta-analysis of 17 facial emotion recognition data sets showed that these age-related difficulties are not uniform. Rather, they are greatest for the emotions of anger, fear, and sadness, comparative to happiness and surprise, with no age-effect found for disgust. Since then, there have been many methodological advances in assessing emotion recognition. The current comprehensive meta-analysis systematically tested the influence of task characteristics (e.g., photographs versus videos). The meta-analysis included 102 data sets that compared facial emotion recognition in older and young adult samples (N = 10,526). With task type combined, the pattern of age-effects across emotions was mostly consistent with the previous meta-analysis (i.e., largest age-effects for anger, fear, sadness; no effect for disgust). However, the magnitude and direction of age-effects were strongly influenced by elements of task design. Specifically, video-based tasks produced relatively moderate age-effects across *all* emotions, which indicates that older adults may not exhibit a positivity effect for facial emotion recognition. For disgust recognition, older adults demonstrated superior accuracy to young adults for the most common image set (Pictures of Facial Affect). However, they were poorer than young adults at recognising this emotion for all other stimulus formats and image sets, which suggests they do no retain disgust recognition. We discuss the implications that such diversity in the age-effects produced by different facial emotion recognition task designs has for understanding real-world deficits and task selection in future emotion recognition studies.

3.3 Introduction

Social cognitive function is the ability to interpret people's emotions and intentions and to respond appropriately. While social cognitive abnormalities are common in neurodegenerative diseases such as Huntington's disease, Alzheimer's disease, and frontotemporal dementia (Kordsachia, Labuschagne, Andrews, & Stout, 2018a; Kordsachia, Labuschagne, & Stout, 2017; Phillips et al., 2010), considerable literature indicates that difficulties are also evident in healthy ageing (Labuschagne, Pedder, Henry, Terrett, & Rendell, 2019; Moran, 2013). Moreover, because of their links with interpersonal functioning, social cognitive abnormalities are a critical predictor of mental health and well-being in older adults (Phillips et al., 2010).

Research into social cognition in ageing has focused predominantly on fundamental social skills, such as the ability to recognise *facial* expressions of emotion, as shown in a meta-analysis of 13 studies (17 datasets) (Ruffman et al., 2008). That pivotal review indicated that age-related differences in facial emotion recognition accuracy were not uniform; that is, older adults were significantly less accurate than young adults in recognising anger, fear, and sadness (*r*s ranged: .27-.34), and marginally, yet still significantly, worse at recognising happiness and surprise (*r*s ranged: .07-.08). No significant age-effect was apparent for disgust recognition, but a trend emerged whereby older adults were more accurate than young adults (r = -.11) (Ruffman et al., 2008).

3.3.1 Then and now: The need for an updated meta-analytic review

Since Ruffman et al.'s (2008) meta-analysis, research into facial emotion recognition in ageing has increased substantially, and many studies have failed to replicate the pattern of age-effects reported by Ruffman et al. (2008). Some studies have reported a null, or non-significant, age-effect for anger, fear or sadness (e.g., Demenescu, Mathiak, & Mathiak,

2014; Fölster et al., 2015; Horning, 2012; Orgeta & Phillips, 2008). Other studies have reported a null effect for happiness (e.g., Ebner et al., 2012; Hot et al., 2013; Keightley et al., 2007), or a small effect in favour of older adults (e.g., Ruffman et al., 2009b). Moreover, the trend identified by Ruffman et al. (2008) for disgust recognition to be slightly enhanced in late adulthood, was contrasted in recent studies that reported a disgust age-effect instead favouring young adults (e.g., Chaby et al., 2015; Demenescu, Mathiak, & Mathiak, 2014; Ngo & Isaacowitz, 2015).

A likely explanation for the mixed findings is the diversity of stimuli and methodological designs used to measure facial emotion recognition. Most of the studies in Ruffman et al.'s (2008) meta-analysis (12 of the 17 datasets) used identical assessment protocols. Specifically, participants were presented with static photographs from the Pictures of Facial Affect (POFA; Ekman & Friesen, 1976), depicting the six basic facial expressions proposed by Ekman (2008) (i.e., anger, disgust, fear, happiness, sadness, and surprise), and were instructed to select a word-label that categorised each expression. A further four datasets used the same task design but with photographs from the Japanese and Caucasian Facial Expressions of Emotion (JACFEE; Matsumoto, 1998). While many researchers still use traditional task designs of this type, other recent studies are using different stimuli and methodological designs.

3.3.2 Models of age differences in emotion perception

Though multiple theories account for age-related declines of emotion perception, these theories largely comprise two overarching mechanisms; biology and motivation. Biologically, general cognitive decline associated with age-related neurodegeneration could account for older adults' emotion perception deficits. Demands are imposed on cognitive processes, such as working memory and processing speed, when viewing and processing facial displays of emotions (Mathersul et al., 2009; Phillips et al., 2008). Thus, emotion

recognition deficits can be inflated when tasks are cognitively demanding. For example, for dynamic expressions, faster transitions in the facial stimuli would likely increase the demands on processing speed. This could incrementally disadvantage older adults due to the well-documented age-related declines in speed of processing (Hedden & Gabrieli, 2004). However, if general cognitive decline fully accounted for older adults' emotion perception difficulties, older adults should demonstrate equal difficulty for all emotional expressions.

Instead, Ruffman et al. (2008) proposed that brain regions specifically associated with the processing of anger, fear, and sadness, such as the orbitofrontal cortex, amygdala, and cingulate cortex (Adolphs, 2002; Murphy et al., 2003; Sprengelmeyer et al., 1998) are regions that are particularly susceptible to age-related volumetric reductions (Lamar & Resnick, 2004). Whereas, the retention of disgust recognition in ageing is explained by the relative sparing of the basal ganglia and insula (Calder et al., 2003; Williams et al., 2006); brain regions implicated in disgust recognition (Calder et al., 2001). However, this explanation for the retention of disgust recognition is somewhat tenuous, given known age-related volumetric reductions for both the insula and parts of the basal ganglia (Persson et al., 2014; Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010).

From a motivational perspective, the trend for older adults to be less impaired in recognising happiness than anger, fear and sadness has been attributed to the 'positivity effect' (e.g., Kellough & Knight, 2012), a well-established pattern of findings showing that older adults demonstrate a preference, through attention and improved task performance, for positive stimuli over negative stimuli (Reed et al., 2014). Several models account for the positivity effect in different ways. According to Socioemotional Selectivity Theory (SST), older adults demonstrate a developmental shift in goals from being future-orientated to present-orientate (Carstensen et al., 2000), and positive stimuli are in the moment more pleasant to attend to (Reed & Carstensen, 2012). Alternatively, the Dynamic Integration

Theory (DIT; Labouvie-Vief, 2009) emphasises the role of cognitive variables. A central tenet of this model is that older adults engage in two integrated processes to maintain emotional well-being (Labouvie-Vief, 2016). The first is *affect optimisation*, an almost automatic tendency to engage in processes that maintain well-being. The second is *affective complexity*, which refers to the ability to coordinate positive and negative feelings into cognitively complex representations. Labouvie-Vief et al. (2007) propose that as older adults' cognitive resources decrease, their affective complexity declines, but they maintain emotional well-being by favouring affective optimisation. Notably, the retention of disgust recognition with age is difficult to reconcile with a positivity effect, regardless of the theoretical model, as disgust would be categorised as a negative expression if a simple positivity/negativity distinction is to be made.

The DIT also highlights the protective function of crystallised intelligence, and maintains that older adults should be able to rely on pre-existing, well-practiced strategies (Labouvie-Vief, 2009). Therefore, lab-based tasks that do not emulate real-world expressions of emotions (i.e., are static and devoid of context), may inflate the age-effects observed by preventing older adults from drawing on their life experience to process the emotional stimuli (Isaacowitz & Stanley, 2011). There is even evidence that including age-relevant context in some facial emotion recognition tasks eliminates age-effects (Richter et al., 2011; Wieck & Kunzmann, 2015). However, it should be noted that these tasks being more relevant means that they are also more motivationally engaging, and thus the impact of crystalised intelligence and increased motivation cannot be disentangled.

A third motivation model, the selective engagement hypothesis, proposes that because older adults' general cognitive abilities are more limited than young adults', to reduce unnecessary mental strain, older adults are more selective of when they engage their limited resources (Hess et al., 2001). Older adults are thought to be more motivated to allocate their

limited resources, and therefore demonstrate superior performances to tasks that have greater personal implications, seem personally relevant, or have social accountability (Richter et al., 2011; Wieck & Kunzmann, 2015). Zhang et al. (2013) were able to eliminate emotion recognition age-effects by inflating participants' perceived closeness to the posers. Notably, their paradigm tested for the influence of motivation separate to the influence of context, thus providing evidence for the selective engagement hypothesis. Though novel tasks can often be intrinsically motivating (Baldassarre & Mirolli, 2013), tasks that more closely emulate real-world experiences tend to be more engaging and meaningful for older adults, relative to tasks lower in ecological validity (Gesn & Ickes, 1999; Hall & Schmid Mast, 2007; Zaki et al., 2008). For example, older adults may be more motivated to allocate cognitive resources to recognising emotion on age-congruent posers (Hess et al., 2001). Alternatively, older adults may be more motivated by tasks involving dynamic stimuli, as they may appear less artificial and more true to life relative to static photographs (Phillips, Slessor, Bailey, & Henry, 2013). Thus, studies using stimuli incorporating these features might be expected to produce weaker age-effects.

This brief review of theories relevant to facial emotion recognition highlights how several aspects of task design could potentially impact the pattern of results produced by different emotion recognition studies. A more recent meta-analysis on facial emotion recognition in healthy ageing (Goncalves et al., 2018) explored the influence of stimulus format on age-effects. Results suggested that age-effects for disgust recognition may be greater when greyscale photographs are used compared to colour, and age-effects for fear recognition may be greater when virtual faces are used compared to human faces; see '3.7 Supplementary Material' for further detail about tasks involving virtual agents. No other stimulus-related moderating factors were identified. However, these findings need to be interpreted with caution because the analysis was based on only 24 empirical studies, and

only one study involved dynamic stimuli. Furthermore, the influence of other task design features, such as the intensity of the emotion, was not considered. Thus, one of the goals of the current meta-analysis was to use broad search terms and inclusion criteria to enable a more comprehensive exploration of the influence of task design on the patterns of age-effects across the literature.

3.3.3 Aims and hypotheses of the current meta-analysis

The purpose of the present meta-analysis was to investigate the impact that variation in task design has on the magnitude and pattern of age effects produced. Moreover, we aimed to determine whether the pattern of age-effects reported by Ruffman et al. (2008) is evident across different facial emotion recognition task designs. Thus, we considered all facial emotion recognition task designs currently used in the healthy ageing literature. However, to limit the length of this meta-analysis, in what is an ever-expanding field, less commonly used task designs (i.e., stimuli involving morphed animations or virtual agents, and tasks that provided participants an either-or choice rather than multiple response options) were included in the meta-analysis but presented as supplementary materials; see '3.7 Supplementary Material'. These tasks had features of interest, but because they are less commonly used, the low *n* values made it challenging to make meaningful interpretations of the results. It was important to control for the number of response options (i.e., two options vs three or more options) because giving participants an 'either-or' choice allows for the use of elimination. This cognitive strategy is less demanding on working memory (Phillips et al., 2008), and therefore tasks allowing the use of elimination may be less confounded by age-related declines in general cognitive ability (Hedden & Gabrieli, 2004).

To further contain our meta-analysis, we chose to only consider age-effects for explicit facial emotion recognition. We, therefore, did not consider facial emotion recognition within the context of multimodal emotion recognition (e.g., Banziger, Mortillaro, & Scherer,

2012; Paulmann & Pell, 2011), or multidimensional ratings such as rating the valence of the expression, the level of arousal, or the attractiveness of the face (e.g., Ebner, 2008). This allowed us to pursue our primary goal of investigating the impact of key task design differences on the pattern of age-effects produced across emotions.

Our first aim was to integrate the current literature and to test whether the pattern of age-effects across emotion types identified by Ruffman et al. (2008) is robust. We hypothesised that (a) older adults would demonstrate *overall* poorer facial emotion recognition than young adults, even with the inclusion of newer stimulus formats and task designs, as this remains the predominant finding reported across individual studies. However, of greater interest was (b) the pattern of age-effects across individual emotion types. We aimed to establish whether the same pattern of age-effects was seen as in previous meta-analyses (Goncalves et al., 2018; Ruffman et al., 2008a). The pattern observed in previous meta-analyses (i.e., retention of disgust recognition) was most parsimoniously explained by variation in the degree of degeneration of different brain regions implicated in emotion recognition. However, if uniform difficulties across all emotions emerged, it would give greater emphasis to the role of general cognitive decline. Conversely, stronger deficits for recognition of negative emotions comparative to positive emotions would fit the motivational models SST and DIT. We also anticipated that different tasks would produce different patterns of age-effects across emotions, however, we were unable to predict specific patterns.

The second aim was to systematically examine how different stimuli used in facial emotion recognition tasks influence the magnitude of the age-effects produced. One of the shortcomings of traditional stimuli is that they are susceptible to ceiling effects, which can limit sensitivity to age differences. Studies that incorporate photographs depicting very intense or exaggerated expressions, frequently report that most young and older adults correctly identify more than 95% of trials for at least one of the emotions displayed, usually

happiness (Campbell et al., 2015; Ebner et al., 2011; Halberstadt et al., 2011). In response, some researchers have digitally altered the static photographs to reduce the intensity of the emotion displayed, thereby producing image sets such as the emotion megamixes in the Facial Expressions of Emotions: Stimuli and Tests (Young et al.). Specifically, the full-intensity photographs are morphed with images depicting either neutral or non-target emotional expressions. The emotions portrayed in the resulting morphed images are presumably more ambiguous, and harder to identify. Thus, tasks involving reduced-intensity photographs would be less susceptible to ceiling effects and better able to discriminate between groups. However, these tasks would also likely place increased load on general cognitive skills, and according to a general cognitive decline model, would disadvantage older adults. We, therefore, hypothesised that (a) stimuli intensity would account for significant variation in age effect magnitudes.

Other researchers, motivated by concerns about the ecological validity of static images (Isaacowitz & Stanley, 2011), have developed tasks that use dynamic (moving) stimuli. Video clips have been created in which either actors or real protagonists relive or demonstrate a target emotion (e.g., Fölster et al., 2015; Grainger et al., 2015; Richter et al., 2011). For (b) video stimuli, two opposing hypotheses could be put forward. According to DIT, life experience should favour older adults when presented with more naturalistic dynamic cues. However, from a general cognitive decline perspective, the fleeting nature of dynamic cues also places more load on rapid attentional processing, which is likely to disadvantage older adults. In line with the Selective Engagement Hypothesis, older adults could also be more motivated by videos due to their realistic nature, or might still find the depictions of emotions by actors too detached from reality to be motivating. Therefore, we were unsure of what magnitude of effects to expect.

In addition to stimulus format, we also identified that (c) *image set* was a variable that warranted exploratory moderator analysis. The POFA is the original and most commonly used set of photographs and depicts black-and-white photographs of young to middle-aged adult Caucasian posers displaying the six basic emotions (Ekman & Friesen, 1976). However, alternative image sets have also been developed that use colour photographs, include a wider variety of ages and racial backgrounds, and have other distinguishing features. For example, the posers in the 'FACES' image set range in age (Ebner et al., 2010), and those in the JACFEE represent two racial backgrounds (Matsumoto, 1998). In the 'NimStim' set of facial expressions (Tottenham et al., 2009), an open and closed mouth version of each expression is included. Ruffman et al. (2008) were limited in terms of analysing variance across image sets because 12 studies in their meta-analysis used the POFA, only four used the JACFEE, and none used alternative image sets such as FACES or NimStim.

3.4 Method

3.4.1 Literature search and study selection

We adhered to PRISMA guidelines for this meta-analysis (Moher, Liberati, Tetzlaff, Altman, & Group, 2010). A search was conducted to identify studies that assessed facial emotion recognition accuracy and reported data for both older adult and young adult samples. The following databases were searched: PsychINFO, MEDLINE Complete, CINAHL, Psychology and Behavioral Science Collection, and Complete Scopus. Final searches were performed in July 2017. Abstracts were initially screened for clear ineligibility (i.e., animal studies, no mention of older adults, literature reviews, or irrelevant measures). A screen of the full-text papers was then conducted by two independent reviewers on papers that passed the initial abstract screen. Differences in eligibility decisions were discussed by the reviewers until consensus was achieved. We checked the reference lists of included studies, but no additional eligible studies were identified.

3.4.2 Inclusion criteria

Studies were eligible for inclusion if: (a) a young and older adult group were included, and the groups were discrete samples rather than a single sample on an age continuum; (b) the young adult sample had a mean age equal ≤30 years; (c) the older adult sample had a mean age ≥65 years, consistent with the minimum and maximum mean ages identified by Ruffman et al. (2008); (d) both samples were healthy adults; (e) the manuscripts were available in full-text format; (f) the manuscripts were published in English, or there was an English translation available; and (g) the statistics published, or provided by the author upon request, could be used to calculate a precise group comparison effect size, for example: sample sizes, means, and standard deviations.

3.4.3 Facial emotion recognition measures

To ensure that the studies had comparable outcomes (i.e., facial emotion *recognition* accuracy), additional inclusion criteria relating to the facial emotion recognition task were applied. Specifically: (a) the variable measured by the task had to be *recognition* of facial emotion rather than the attribution of a rating of valence (i.e., the degree to which the expression is positive or negative) or a rating of arousal. A caveat here is that studies that used valence ratings to formulate a recognition accuracy score were included (e.g., Richter et al., 2011); (b) the stimuli were human faces or a virtual agent, not a line drawing (cartoon) or emoticon; (c) the task measured facial emotion recognition in isolation from alternative modalities (i.e., voices or bodies expressing emotion), and thus studies were excluded if they required participants to match facial expressions to the same emotion portrayed by other modalities; (d) the participants were instructed to classify or label the emotion displayed rather than discriminate between emotions (e.g., to decide whether two faces expressed the same or different emotions); and (e) the stimuli included at least two of the six universal emotions identified by Ekman, rather than only one emotion alongside a neutral expression,

so that the task required participants to recognise emotion types rather than determine the presence or absence of emotion.

3.4.4 Search terms

Search terms related broadly to the action of recognising or interpreting emotion, combined with various terms related to facial stimuli or facial expressions, in addition to commonly used labels for ageing or older adults. For example, for databases hosted by Ebsco the search terms were: [(recogni* OR perception OR perceive OR process* OR label* OR decod* OR identif* OR communicat*) N2 emotion*] OR [(recogni* OR perception OR perceive OR process* OR label* OR decod* OR identif* OR communicat*) N2 expression*] OR [mood N2 recognition] OR ["affect recognition"] OR ["emotion* decision making"] OR ["social decision making"] AND [facial OR emotion* OR face* OR photograph*] AND [elderly OR (old N2 age) OR "older adult" OR "older people" OR "older participants" OR "age related" OR aging]. Relevant subject headings were also identified and added to the search (e.g., "emotional intelligence", "facial emotions and expressions", "older people").

3.4.5 Data extraction

For each study, the demographic data extracted were; the mean age of the young and older adult groups, and the number of participants in each group. For facial emotion recognition, all reported age-effects (e.g., *r*, *d*) were extracted. If this data was unavailable, the mean accuracy and standard deviation, or error, were extracted for each age group. Otherwise, alternate statistics from which effect sizes could be precisely calculated (e.g., *t*, χ^2) were extracted. The following data were also obtained for each study; nature of stimuli posers (e.g., human, virtual agent); format of stimuli used (e.g., full-intensity static photographs, reduced-intensity static images, morphed-animations); task format (e.g., forced-

choice labelling, go-no-go); type of photograph (e.g., greyscale, full colour); number of response options included in the task; and image set from which stimuli was obtained.

The first author extracted all data and, if required, contacted the first and/or corresponding author of each manuscript to request missing data. In instances where the same data were published across multiple publications, we selected the publication that reported on the largest sample or that reported the most relevant statistics. Where the degree of sample overlap was unclear, authors were contacted to confirm whether the participants in their multiple manuscripts were of the same cohort. In '3.7 Supplementary Material', we provide a list of the publications that were excluded from this meta-analytic review because either the necessary data to calculate effect sizes was not available or the participant data overlapped with another publication.

Where articles reported data from more than two discrete age groups (e.g., Mill et al., 2009), results were collapsed for the age groups that yielded a sample within the eligibility age restraints. Where studies reported more than one facial emotion recognition outcome, i.e., a static task and a dynamic task (e.g., Grainger, 2015) or the same measure under varying conditions, i.e., familiar vs. unfamiliar faces (e.g., Stanley & Isaacowitz, 2015), each outcome was included, and the dependency between the outcomes was accounted for in the multivariate meta-analysis model. For studies that reported raw hit rates and had also calculated a post-hoc unbiased hit rate (i.e., Fölster et al., 2015; Orgeta, 2010; Zhao, Zimmer, Shen, Chen, & Fu, 2016), the raw scores were used so that any difference in the age-effects detected across studies could be attributed to differences in task design. Given that only four of the included studies reported unbiased hit rates, we were unable to contrast raw score effects versus unbiased hit rates effects.

3.4.6 Data analyses

Comprehensive Meta-Analysis software was used to generate effect sizes as *Hedge's g* for each outcome of each study (i.e., each emotion and measure). Multivariate meta-analysis models were then fitted to these effect sizes using the rma.mv function of the 'metafor' package (Viechtbauer, 2010) in the statistical software environment R (R Core Team, 2018). Random-effect models were used for all analyses because this type of modelling is better suited for heterogeneous effect distributions (Lipsey & Wilson, 2001). Random effects were specified on both the outcome (emotion and measure) and study level. By specifying random effects at both of these grouping levels it meant that a variance-covariance matrix was generated whereby the same random effect was assigned to effects that were dependent, whereas effects that were independent were assigned a different random effect. Knapp and Hartung's (Knapp & Hartung, 2003) adjustment was applied to all models to reduce the number of unjustified significant results (Assink & Wibbelink, 2016). Robust variance estimations and robust *t*-tests for each of the coefficients were calculated, due to the inclusion of dependent effect sizes (Tipton & Pustejovsky, 2015), using the 'clubsandwich' package for R (Pustejovsky & Tipton, 2017). Wald-tests were then used to conduct the *F*-tests comparing variance across groups of coefficients (i.e., all the coefficients representing a stimulus format). To correct for sampling error, the inverse of the model-implied variance-covariance matrix was used to weight studies.

To ascertain the degree to which older adults differ from young adults in their overall facial emotion recognition accuracy (Aim 1a), a three-level meta-analytic model was fitted to the data, with the intercept representing overall facial emotion recognition. Though we initially tried to fit a model to the data that would allow between-study heterogeneity to vary depending on emotion type, task, or combination of emotion type and task, and moderate for emotion type, this model did not converge. Notably, most papers reported multiple facial

emotion recognition age-effects. Specifically, an age-effect for each emotion and task (i.e., dynamic task and static task). If each study had reported an age-effect for every emotion this would have been a simple model to fit. However, this was a complex data structure, with studies reporting different combinations of emotions and tasks, and multiple estimates for the same combination of emotion and task (i.e., two measures of dynamic anger). Thus, the model we report on for overall emotion recognition was useful only as a means of estimating as single aggregate effect size as could not moderate for emotion type.

As the recognition of different emotions are arguable different outcomes (e.g., anger recognition accuracy is not the same as happiness recognition) we decided that a more appropriate way to approach this complex data structure was to separate data by emotion type. Thus, age-effects for anger recognition, for example, were only modelled with other anger effects. This approach improved the fit of the models while still allowing us to qualitatively observe the pattern of age-effects produced across emotions (Aim 1b). To analyse the moderating effect of different stimuli on age-effect magnitudes (Aim 2), in each emotion model we also included stimulus format (e.g., full-intensity photographs, reduced-intensity photographs, videos) and image set (e.g., POFA, JACFEE, FACES) as moderators. Note, however, that the number of response options (two vs. 3+) was included as a third moderator, to account for differences in the cognitive load of tasks involving only two response options compared to those that gave participants three or more response options; see '3.7 Supplementary Material' for further detail.

While each study had used one of five different stimulus format, with each subgroup well represented, this was not the case for the different image sets. The studies contributing to this meta-analysis used stimuli from 19 different image sets, and that is not including the ten studies that created their own stimuli. The most commonly used stimuli were: POFA (k = 24), FACES (k = 9), JACFEE (k = 8), NimStim (k = 7), Penn Emotion Recognition Test (ER-

40; Gur et al., 2001) (k = 3), Diagnostic Analysis of Nonverbal Behaviour (DANVA 2; Nowicki & Duke, 2001) (k = 3), and Montreal Set of Facial Displays of Emotion (MSFDE; Beaupré & Hess, 2005) (k = 3). We initially tried coding all studies by their image set used, even if they were the only study to use it, however, this resulted in too many single k subgroups and the model was over-fitted. We, therefore, chose to only consider image sets that had been used by at least four of the studies included in this meta-analysis and coded the remaining image sets as 'other'.

We used Wald tests to test the moderating effect of each variable while accounting for the other moderators. For example, the moderating effect of stimulus format while adjusting for image set used and the number of response options included. The individual robust t-tests within each model provided a means of statistically contrasting the effect estimates. For stimulus format, we contrasted the effects of full-intensity photographs and reduced-intensity photographs (Aim 2a), and full-intensity photographs and videos (Aim 2b). For image set (Aim 2c), we contrasted the effects of POFA and FACES, POFA and JACFEE, and POFA and NimStim. These contrasts also adjusted for the other moderating variables. However, when reporting the effect estimates for each task type across emotions (i.e., each stimulus format and each image set) (Aim 1b), we reported effects collapsed across the other moderators. While it would have been interesting to report effects for combinations of moderators (e.g., studies that used reduced-intensity photographs sources from the FACES database and included multiple response options), most combinations of moderators were only represented by one or two effects. Such small k values made this data uninterpretable.

3.5 Results

3.5.1 Included articles

The initial literature search yielded a total of 5,327 articles. Removing duplicates reduced this to 3,785 articles. The titles and abstracts were screened, and 371 articles were retained and subjected to a full-text review. Ultimately, 81 studies were deemed to meet inclusion criteria; Table 3.1 for demographic information. Figure 3.1 summarises the screening process.

Table 3.1

Demographic information for each study included in the meta-analysis including participant characteristics and details of task/stimuli.

Study/Sub-study		Participant Characteristics				Facial Emotion Recognition Task				
		n		Age (*M*)						
		Y	O	Y	O	Image set	Col.	Stimulus format	Task Type	No. Emo.
Allen, Lien, and Jardin (2017)	1.	20	20	21.7	68.1	NimStim	Y	Full-intensity Photographs Reduced-intensity Photographs	Labelling (2 labels)	2
	2.	22	22	21.7	68	NimStim	Y	Full-intensity Photographs Reduced-intensity Photographs	Labelling (2 labels)	2
Altamura et al. (2016)		20	20	24	71.2	KDEF	Y	Morphed-animations	Labelling (2 labels)	2
Baena, Allen, Kaut, and Hall (2010)		39	39	23.67	69.9	-	-	Virtual Agents	Labelling	3
Bailey et al. (2009)		34	33	19.7	73.7	POFA	N	Full-intensity Photographs	Go-no-go	2
Beer, Fisk, and Rogers (2010)		42	42	19.74	72.48	MSFDE	N	Reduced-intensity Photographs	Labelling	3+N
						Wilson, Loffler, and Wilkinson (2002)	N	Virtual Agents[a]	Labelling	3+N
						Created for study	N	Virtual Agents	Labelling	3+N
Calder et al. (2003)	1.	24	24	25	65.08	POFA	N	Full-intensity Photographs	Labelling	6

	2a.	73	58	24.3	65.24	POFA		Full-intensity Photographs	Labelling	6
	2b.	28	23	23.93	66.48	POFA (Emotion Hexagon)		Reduced-intensity Photographs	Labelling	
Campbell et al. 2014		17	17	19.68	72.07	FACES	Y	Full-intensity Photographs	Labelling	5+N
Campbell, Murray, Atkinson, and Ruffman (2015)		32	32	20.4	71	FACES	Y	Full-intensity Photographs	Labelling	5
Casares-Guillen, Garcia-Rodriguez, Delgado, and Ellgring (2016)		40	24	26.9	75.38	Created for study	Y	Virtual Agents	Labelling	6
Chaby, Boullay, Chetouani, and Plaza (2015)		31	31	25.8	67.2	KDEF	Y	Full-intensity Photographs	Labelling	5+N
Chaby, Hupont, Avril, Luherne-du Boullay, and Chetouani (2017)		22	22	22.18	70.4	FACES	Y	Full-intensity Photographs	Labelling	5+N
Circelli, Clark et al. (2013)		16	16	19.2	68.9	POFA	N	Full-intensity Photographs	Labelling	6+N
Davis and Chute (2002)		16	16	20.6	69.1	ER-40	Y	Reduced-intensity Photographs	Labelling	4+N
Demenescu et al. (2014)		21	15	25.76	63.8	ER-40	Y	Full-intensity Photographs	Labelling	5+N
Di Domenico et al. (2015)		40	40	23.63	70.25	KDEF	Y	Morphed-animations	Labelling (2 labels)	2
Ebner and Johnson (2009)		32	24	19.3	74.8	FACES	Y	Full-intensity Photographs	Labelling	2+N
Ebner et al. (2010)		30	20	25.95	73.59	FACES	Y	Full-intensity Photographs	Labelling	5+N

Ebner et al. (2011)		46	33	22.62	73.52	FACES	Y	Full-intensity Photographs	Labelling	5+N
Ebner, Johnson, and Fischer (2012)		30	30	25.1	68.2	FACES	Y	Full-intensity Photographs	Labelling	2+N
Fölster et al. (2015)		30	30	23.93	70.33	Created for study	N	Videos	Labelling	5
Grainger et al. (2015)	1.	42	39	26	74	FEEST* (Morphed Continua)	N	Morphed-animations	Labelling	6
						FEEST* (Ekman 60)		Full-intensity Photographs		
						FEEST* (Emotion Megamixes)		Reduced-intensity Photographs		
	2.	40	44	18.48	73.5	ADFES	Y	Videos	Labelling	7
								Full-intensity Photographs		
Halberstadt et al. (2011)		60	61	20.5	70.5	FEEST*	N	Full-intensity Photographs	Labelling	6
Hall (2001)		17	33	-	-	NYEB	N	Full-intensity Photographs	Labelling	8
Haskins (2013)		66	61	18.82	69.66	DANVA 2	Y	Reduced-intensity Photographs	Labelling	4
Henry et al. (2008)		30	30	19.4	76.9	FEEST*	N	Full-intensity Photographs	Labelling	6
Horning (2012)		289	162	23.31	74.48	Created from POFA	N	Morphed-animations	Labelling	6
Hühnel, Fölster, Werheid, and Hess (2014)		38	37	23.7	71.4	Fölster et al. (2015)	Y	Videos	Intensity rating	4
	1.	25	25	22.64	66.96	FEEST*	N		Labelling	6

Hunter, Phillips, and MacPherson (2010)	2.	20	20	20	70.55	POFA	N	Full-intensity Photographs		4
Isaacowitz et al. (2007)		189	78	27.05	71.9	POFA	N	Full-intensity Photographs	Labelling	6+N
Keightley, Winocur, Burianova, Hongwanishkul, and Grady (2006)		30	30	25.7	69.6	JACFEE	Y	Full-intensity Photographs	Labelling	7+N
Keightley, Chiew, Winocur, and Grady (2007)		10	11	27.2	72.5	JACFEE	Y	Full-intensity Photographs	Labelling	6+N
Kessels, Montagne, Hendriks, Perrett, and de Haan (2014)		124	12	27.22	69.5	Perrett Lab Emotion Recognition Task[b]	N	Morphed-animations	Labelling	6
Krause (2015)		19	52	-	-	NYEB	N	Full-intensity Photographs	Labelling	8
Krendl and Ambady (2010)		36	42	19.8	75.8	DANVA2	Y	Reduced-intensity Photographs	Labelling	4
Krendl, Rule, and Ambady (2014)		30	32	23.1	70.7	DANVA2	Y	Reduced-intensity Photographs	Labelling	4
Larcom (2014)		80	80	18.97	72.23	NimStim	Y	Full-intensity Photographs	Labelling	3
Leime, Rique Neto, Alves, and Torro-Alves (2013)		19	9	20.1	74.7	NimStim	Y	Reduced-intensity Photographs	Labelling	4
Liao, Wang, Lin, Chan, and Zhang (2017)		31	30	23.52	67.77	Chinese Facial Affect	N	Full-intensity Photographs	Labelling	5+N
Nakamura, Osonoi, and Terauchi (2010)		30	30	21.37	70.3	Chinese Facial Affect	N	Full-intensity Photographs	Go/no-go	2+N

MacPherson, Phillips, and Della Sala (2002)		30	30	28.8	69.9	JACFEE	Y	Full-intensity Photographs	Labelling	7
MacPherson, Phillips, and Della Sala (2006)		29	29	29	69.8	JACFEE	Y	Full-intensity Photographs	Labelling	7
Maki, Yoshida, Yamaguchi, and Yamaguchi (2013)		25	17	18.9	76.8	DB99[c]	Y	Reduced-intensity Photographs	Expression matching	6
Maria Sarabia-Cobo, Navas, Ellgring, and Garcia-Rodriguez (2016)		50	57	28.5	78.19	Created for study	Y	Virtual Agents	Labelling	6
Marquardt, Levitt, Sherrard, and Cannito (2014)		20	20	26	65.4	Created for study	-	Videos	Labelling	4
McDowell, Harrison, and Demaree (1994)		30	30	19.47	74.03	POFA	N	Full-intensity Photographs	Labelling	4+N
Mienaltowski, Corballis, Blanchard-Fields, Parks, and Hilimire (2011)		16	15	19.94	69.53	NimStim	N	Reduced-intensity Photographs	Labelling	3+N
Mienaltowski et al. (2013)		37	40	19.23	66.57	MSFDE	N	Reduced-intensity Photographs	Labelling (2 labels)	4
Mill et al. (2009)		358	37	-	-	JACFEE	Y	Full-intensity Photographs	Labelling	7+N
Murphy and Isaacowitz (2010)		50	41	19.34	72.04	DANVA2	Y	Reduced-intensity Photographs	Labelling	4
Ngo and Isaacowitz (2015)	1.	30	30	19.64	67.48	FACES	Y	Full-intensity Photographs	Labelling	3
	2.	41	40	19.87	73.54					2+N
Noh and Isaacowitz (2013)		37	47	20.38	73.49	POFA	N	Full-intensity Photographs	Labelling (2 labels)	2

Orgeta and Phillips (2008)	40	40	20.8	69.83	FEEST* (Ekman 60)	N	Full-intensity Photographs	Labelling	6
					FEEST* (Emotion Megamixes)	N	Reduced-intensity Photographs	Labelling	6
Orgeta (2010)	40	40	20.8	69.73	FEEST* (Ekman 60)	N	Full-intensity Photographs	Labelling	6
Phillips et al. (2002)	30	30	29.9	69.2	POFA	N	Full-intensity Photographs	Labelling	6
Phillips and Allen (2004)	42	49	21.31	66.8	FAN	N	Reduced-intensity Photographs	Intensity rating	4
Pollock, Khoja, Kaut, Lien, and Allen (2012)	14	14	19	70	NimStim	Y	Full-intensity Photographs Reduced-intensity Photographs	Labelling (2 labels)	2
Rauers, Blanke, and Riediger (2013)	100	100	25.94	74.2	FACES	Y	Full-intensity Photographs	Labelling	5+N
Richter et al. (2011)	48	35	23.33	70.37	Created for study	-	Videos	Intensity rating	3
Roring, Hines, and Charness (2006)	20	20	23	71	POFA	N	Full-intensity Photographs	Discrimination	6
Ruffman, Halberstadt, and Murray (2009a)	20	20	20.6	72.3	FEEST* (Ekman 60)	N	Full-intensity Photographs	Labelling	6
	60	61	20.5	70.5					
Ruffman, Ng, and Jenkin (2009b)	30	30	19	69	NimStim	N	Full-intensity Photographs	Labelling	3
Sasson et al. (2010)	2392	318	-	-	ER-40	Y	Full-intensity Photographs Reduced-intensity Photographs	Labelling	4+N

Stanley and Blanchard-Fields (2008)		171	193	20.6	70.72	MSFDE	N	Full-intensity Photographs	Labelling	5
Stanley and Isaacowitz (2015)		51	52	21.35	74.96	POFA	N	Full-intensity Photographs	Labelling	6+N
		47	44	-	-	Created for study	-	Videos	Labelling	5+N
Sullivan and Ruffman (2004a)	1.	31	30	26	72	POFA	N	Morphed-animations	Labelling	4
Sullivan and Ruffman (2004b)		24	24	30	73	POFA	N	Full-intensity Photographs	Labelling	6
Sullivan et al. (2007)	1.	30	30	23	72	POFA	N	Full-intensity Photographs	Labelling	6
	2.	27	27	23	73					
Sullivan, Campbell, Hutton, and Ruffman (2017)		57	57	20	70	POFA	N	Full-intensity Photographs	Labelling	6
Suzuki, Hoshino, Shigemasu, and Kawamura (2007)		34	34	20.6	69.7	JACFEE	N	Full-intensity Photographs	Labelling	6
Suzuki and Akiyama (2013)		36	36	21.4	69.4	JACFEE + POFA	N	Full-intensity Photographs	Labelling	6
Svärd , Wiens, and Fischer (2012)		19	20	26.35	73.7	POFA	N	Full-intensity Photographs	Labelling	2+N
Sze, Goodkind, Gyurak, and Levenson (2012)		76	74	22.99	66.38	NJIT	Y	Full-intensity Photographs Reduced-intensity Photographs	Labelling	5
Szymkowicz, Persson, Lin, Fischer, and Ebner (2016)		30	30	25.13	68.27	FACES	Y	Full-intensity Photographs	Labelling	2+N
Weiner (2006)		10	10	26	69.5	POFA	N	Full-intensity Photographs	Labelling	5

Werheid et al. (2010)		20	20	23.56	66.2	KDEF + AR Face database + own database	Y	Full-intensity Photographs	Labelling	2+N
Wong et al. (2005)		20	20	19.2	69.5	POFA	N	Full-intensity Photographs	Labelling	6
Wieck and Kunzmann (2017)	1.	100	100	23.98	68.41	Created for study	-	Videos	Intensity rating	2
	2.	50	50	21.78	69.28					
Williams et al. (2009)		176	360	-	-	ER-40	Y	Full-intensity Photographs	Labelling	5+N
Zhang et al. (2013)		18	15	-	-	Wang & Markham (1999)	N	Full-intensity Photographs	Labelling	3
Zhao, Zimmer, Shen, Chen, and Fu (2016)		30	31	22.7	71.9	NimStim	Y	Full-intensity Photographs	Labelling	6
Ziaei et al. (2016)		21	21	20.65	69.75	FEEST* (Ekman 60)	N	Full-intensity Photographs	Labelling	6

Note. Col. = Colour (Y/N), No. Emo. = Number of emotions included in task, +N = plus neutral expression, NYEB = New York Emotion Battery, MSFDE = Montreal Set of Facial Displays of Emotion, KDEF = Karolinska Directed Emotional Faces, ER-40 = Penn Emotion Recognition Test, ADFES = Amsterdam Dynamic Facial Expression Set, JACFEE = Japanese and Caucasian Facial Expressions of Emotion, POFA = Pictures of Facial Affect (this includes Ekman 60 and CATS), NJIT = New Jersey Institute, FAN = Florida Affect Naming task (subtest 3).

*FEEST is a digitalised version of the POFA and was therefore combined when appropriate.

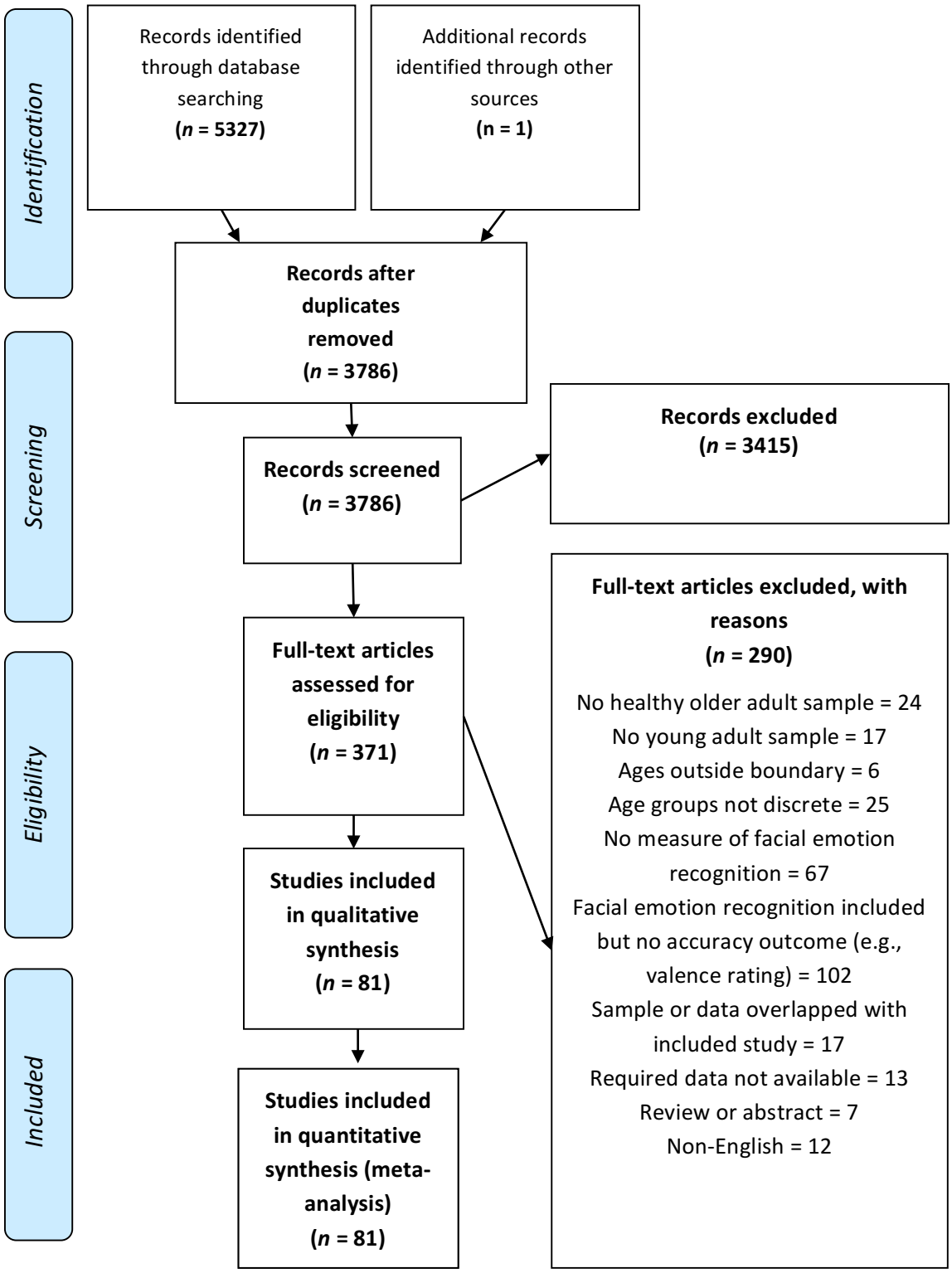
[a] Coded as virtual agent for the purpose of this meta-analysis but referred to as synthetic faces in original article.

[b] For details see Frigerio, Burt, Montagne, Murray, and Perrett (2002).

[c] Created by Advanced Telecommunications Research Institute International, Inc. Nara, Japan

Figure 3.1

Summary of search and screening process.



3.5.2 Overall facial emotion recognition age-effect (Aim 1a)

From the 81 publications, we examined a total of 102 facial emotion recognition tasks. Full-intensity static photographs were the most commonly used stimulus format (k = 64), but other stimulus formats represented were: reduced-intensity photographs (k = 19), videos (k = 8), morphed-animations (k = 6), and virtual agents (k = 5); see '3.7 Supplementary Material' regards the latter two stimulus formats.

When all facial emotion recognition age-effects were fitted to a model, regardless of task design used, a significant overall age-effect emerged that was moderate in magnitude (g = -0.40, p <.001), Supplementary Table 3.1, with older adults demonstrating poorer overall facial emotion recognition accuracy than young adults.

3.5.3 Patterns of facial emotion recognition age-effects across emotion types (Aim 1b)

As anticipated, when we separated the data by emotion type, the estimated average effects varied across emotion; Table 3.2 and Supplementary Table 3.1. The direction of the estimated average effects for each emotion indicated that older adults were significantly less accurate than young adults across all emotions except disgust, for which no age-effect emerged. The magnitude of the effects differed across emotion type. A moderate-to-large age-effect emerged for sadness (g = -0.66); moderate age-effects for fear (g = -0.61) and anger (g = -0.48), a small-to-moderate age-effect for surprise (g = -0.34), a small age-effect for happiness (g = -0.18), and there was no significant age-effect for disgust (g = -0.11, p = .23).

This pattern of results is somewhat consistent with the findings of Ruffman et al. (2008) who also detected moderate effects for anger and fear, a small effect for happiness, and a non-significant effect for disgust. However, Ruffman et al. (2008) reported age-effects for sadness and surprise that were notably smaller than those in the present results. For

disgust, Ruffman et al. (2008) reported a trend (that was approaching significance) towards older adults being more accurate than young adults, which our results did not replicate. Our results were more consistent with the findings of Goncalves et al. (2018) who also observed a larger age-effect for surprise than Ruffman et al. (2008) and reported no significant age-effect for disgust.

Although no age-effect emerged for disgust, 30 of 62 tasks that included disgust did produce significant age-effects for disgust recognition, and many of these age-effects were substantial in magnitude (i.e., moderate and large effects). Inspection of the forest plot (Supplementary Figure 3.2) revealed that the significant effects for disgust ran in both positive and negative directions (e.g., 35.48% favoured older adults). Thus, the overall age-effect for disgust seems to have obscured a pattern of results with positive effects counteracting negative effects. While all other emotion types were also associated with significant heterogeneity (*Q*), Supplementary Table 3.1, for each emotion except disgust, the study effects demonstrated little variance in direction; Supplementary Figures 3.1 and 3.3-3.6. Thus, for anger, fear, happiness, sadness and surprise the significant heterogeneity within samples is likely to have impacted the overall effect magnitudes but the effect direction (i.e., older adults being less accurate) appears robust.

Next, we explored the pattern of effects produced across emotion type for the different task designs, i.e., the stimulus format and image set used.

3.5.3.1 Stimulus formats

In terms of patterns across emotions, when full-intensity photographs were considered, the exact same pattern of age-effects emerged as to when all stimulus formats were collapsed; Table 3.2. Specifically, the largest age-effect was for sadness, followed by fear, then anger and then surprise, the smallest age-effect was for happiness, and no age-effect was present for disgust.

However, this pattern of age-effects was not replicated across any other stimulus format; Table 3.2. Some of the effects failed to reach significance and were likely underpowered due to small *n* values. However, when reduced-intensity photographs were considered, the largest age-effect was for surprise (g = .88). Furthermore, when videos were considered, a unique pattern emerged where the effect estimates for *all* emotion types were of similar magnitudes (g ranged from -0.30 to -0.76).

The most notable differences were for disgust. As noted, no age-effect emerged for disgust when all stimulus formats were considered. However, only full-intensity photographs replicated this finding (g = -0.05, p = .63). Conversely, age-effects emerged for disgust recognition for alternative stimulus formats. Videos produced a moderate-to-large significant age-effect (g = -0.76, p = .01), and reduced-intensity photographs produced a small-to-moderate age-effect (g = -0.42) that was, approaching significant (p = .08). In both instances, older adults demonstrated superior accuracy to young adults. These conflicting age-effects for disgust recognition are obscured when all stimulus formats are considered together.

Table 3.2

Age-effect size estimates across emotion types, first with stimulus format collapsed, and then grouped by stimulus format.

Stimulus Format/Emotion	Estimated Random Effect			Between
	n	*M* [95% CI]	p^a	p^b
Anger[c]	94	0.48 [-0.60, -0.36]	<.001	
Full-Intensity Photographs	60	0.48 [-0.61, -0.37]	<.001	-
Reduced-Intensity Photographs	17	0.47 [-0.65, -0.28]	<.001	.62
Videos	8	0.50 [-1.00, 0.01]	.05	.83
Disgust[c]	61	0.11 [-0.30, 0.07]	.23	
Full-Intensity Photographs	49	0.05 [-0.24, 0.15]	.63	-
Reduced-Intensity Photographs	5	0.42 [-0.93, 0.08]	.08	.20
Videos	4	0.76 [-1.23, -0.30]	.01	.27
Fear[c]	69	0.61 [-0.76, -0.47]	<.001	
Full-Intensity Photographs	48	0.65 [-0.86, -0.43]	<.001	-
Reduced-Intensity Photographs	13	0.54 [-0.71, -0.37]	<.001	.24
Videos	2	0.30 [-1.82, 1.22]	.24	.77
Happiness[c]	85	0.18 [-0.26, -0.10]	<.001	
Full-Intensity Photographs	55	0.13 [-0.24, -0.02]	.02	-
Reduced-Intensity Photographs	15	0.20 [-0.35, -0.05]	.01	.46
Videos	6	0.56 [-0.94, -0.18]	.02	.08
Sadness[c]	76	0.66 [-0.80, -0.52]	<.001	
Full-Intensity Photographs	47	0.70 [-0.89, -0.50]	<.001	-
Reduced-Intensity Photographs	14	0.61 [-0.91, -0.32]	<.001	.57
Videos	8	0.48 [-0.64, -0.33]	<.001	.84
Surprise[c]	40	0.35 [-0.55, -0.15]	.001	
Full-Intensity Photographs	31	0.30 [-0.54, -0.05]	.02	-
Reduced-Intensity Photographs	3	0.88 [-2.12, 0.36]	.09	.02
Videos	2	0.32 [-0.37, -0.27]	.01	.37

Note. *n* = number of effect size estimates. A negative effect size indicates that older adults are worse than young adults. CI = confidence interval. [a] Significance of the random effect, [b] significance of the difference between the contrasts (compared to full-intensity photographs) after adjusting for the number of response options and image set, [c] effect estimate for the emotions with stimulus format collapsed.

3.5.3.2 Image sets

We also observed variance in the patterns of effects produced across emotions by the different image sets; see Table 3.3. Once again the most notable difference was the age-effect for disgust. Specifically, stimuli from the POFA produced a significant disgust effect in favour of older adults (g = 0.30, p = .02). However, the POFA stimuli were the only image set to produce this finding. In contrast, stimuli from the FACES and JACFEE image sets produced disgust effects in favour of young adults, though only the FACES effect was approaching significance (g = -0.40, p = .06, and g = -0.29, p =.35, respectively). While the effect produced by the 'other' image sets, is limited in terms of interpretation due to it being a culmination of many varied image sets, it does provide an indication of the remaining literature. The combined estimated disgust effect for all the image sets coded as 'other' also significantly favoured young adults (g = -0.56, p <.001). Thus, an age-effect for disgust favouring younger adults was the dominant finding.

In addition, the small age-effect for happiness that emerged across the different stimulus formats and when all studies were considered together, was not present for all image sets. Neither the FACES and NimStim stimuli produced an age-effect for happiness (g = 0.02, p = .83, and g = 0.07, p = .66, respectively). Yet, the FACES and NimStim stimuli both produced age-effects for sadness that were notably larger than the effect that emerged when all studies were considered together (g = -1.01, p = .12, and g = 1.19, p = .01, respectively). These image sets, therefore, do not appear to favour older adults across all emotions, rather they produce a pattern of effects whereby no effect emerges for happiness but a very large age-effect, in favour of young adults, emerges for sadness.

Table 3.3

Age-effect size estimates across emotion types, grouped by image set.

Image Set/Emotion	Estimated Random Effect			Between
	n	*M* [95% CI]	p^a	p^b
Anger				
POFA	34	0.36 [-0.63, -0.09]	.01	-
FACES	10	0.60 [-0.96, -0.25]	.01	.28
JACFEE	8	0.64 [-0.97, -0.30]	.003	.18
NimStim	11	0.33 [-0.75, 0.09]	.10	.49
Other	24	0.55 [-0.75, -0.37]	<.001	.15
Disgust				
POFA	30	0.30 [0.07, 0.54]	.02	-
FACES	7	0.40 [-0.82, 0.02]	.06	.01
JACFEE	8	0.29 [-0.99, 0.41]	.35	.09
NimStim	2	0.12 [-4.81, 4.57]	.80	.47
Other	12	0.56 [-0.70, -0.41]	<.001	<.001
Fear				
POFA	30	0.55 [-0.79 -.031]	<.001	-
FACES	7	1.35 [-2.80, 0.10]	.06	.20
JACFEE	8	0.54 [-0.78, -0.29]	.001	.53
NimStim	2	0.88 [-2.17, 0.41]	.10	.38
Other	12	0.50 [-0.65, -0.36]	<.001	.57
Happiness				
POFA	30	0.14 [-0.28, 0.01]	.06	-
FACES	8	0.02 [-0.16, 0.20]	.83	.15
JACFEE	8	0.34 [-0.88, 0.20]	.18	.44
NimStim	10	0.07 [-0.39, 0.54]	.66	.79
Other	23	0.28 [-0.38, -0.18]	<.001	.33
Sadness				
POFA	30	0.55 [-0.80, -0.31]	<.001	-
FACES	5	1.10 [-2.67, 0.46]	.12	.40
JACFEE	8	0.96 [-1.24, -0.69]	<.001	.06
NimStim	4	1.19 [-1.80, -0.57]	.01	.07

Other	22	0.55 [-0.73, -0.38]	<.001	.86
Surprise				
POFA	26	0.29 [-0.55, -0.01]	.04	-
JACFEE	8	0.44 [-0.91, 0.04]	.07	.87
Other	5	0.49 [-0.79, -0.20]	.01	.09

Note. n = number of effect size estimates. A negative effect size indicates that older adults are worse than young adults. CI = confidence interval. [a] Significance of the random effect, [b] significance of the difference between the contrasts (compared to POFA) after adjusting for the number of response options and stimulus format.

In summary, when all task designs are collapsed, the pattern of results across emotions is mostly consistent with Ruffman et al. (2008). However, when we controlled for stimulus format, this pattern was only present for full-intensity photographs. Considering that full-intensity photographs were the most commonly used stimulus format, it appears that the pattern of age-effects observed when all stimulus formats were considered together, was strongly influenced by the use of full-intensity photographs and is not robust. There may be less variation in older adults' ability to recognise different emotion types than previously thought, with video-based tasks producing age-effects of similar magnitude across all emotion types. Furthermore, age-effects were present for disgust, but the magnitude, direction, and significance depended on stimulus format and image set. Initially, older adults were less accurate at recognising disgust on reduced-intensity photographs and videos but not full-intensity photographs. However, when we considered image sets, we discovered that older adults were *more* accurate recognising disgust than young adults on the POFA stimuli, but less accurate on stimuli from all other image sets. Since the effect estimates for each image set are primarily comprised of effects from studies that used full-intensity photographs, it is likely that the null disgust effect for full-intensity photographs is not robust and instead obscures a pattern of results whereby older adults are less accurate than young adults at

recognising disgust on the majority of image sets, but significantly more accurate than young adults for the POFA stimuli.

3.5.4 Moderator effects of task features (Aim 2)

After establishing that there are differences in the patterns of effects across emotions produced by different task types, next we tested the significance of the variance in the magnitude of age-effects produced by different task types. The combined variance accounted for by the stimulus format, image set, and number of response options was significant for disgust (p <.001), fear (p <.001), happiness (p = .02), and surprise (p <.001). However, it was not significant for anger (p =.30) or sadness (p = .36); Supplementary Table 3.2.

3.5.4.1 Stimulus formats (Aim 2a + 2b)

After adjusting for the image set used in the task, and the number of response options included, stimulus format was not a unique moderator of age-effects in its own right for any of the emotions; Supplementary Table 3.2.

To identify whether reduced-intensity photographs and videos produced significantly larger or smaller age-effects than static full-intensity photographs, the estimated average effects of the alternative stimulus formats were contrasted with the estimated average effects produced by full-intensity photographs; Table 3.2. Once again, for both contrasts, we adjusted for the image set used and the number of response options included.

Contrary to our expectation, the estimated effects for reduced-intensity photographs was only significantly larger than full-intensity photographs for surprise (g = -0.88 vs. g = -0.30, p = .02). The difference between reduced and full-intensity photographs was non-significant for all other emotions (p ranged .24 to .62). Similarly, studies that used videos only produced effects that were larger than those that used full-intensity photographs for happiness, with the difference in effect magnitudes approaching significance (g = -0.56 vs. g

= -0.13, p = .08). For all other emotions, the magnitude of the age-effects produced by videos and full-intensity photographs did not significantly differ (p ranged .27 to .84).

3.5.4.2 Image sets (Aim 2c)

After adjusting for the number of response options and stimulus format, the variance in effect magnitudes produced by the different image sets was significant for disgust (p = .06). However, this was non-significant for all other emotions (p ranged from .11 to .61); Supplementary Table 3.2.

To explore whether newer image-sets produced significantly larger or smaller age-effects than the original facial emotion recognition image set, the estimated average effects of the FACES, JACFEE, and NimStim were contrasted with the estimated average effects produced by the POFA stimuli; Table 3.3. Once again, for these contrasts, we adjusted for stimulus format used in the task, and the number of response options included.

For disgust, the FACES stimuli produced an age-effects that were significantly different from the small age-effect produced by the POFA in favour of older adults (g = -0.40 vs. g = 0.30, p = .01). The effect produced by the FACES stimuli was larger in magnitude than the POFA effect, and in conflicting direction. The same pattern emerged between the JACFEE and the POFA for disgust, and the difference was approaching significance (g = -0.29 vs. g = 0.30, p = .06). For sadness, the difference in age-effect magnitude produced by both the JACFEE and the NimStim image sets, compared to the POFA was also approaching significance (g = -0.96 vs. g = -0.55, p = .06, and g = -1.19 vs. g = -0.55, p = .07). Both the JACFEE and NimStim produced larger age-effects for sadness than the POFA. For all other emotions, the contrasts did not approach significance (p ranged .15 to .79).

In summary, the three moderators (i.e., stimulus format, image set, and the number of response options) when combined accounted for significant variance in the age-effects

produced across studies. However, only the image set used accounted for unique variance, once stimulus format and the number of response options were adjusted for, and this was only for disgust. The results suggest that older adults demonstrate difficulty recognising disgust on all stimuli and image sets, expect the POFA for which they are more accurate than young adults. Image set being a unique moderator for disgust further supports the premise that it is the POFA stimuli specifically, rather than stimulus format, that is leading to an overall null-effect emerging for disgust. There was some variance in the effect magnitudes produced by the different stimuli formats and image sets across the other emotions. Specifically, reducing the intensity of photographs produce an age-effect that was larger than full-intensity photographs for surprise. Additionally, using dynamic videos instead of static full-intensity photographs, produced a larger age-effect for happiness. Lastly, the FACES and JACFEE image sets produced larger age-effects for sadness than the POFA.

3.5.5 Publication bias

A bias that occurs when one estimates an average effect from the effects of published data, is that any unpublished findings are omitted. This "file drawer problem" (Rosenthal, 1979) can inflate the observed effects given the likelihood that many of the findings that have not been published are non-significant. We tested for publication bias using Egger's regression test (Sterne & Egger, 2005), by running each of the emotion-specific multilevel models with sampling variance as a moderator. If the intercept significantly deviated from zero, it indicated bias whereby the larger the deviation the greater the bias. We detected significant bias for anger and happiness (both $p = .01$).

To investigate the threat that this bias posed to our findings, we used Orwin's N_{es} to calculate the estimated average effects that would be produced if the number of effects was doubled, and all of the added study effects averaged zero. For anger, adding 94 study effects averaging zero would reduce the estimated average effect to $g = -0.23$. For happiness, adding

85 study effects averaging zero would reduce the observed estimated average effect to g = -0.08. Thus, even after accounting for publication bias, the direction of the effects appears robust (i.e., favours young adults), but the magnitude is reduced. However, it is worth noting that the results from the current meta-analysis revealed that task characteristics influence the magnitude of age-effects produced. The Orwin's N_{es} calculation does not account for effects clustered by task differences. While publication bias may have inflated the overall estimated effects observed for anger and happiness, these effects would still be a culmination of differing effect magnitudes across task types.

3.6 Discussion

This meta-analysis had two aims. The first was to identify whether the pattern of age-effects across emotions observed by Ruffman et al. (2008) is robust given the recent increase in quantity and diversity of facial emotion recognition studies in ageing. The second was to examine the influence of specific characteristics of task designs on age-effect magnitudes for facial emotion recognition.

With respect to the first aim, the current study substantially extends the previous meta-analyses, which included 17 data sets (Ruffman et al., 2008) and 25 data sets (Goncalves et al., 2018), by integrating and examining results from nearly four times as many studies (total k = 81; total n = 102). Despite the substantial increase in studies, when all stimuli and task types were considered together, a similar pattern of age-effects emerged for the current literature as in the previous meta-analyses (Goncalves et al., 2018; Ruffman et al., 2008). That is, older adults demonstrated the strongest facial emotion recognition difficulties for sadness, fear, and anger; less difficulty for happiness; and no age-related difficulty for disgust. However, and more importantly, when we considered each task design separately, we found that this pattern of effects was not robust. Rather, it was present only when full-intensity photographs were used. For reduced-intensity photographs, the strongest age-related

difficulty was for surprise. For videos, there was minimal difference in the magnitude of age-effects across emotions. A rather interesting finding emerged for disgust, where not only the magnitude but the direction of the age-effect was dependent on the image set used.

In line with our second aim, when we examined methodological features of task designs, we identified some systematic variance in the effect magnitudes linked to the stimulus format, and the image set used, but this was restricted to certain emotions. Reducing the intensity of photographs only increased the age-effect magnitude for surprise. Moreover, using dynamic stimuli (i.e., videos instead of static full-intensity photographs) only increased the age-effect magnitude for happiness. Finally, using newer image sets, compared to the original POFA image set, increased the age-effect magnitude for disgust and sadness only. Below, we discuss several key highlights from our meta-analysis that have implications for future research.

3.6.1 Selective retention of disgust recognition: Real-world phenomenon or function of task design?

One of the key aspects of the pattern of effects reported by Ruffman et al. (2008) was that no significant age-effect emerged for disgust. Moreover, there was a trend towards older adults demonstrating superior disgust recognition than younger adults. This finding suggested that older adults retained the ability to recognise disgust. In the current meta-analysis, when all stimuli were considered together, no significant age-effect emerged for disgust recognition. However, further investigation revealed that this null-effect was the culmination of disgust effects of conflicting directions, with the direction of the effects systematically related to the stimuli used. We conclude that, across the literature, the dominant finding is that older adults demonstrate significant difficulty recognising disgust.

Initially, older adults were substantially *less* accurate than young adults at recognising disgust on videos and reduced-intensity photographs but not full-intensity photographs. However, when we explored the influence of image set used, we discovered that older adults were significantly superior to young adults at recognising disgust on POFA stimuli, but inferior for all other image sets. Since image set was a significant moderator of disgust effects even after adjusting for stimulus format used, it appears that it is image set, rather than stimulus format, that influences the direction of the disgust age-effect. This discovery provides an explanation for why Ruffman et al. (2008) detected a trend towards older adults being superior at disgust recognition, given that the majority of the studies reviewed by Ruffman et al. (2008) used the POFA image.

We highlight that the POFA image set, which resulted in older adults having superior disgust recognition to young adults in the current meta-analysis, consists of black-and-white Ekman faces (Ekman & Friesen, 1976). The latter could aide older adults' performance given their greater familiarity with black-and-white images. Additionally, age-related declines in colour vision (Owsley, 2011) may also impact on older adults' ability to distinguish subtle facial features on coloured stimuli. However, the conflicting effect direction was observed only for disgust, and thus it is unclear what is unique about the disgust faces in the POFA image set.

One difference between disgust and other emotions is that disgust is easier to recognise from the mouth region rather than the eye region (Calder et al., 2000). There is evidence that when processing faces, that older adults typically attend relatively more to the lower half of the face, whereas young adults attend more to the upper half (Circelli et al., 2013; Grainger et al., 2015; Murphy & Isaacowitz, 2010; Sullivan et al., 2007). Thus, one possibility is that disgust images from the POFA, more clearly depict disgust in the mouth region compared to other images sets, as this would advantage older adults. However, further

research is required to determine what is unique about the POFA disgust faces that produced a recognition age-effect in favour of older adults.

Despite being unable to conclude why the faces depicting disgust from the POFA image set gave older adults an advantage over young adults, the finding that older adults demonstrate difficulty recognising disgust across all other stimuli has theoretical implications. One of the reasons that Ruffman et al. (2008) dismissed the general cognitive decline model was because of their finding that disgust recognition was selectively preserved in ageing. Thus, a neuropsychological model that highlighted the relative sparing from age-related neurodegeneration of the brain regions associated with disgust recognition (i.e., the basal ganglia and insula), was proposed as a better model for explaining older adults' emotion recognition difficulties. However, our finding that older adults have difficulty recognising disgust on most stimuli is more in keeping with widespread neurodegeneration, and in turn general cognitive decline. Notably, age-related reductions in volume have been documented for both the basal ganglia and insula (Casse-Perrot et al., 2007; Raz & Rodrigue, 2006).

3.6.2 Positivity effect in facial emotion recognition: Real-world bias or function of task design?

A second key aspect of the pattern of effects reported by Ruffman et al. (2008) was a markedly smaller age-effect for happiness recognition, compared to anger, fear, and sadness. It was unclear whether older adults genuinely retained their ability to recognise happy expressions, or whether the smaller age-effect produced for this emotion was a product of task design. Specifically, ceiling effects occur more often for happiness recognition, than for other emotions, which seems particularly likely to limit any age-effect produced for happiness.

In the current meta-analysis, older adults demonstrated roughly moderate age-differences across *all* emotions for video stimuli. Moreover, the happiness effect produced by videos was significantly larger in magnitude than that of full-intensity photographs. This finding suggests that older adults' greater difficulty recognising anger, sadness, and fear, compared to happiness, is in part a function of task design and thus it is unclear whether this pattern represents a real-world phenomenon. Videos may produce more consistent age-effects across emotion types because the emotions are more realistic, and thus subtler, which could have balanced the sensitivity across emotion types (Grainger et al., 2015). However, this does not account for our finding that reduced-intensity photographs, which are also subtler that full-intensity photographs, still produced an age-effect for happiness that was smaller in magnitude than for all other emotion types.

One reason that reducing the intensity of expressions is theorised to increase task sensitivity, is that it may decrease the chance that one or both age groups will perform at ceiling (Hess, Blairy, & Kleck, 1997). We visually inspected young and older adults' accuracy means for recognition of full- and reduced-intensity photographs of happiness, and noted that ceiling effects were indeed less commonly reported when reduced-intensity photographs were used. A decrease in ceiling effects without a concomitant increase in age-effects suggests older adults genuinely find happiness easier to recognise than anger, fear, and sadness on reduced-intensity photographs.

The neuropsychological model may provide an explanation for why older adults found static expressions of subtle happiness as easy to recognise as static expressions of overt happiness, yet found dynamic expressions of happiness, which are likely subtle, more difficult. First, there is evidence that people with damage to the orbitofrontal cortex, an area of the prefrontal cortex known to deteriorate in ageing (Lamar & Resnick, 2004), exhibit impairments recognising subtle facial expression of happiness, yet are able to recognise overt

facial expressions (Willis, Palermo, McGrillen, & Miller, 2014). Second, there is evidence that different neural regions are activated when recognising facial expressions on static versus dynamic stimuli, specifically for happiness, with the pre-frontal cortex being one area that is activated differently for recognition of dynamic versus static expressions (Kessler et al., 2011; Kilts, Egan, Gideon, Ely, & Hoffman, 2003). Therefore, one possibility is that there is an interaction between how overt versus subtle, and dynamic compared to static stimuli, are processed at a neural level. However, further work is needed that clearly maps how the different patterns of age-effects across the different emotions and stimulus formats map onto changes in the ageing brain to directly test this possibility. Indeed, further work is needed more broadly that shows a direct link between the process of emotion recognition and the functional and structural integrity of specific cerebral regions, as research showing these relationships is surprisingly limited.

Although it remains unclear whether older adults genuinely find happiness easier to recognise than the other emotion types, the different patterns of age-effects produced by video-based tasks fails to support there being an age-related positivity effect.

3.6.3 Dynamic stimuli: Implications for real-world estimations

As noted earlier, it is possible to generate two opposing hypotheses for how the magnitude of age-effects might differ for dynamic relative to static stimuli. Life experience and greater motivation might particularly benefit older adults' performance when presented with naturalistic dynamic cues, thus reducing the magnitude of the age-effects for dynamic (vs. static) stimuli. Alternatively, from a general cognitive decline perspective, the additional processing speed demands associated with dynamic stimuli might *disadvantage* older adults, thus increasing the magnitude of the age-effects for dynamic (vs. static) stimuli.

Except for happiness, after adjusting for image set used, the effects produced by videos did not differ significantly in magnitude from the effects produced by full-intensity photographs. Perhaps older adults were both advantaged by the naturalistic cues and disadvantaged by the processing speed demands of dynamic stimuli. However, in terms of patterns of effects across emotions, videos produced relatively moderate age-effects across all emotions, in contrast to mix of small and large age-effects produced by static full-intensity photographs, which suggests uniform emotion recognition difficulties.

It has been suggested that the frequent use of static photographs to assess facial emotion recognition in healthy ageing has led to an overestimation of the level of difficulty that older adults experience in real-world scenarios (Isaacowitz & Stanley, 2011). However, our findings suggest that, except for happiness, videos typically produced age-effects that were not significantly different in magnitude from full-intensity photographs. It may be that video-based tasks also overestimate real-world difficulties since the use of actors (e.g., Grainger, 2015) and limited context makes the ecological validity of videos questionable. Thus, more ecologically valid video-based tasks could still advantage older adults. Too few video-based tasks used real protagonists and/or contextual stimuli (e.g., Richter et al., 2011; Wieck & Kunzmann, 2017) for us to systematically explore the influence of these task features. However, the extent to which older adults find video stimuli less artificial and therefore more engaging would likely depend on whether the emotions depicted were authentic portrayals. Older adults may also find stimuli that is embedded in context, particularly when age-relevant, more motivating to attend to relative to stimuli devoid of context (Wieck & Kunzmann, 2015). Settings with context may also be less cognitively demanding, as they would allow older adults to rely more on crystallised recognition abilities, a protective factor for facial emotion recognition (Labouvie-Vief, 2009).

This potential discrepancy between real-world ability and performance on laboratory-based tasks highlights the need for further research to confirm the validity of facial emotion recognition tasks. The results of the current meta-analysis provide evidence that the tasks being used currently are incredibly diverse in terms of the age-effect magnitudes and patterns produced. While the pattern of effects reported by Ruffman et al. (2008) captures the pattern of effects produced by full-intensity photographs from the POFA, our results suggest that this pattern is not seen when other tools are used, and thus may not reflect real-world ability.

3.6.4 Limitations

As with all meta-analyses, this meta-analysis was limited by the availability of specific data. While we were able to ascertain that reduced-intensity photographs were more sensitive to age-effects for surprise than full-intensity photographs, we were unable to systematically examine the relative sensitivity of specific intensity levels (i.e., 50% vs. 60% vs. 75%). Furthermore, there were task features that likely advantage or disadvantage older adults that we were not able to explore, such as the duration that the stimuli were presented for, the presence or absence of context, and the inclusion of non-lexical vs. lexical labelling. We recommend that researchers consider these features in future study designs given that we have identified that many age-effects in facial emotion recognition are systematically linked to task design. Furthermore, we only included facial emotion recognition accuracy outcomes and did not consider reaction time and efficiency outcomes. In addition, this meta-analysis focused on explicit facial emotion recognition and we, therefore, did not consider tasks that involved multimodal emotion recognition, valence ratings, or passive viewing of the stimuli. All of the tasks included in the meta-analysis required cognitive control processes beyond recognition of emotion, and these other cognitive processes may modulate the age-effects observed. With the increasing trend towards unbiased hit-rates being calculated *post-hoc* (e.g., Fölster et al., 2015), an interesting follow-up review would be to examine the difference

that this makes to the pattern of age-effects produced. However, there were not enough studies that reported unbiased hit-rates to include this as a moderator in the current meta-analysis.

3.6.5 Summary and conclusions

This meta-analysis provides important clarification on how age influences the recognition of basic emotions, as well as on the influence of specific task features and task designs in understanding these effects. Similar to Ruffman et al.'s (2008) earlier meta-analysis, the current results show that, when all studies are considered together, older adults exhibit particular difficulty recognising anger, sadness, fear, lesser difficulty recognising surprise and happiness, and no difficulty recognising disgust. However, the current results also show that this is true only for full-intensity photographs. By contrast, for videos, older adults demonstrate relatively moderate age-differences across all emotions. For reduced-intensity photographs, older adults demonstrate moderate or large age-deficits for all emotions except happiness, with the reduction of intensity significantly increasing the effect magnitude for surprise. In further contrast to the meta-analysis by Ruffman et al. (2008), we found that older adults do exhibit difficulty in recognising disgust, but that this effect is masked by the superior accuracy of older adults compared to young adults recognising disgust for the most commonly used POFA stimuli.

The complexity of the pattern of age-effects identified in this meta-analysis has implications for models of healthy ageing, but also more broadly for researchers interested in assessing emotion recognition in other populations. This is because the key finding to emerge is that the magnitude, specificity, and even the direction of age-effects of emotion recognition are not only dependent on the specific emotion being assessed but also the manner in which the recognition of these emotions is assessed. Therefore, methodological features are a critical consideration in any study seeking to assess this construct, with far-reaching

implications not only for the field of gerontology but also more broadly for researchers interested in the assessment of social cognition and the many clinical disorders where social cognitive impairment is a core and debilitating problem.

3.7 Supplementary Material

3.7.1 Introduction

In the main manuscript, we review the common task designs used to test facial emotion recognition in healthy ageing. While our aim was to test the robustness of the pattern of effects reported by Ruffman et al. (2008) across all facial emotion recognition tasks, as the field is vast, there have been adjustments made to facial emotion recognition tasks that we were unable to details in our main review. We included these task features in our analyses, however, we present them here to reduce the overall length of the manuscript and because these subgroups were likely underpowered (i.e., were less commonly used and thus had low *k* values). We have outlined these features below, detailed their estimated effects, and discussed potential implications of the findings. However, caution is needed when interpreting these findings due to the low *n* values in each subgroup. While the findings of these additional task designs provide some interesting insights, we do not believe that their implications contrast with any of the conclusions drawn in the main manuscript. We provide this supplementary material to aide with task selections and to ensure we consider the entire facial emotion recognition and ageing field in our review.

In this supplementary material, we have also included tables detailing additional data. These are provided after the discussion. Supplementary Table 3.1 provides the heterogeneity of each emotion model without moderators, and Supplementary Figures 3.1 through to 3.5 show the forest plots for these effects. Supplementary Table 3.2 shows the moderating effects of each variable.

3.7.1.1 Additional adjustments to facial emotion recognition tasks

Number of response options. Due to concerns that providing participants with multiple response options, typically multiple lexical labels, places too great of cognitive

demand, many studies have presented participants with fewer than six facial expressions to choose from. Recent studies have even reduced the number of response options in any given item to only two, to give participants an 'either-or' choice that allows for the use of elimination, a cognitive strategy that is less demanding on working memory (Phillips et al., 2008). Tasks with two response options include Go-no-go tasks (i.e., 'click for happy, do not click for sad'), tasks where the target emotion is present or absent (i.e., 'click left key if expression is happy, click right key if expression is not happy'), facial discrimination (i.e., 'which of these two faces is happy?'), and those including two labels (i.e., 'is this face happy or sad?') (e.g., Ebner et al., 2013; Roring et al., 2006). Given the well-established age-related decline of working memory, tasks involving fewer responses may be less confounded by age-related declines in general cognitive ability (Hedden & Gabrieli, 2004), and may produce smaller age-effects.

Morphed animations. A second form of dynamic stimuli, referred to as morphed-animations, has also been developed, which emulates the moving nature of videos but uses pre-existing static stimuli and digital morphing software to create a dynamic stimulus. For the latter, photographs displaying target emotions are digitally morphed from either photographs of neutral faces (e.g., Altamura et al., 2016; Di Domenico et al., 2015; Grainger et al., 2015) or from photographs of other, non-target emotions (e.g., Sullivan & Ruffman, 2004a). These animations serve to create a morphing sequence in which the target expression is initially absent but then slowly emerges. Only one study in the Ruffman et al. (2008) review used morphed-animations (i.e., Sullivan & Ruffman, 2004a).

Virtual agents. There are also images that do not involve human posers, but rather are digital productions of virtual agents. The creation of digital avatars has a number of important strengths, in particular greater experimental control with respect to the features of the expressions. However, the use of this stimulus format may disadvantage older adults who,

due to generational differences, are not familiar with or as motivated by artificial computer-based images. In a recent meta-analysis, Goncalves et al. (2018) found that this stimulus feature (i.e., virtual versus human faces) did not moderate facial emotion recognition age-effects. However, they categorise the stimuli used in Hunter et al. (2010) as virtual agents, whereas the stimuli used in this study (images from the FEEST) involved digitally manipulated human faces that would arguably be more accurately categorised as reduced-intensity human photographs; see level of intensity section below for further details. We, therefore, re-test the moderating effect of virtual versus human faces in our larger set of data, with the stimuli used in Hunter et al. (2010) coded as reduced-intensity photographs.

3.7.1.2 Hypotheses

In the main manuscript, our first aim was to examine the pattern of effects produced across emotions by different facial emotion recognition tasks, however, we noted that we were unable to predict what patterns would emerge for each task design. Our second aim was to identify if different task features were significant moderators of the magnitude of age-effects produced, and to contrast newer stimulus formats with traditional full-intensity photographs.

In relation to the first aim, here, we were interested in the patterns of effects produced across emotions for tasks involving two response options, three or more response options, morphed animations, and virtual agents (Supplementary Aim 1).

In relation to the second aim, with respect to the moderating effect of the number of response options (Supplementary Aim 2a), we hypothesised that this task feature would be a significant moderator and studies that included at least three response options would produce larger age-effects than studies that only included two response options. As noted earlier, this was based on a higher number of response options imposing greater cognitive load.

For the different stimulus formats, we tested the overall moderating effect of this variable in the main manuscript. Here, we contrasted morphed animations (Supplementary Aim 2b) and virtual agents (Supplementary Aim 2c) with full-intensity photographs. For morphed animations, as with videos, two opposing hypotheses could be put forward about whether these stimuli would differ in age-sensitivity from static stimuli. Although life experience should favour older adults when they are presented with more naturalistic dynamic cues, the fleeting nature of dynamic cues also places more load on rapid attentional processing, which is likely to disadvantage older adults. Morphed animations, being arguably less natural than videos due to being digital morphing sequences between static images, would not necessarily provide older adults with the opportunity to rely on life experience. Furthermore, the final frame of morphed animations is often a full intensity-photographs. Thus, morphed animations were expected to behave more similarly to full-intensity photographs, in terms of the pattern and magnitude of age-effects produced, than videos. For virtual agents, we hypothesised that these stimuli would produce significantly larger age-effects than their human full-intensity photograph counterparts due to generational differences in familiarity with virtual stimuli. Specifically, we reasoned that older adults would be less familiar with, and less motivated by, digital faces than young adults. Goncalves et al. (2018) also explored the influence of virtual faces versus human faces and observed a moderating effect for fear. Specifically, that there were larger age-effects for fear recognition on virtual faces compared to human faces. However, this finding needs to be interpreted with caution because the influence of other task design features, such as the number of response options and intensity of the emotion, was not considered, and the FEEST stimuli should arguably be coded as reduced-intensity photographs rather than virtual agents.

3.7.2 Method

As noted in the main manuscript, publications were excluded if the necessary data to calculate effect sizes was not available, or was not made available, upon request (Allen & Brosgole, 1993; Garcia-Rodriguez et al., 2011; Garcia-Rodriguez, Fusari, Rodriguez, Hernandez, & Ellgring, 2009; Hot et al., 2013; Malatesta, Izard, Culver, & Nicolich, 1987; McDowell et al.,1994; Moreno, Borod, Welkowitz, & Alpert, 1993; Prodan, Orbelo, & Ross, 2007; Rossignol, Bruyer, Philippot, & Campanella, 2009; Svärd et al., 2012; Weidner, 2014; West et al., 2012; Williams et al., 2009). The following publications were also excluded, but due to overlapping participant data (Beer, Fisk, & Rogers, 2009; Beer, Smarr, Fisk, & Rogers, 2015; Gunning-Dixon et al., 2003; Horning, 2012; Lynchard, 2012; Smarr, Fisk, & Rogers, 2011; Sze, 2014; Voelkle et al., 2014).

While the methodology is detailed in the main manuscript, here we outline the methods relating specifically to the supplementary hypotheses. The number of response options included in the task (coded as two or three plus) was incorporated as a moderator in all models. However, here we tested the moderating effect of number of response options (Supplementary Aim 2a), while adjusting from stimulus format and image set, to identify if studies that included three or more response options produced larger effects than those that included only two; Supplementary Table 3.3. Note, that we were only able to test this contrast for anger, happiness, and sadness because fear, disgust, and surprise were not represented in the studies that included only two response options. We also contrasted the estimated effects produced by morphed animations and virtual agents to those produced by full-intensity photographs (Supplementary Aim 2a and 2b); Supplementary Table 3.4.

3.7.3 Results

3.7.3.1 Supplementary Aim 1: Patterns across emotions

The overall effect estimates (i.e., collapsed across stimuli) for studies grouped by the number of response options can be found in Supplementary Table 3.3, however, we are unable to discuss the patterns across emotions for task features, as only three emotions were represented.

When virtual agents were used (Supplementary Table 3.4), a similar pattern emerged as when full-intensity human photographs were considered, whereby the largest effect was found for sadness (g = -0.83), then fear (g = -0.77) and then anger (g = -0.61), and the smallest effect for happiness (g = -0.14); there were no estimated effects for disgust or surprise.

When morphed animations were used (Supplementary Table 3.4), a similar pattern also emerged, whereby the largest effect was for sadness (g = -0.65), then fear (g = -0.61), and then anger (g = -0.48), and the smallest effects were for happiness (g = -0.27) and surprise (g = -0.18). However, morphed animations produced a small age-effect for disgust recognition (g = .23), that while non-significance (p = .13) was in favour of older adults.

3.7.3.2 Supplementary Aim 2a: Moderating effects of number of response options

There were 13 (12.15%) datasets that included two response options in their facial emotion recognition task, thus giving participants an ‘either or’ choice rather than a choice between multiple emotion labels. The remaining 76 datasets included three or more response options. With each dataset coded as either two options or three plus options, and after adjusting for stimulus format used, number of response options was a significant moderator of the age-effects produced for recognition of anger (p = .05), with studies that included at least three response options producing significantly larger age-effect (g = -0.53 vs g = -0.07);

Supplementary Table 3.3. Number of response options was not a significant moderator for happiness (p = .24) or sadness (p = .27). However, this finding needs to be interpreted with caution as only four datasets for tasks involving two response options involved sadness.

Happiness was well represented (k = 11), so we can, therefore, conclude that older adults found recognition of happiness as easy in tasks involving multiple response options as they do in tasks involving only two response options, but found anger recognition easier in tasks that give them an either-or choice than in tasks involving more response options. More data is required to make conclusions about the other emotion types, as these emotions could not be analysed due to lack of representation in studies that included two response options.

3.7.3.3 Supplementary Aim 2b: Morphed animations vs. static photographs

To test our hypothesis that older adults would find morphed animations as easy to recognise as full-intensity photographs, we contrasted the coefficient representing morphed animations with the intercept that represented full-intensity photographs. Across all emotions, the differences observed between the effect magnitudes produced by studies that used morphed-animations compared to full-intensity photographs were non-significant (p ranged from .18 to .87); Supplementary Table 3.4. However, this finding needs to be interpreted with caution as the age-effects produced by morphed animations were likely underpowered due to few studies utilising this stimulus format. The largest difference in the effects produced by morphed-animations compared to full-intensity photographs was for disgust (g = 0.23 vs. g = -0.06), for which morphed-animations produced the larger effect.

3.7.3.4 Supplementary Aim 2c: Virtual agents vs. human faces

To test our hypothesis that older adults would perform more poorly than young when recognising emotions displayed by virtual agents compared to human faces, we compared the coefficient that represented virtual agents with the intercept that represented full-intensity photographs. In contrast with our expectation, there were no significant differences between

the age-effects produced by virtual agents and those produced by full-intensity human photographs for any of the emotions (*p* ranged from .17 to .96); Supplementary Table 3.4. Again, the presence of small *k* values may have underpowered this analysis.

3.7.4 Discussion

In terms of the number of response options, facial emotion recognition tasks that involve at least three response options showed the largest age-effects. This is not surprising given that choosing between multiple options imposes greater cognitive demands than 'either- or' choices. Cognitively demanding tasks disadvantage older adults compared to young adults due to age-related declines in working memory and other broader cognitive operations (Hedden & Gabrieli, 2004). Older adults may also be less motivated to allocate cognitive resources to tasks that are more cognitively demanding.

In the main manuscript, we highlighted that the pattern of effects across emotions that was reported by Ruffman et al. (2008), was only present for stimuli from the POFA. Here we note that virtual agents also produced a similar pattern of effects; smaller age-effects for happiness, than for anger, fear, sadness, and surprise. Disgust was not represented by virtual agents, so we are unable to identify if older adults demonstrated superior disgust recognition, compared to younger adults, on virtual stimuli.

In the main manuscript, we also discussed the pattern reported by Ruffman et al. (2008) for older adults to demonstrate less difficulty recognising happiness than anger, fear, and sadness. In the main manuscript, we highlighted how our findings go against the premise of a positivity effect. Here, we highlight that a positivity effect was present for morphed animations and virtual agents. However, for morphed animations, it is worth noting that if the animations transition to full-intensity expression of happiness, the task may be no more difficult than tasks involving full-intensity photographs. Thus, morphed animations may lack

the sensitivity to facial emotion recognition accuracy age-effects that full-intensity photographs do. These tasks do have the added benefit, however, of also considering reaction time, or intensity at which expression was recognised and can encourage participants to respond as soon as they identify the emotion. It may be that when reaction time, or intensity at which expression was recognised, is considered that larger age-effects emerge for happiness recognition.

A pattern consisted of a positivity effect was also present for virtual agents. However, it may be that happiness, being the only uniquely positive emotion typically included in facial emotion recognition tasks, is not easily mistaken for alternative expressions even when virtual agents (for which older adults were typically disadvantaged) are used. Facial emotion recognition tasks typically include various negative emotions (i.e., anger, fear, sadness), which makes the recognition of happiness a qualitatively different task. While negative emotions are easily mistaken for each other, it is less likely that a positive expression such as happiness would be mistaken for a negative expression. Indeed, when we considered number of response options as a moderating variable, inclusion of multiple response options did not make happiness any more difficult for older adults to recognise than when only two response options were provided. Considering the additional response options are typically negative emotion labels, this supports the argument that happiness is not easily mistaken for negative emotions. For facial emotion recognition tasks to be more sensitive to age-effects recognising happiness, other positive expressions, which could readily be mistaken for happiness, would need to be included. Thus the stronger positivity effect observed for virtual agents, may not be evidence of a real-world bias, but could still be a function of task design.

It is also important to note the using only two response options, virtual agents, or morphed animations has limitations. While including multiple response options may also conflate emotion recognition deficits with deficits in other cognitive skills, it remains unclear

whether tasks only involving two response options accurately quantify real-world deficits. In real-world recognition scenarios, older adults are not provided with an 'either-or-choice'. Tasks that included only two responses, such as Go-No-Go tasks, may, therefore, be more suited for specific study types, such as brain imaging studies that require simpler task designs or that are known to activate certain brain regions. For virtual agents, tasks involving these stimuli are arguably not measuring recognition of human expressions of emotions. It seems likely to reflect generational differences of being exposed less to virtual stimuli than young adults, and/or finding digital stimuli less engaging. To avoid generational confounds, we suggest limiting the use of these stimuli to research questions relating specifically to older adults' recognition of emotion on virtual agents. For morphed animations it may be more useful to consider facial emotion recognition efficiency (i.e., both the speed and accuracy of recognition), rather than just accuracy. Otherwise, the final frame of animation may be overly utilised by participants, particularly if it is a full-intensity expression.

Supplementary Table 3.1

Effect size estimates (young versus older) for overall facial emotion recognition and each individual emotion.

Emotion	Random Effect			Heterogeneity
	n	*M* [95% CI]	*p*	*Q*
OVERALL	463	-0.40 [-0.47, -0.33]	<.001	2655.74***
Anger	94	-0.48 [-0.61, -0.37]	<.001	494.42***
Disgust	61	-0.11 [-0.30, 0.07]	.23	477.08***
Fear	69	-0.61 [-0.76, -0.47]	<.001	414.45***
Happiness	85	-0.18 [-0.26, -0.10]	<.001	205.31***
Sadness	76	-0.66 [-0.80, -0.52]	<.001	472.57***
Surprise	40	-0.34 [-0.54, -0.15]	.002	181.96***

Note. *n* = number of effect size estimates. A negative effect size indicates that older adults are worse than young adults. CI = confidence interval. Q = heterogeneity statistics.

*** p <.001.

Supplementary Table 3.2

Results of moderator tests for stimulus format and image set, with tests of residual heterogeneity of these models.

	Test of Moderator			Q^a
	f	*df*	*p*	
All moderators				
Anger	1.21	9,84	.30	439.98***
Disgust	5.73	9,52	<.001	197.70***
Fear	14.16	9,59	<.001	319.67***
Happiness	2.42	9,75	.02	160.45***
Sadness	1.13	9,66	.36	374.65***
Surprise	10.02	7,32	<.001	144.07***
Number of responses				
Anger	5.33	8.42	.05	
Happiness	1.59	8.08	.24	
Sadness	1.66	2.64	.30	
Stimulus format				
Anger	0.15	7.62	.96	
Disgust	1.79	2.84	.34	
Fear[b]	1.28	1.42	.34	
Happiness	1.25	7.1	.48	
Sadness	0.18	7.02	.94	
Surprise[b]	21.7	0.79	.23	
Image set				
Anger	0.69	21.1	.61	
Disgust	5.39	4.72	.05	
Fear	1.01	5.19	.48	
Happiness	1.84	18.5	.16	
Sadness	1.99	11.3	.16	
Surprise	6.58	2.32	.11	

Note. [a] Test of residual heterogeneity after accounting for variance of included moderators, [b]videos and virtual agents not included in this analysis due to *k* values of two or less.

Supplementary Table 3.3

Facial emotion recognition age-effect size estimates grouped by number of response options included in the task.

Number of Response Options	Random Effect			Between
	n	*M* [95% CI]	p^a	p^b
Anger				
2	12	-0.07 [-0.46, 0.32]	.68	.05
3+	82	-0.53 [-0.64, -0.42]	<.001	
Happiness				
2	11	0.11 [-0.32, 0.55]	.52	.24
3+	73	-0.21 [-0.29, -0.14]	<.001	
Sadness				
2	4	0.03 [-2.16, 2.22]	.95	.30
3+	71	-0.70 [-0.87, -0.53]	<.001	

Note. n = number of effect size estimates. A negative effect size indicates that older adults are worse than young adults. CI = confidence interval. [a] Significance of the random effect, [b] significance of the difference between the contrasts after adjusting for number of response options and image set.

Supplementary Table 3.4

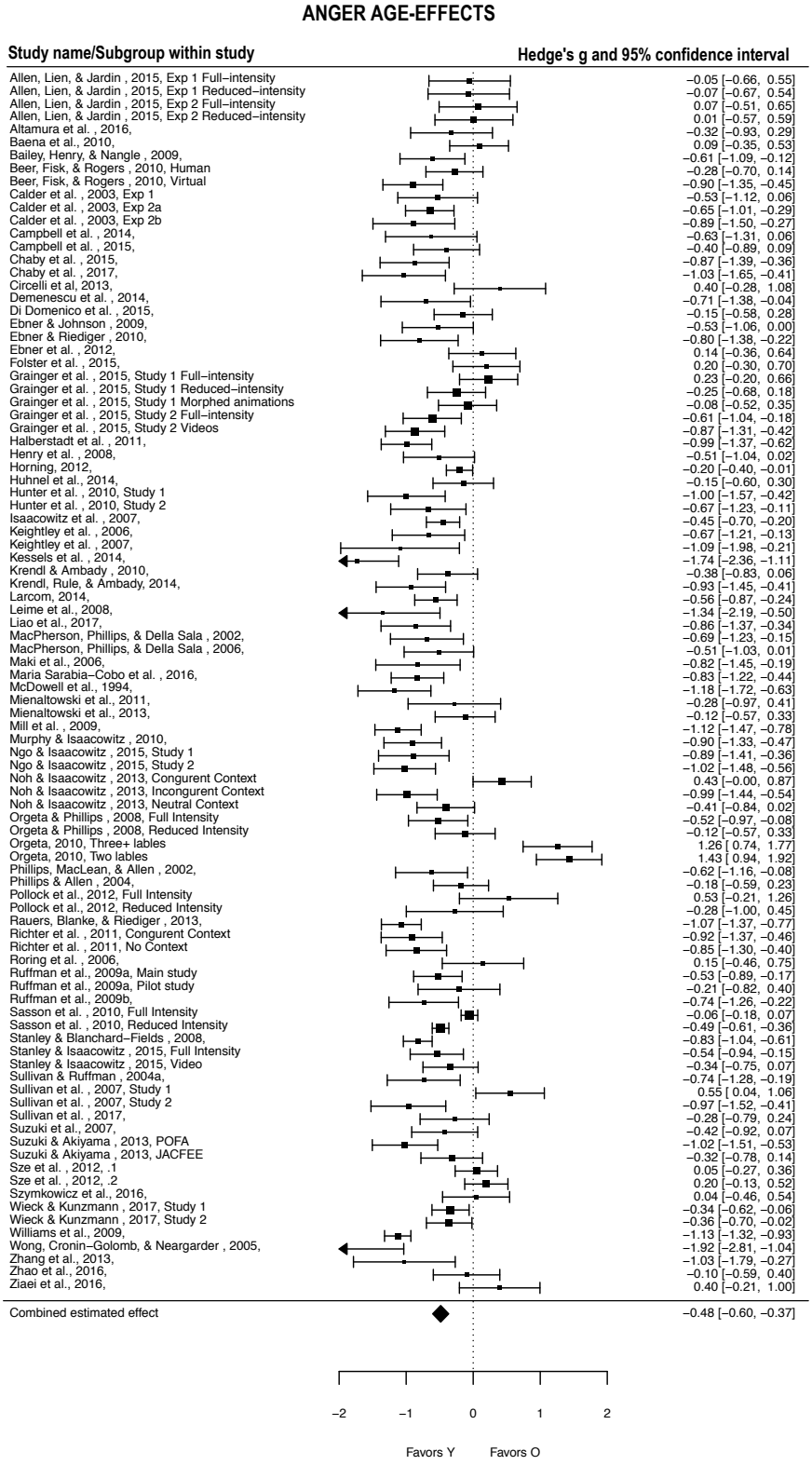
Age-effect size estimates across emotion types, grouped by morphed animations and virtual agents.

Stimulus Format/Emotion	Estimated Random Effect			Between
	n	*M* [95% CI]	p^a	p^b
Anger				
Morphed Animations	6	0.48 [-1.06, 0.09]	.08	.54
Virtual Agents	3	0. 62 [-2.00, 0.77]	.20	.96
Disgust				
Morphed Animations	3	.23 [-0.16, 0.61]	.12	.36
Fear				
Morphed Animations	4	0.61 [-1.29, 0.08]	.07	.29
Virtual Agents	2	0.77 [-3.39, 1.86]	.17	.17
Happiness				
Morphed Animations	6	0.27 [-0.53, -0.003]	.05	.18
Virtual Agents	3	0.14 [-0.81, 0.52]	.45	.66
Sadness				
Morphed Animations	4	0.65 [-1.11, -0.20]	.02	.76
Virtual Agents	3	0.83 [-3.08, 1.40]	.25	.84
Surprise				
Morphed Animations	3	0.18 [-0.61, 0.25]	.22	.90

Note. n = number of effect size estimates. A negative effect size indicates that older adults are worse than young adults. CI = confidence interval. [a] Significance of the random effect, [b] significance of the difference between the contrasts (compared to full-intensity photographs) after adjusting for number of response options and image set.

Supplementary Figure 3.1

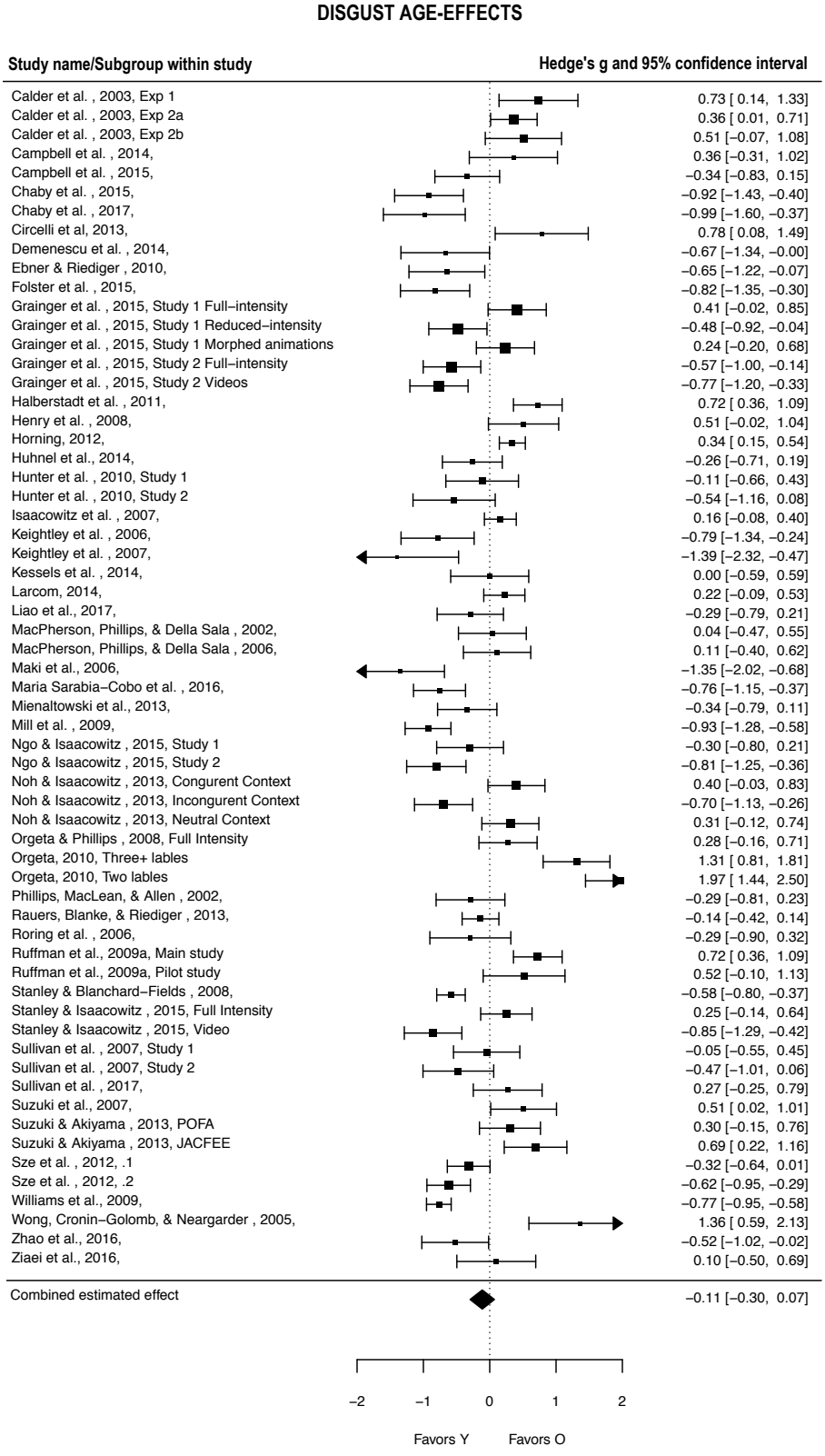
Age-effects for anger recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

Supplementary Figure 3.2

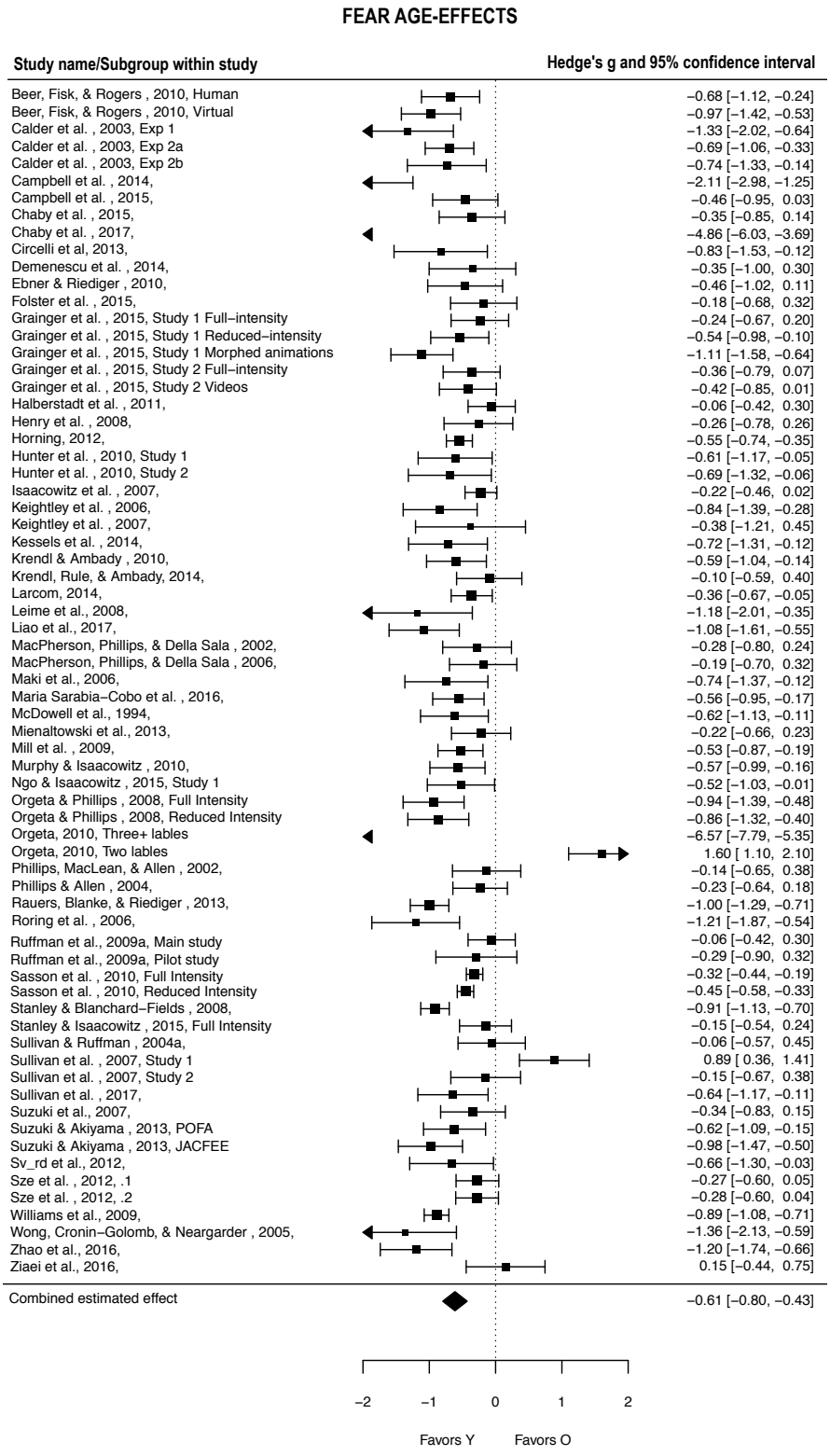
Age-effects for disgust recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

Supplementary Figure 3.3

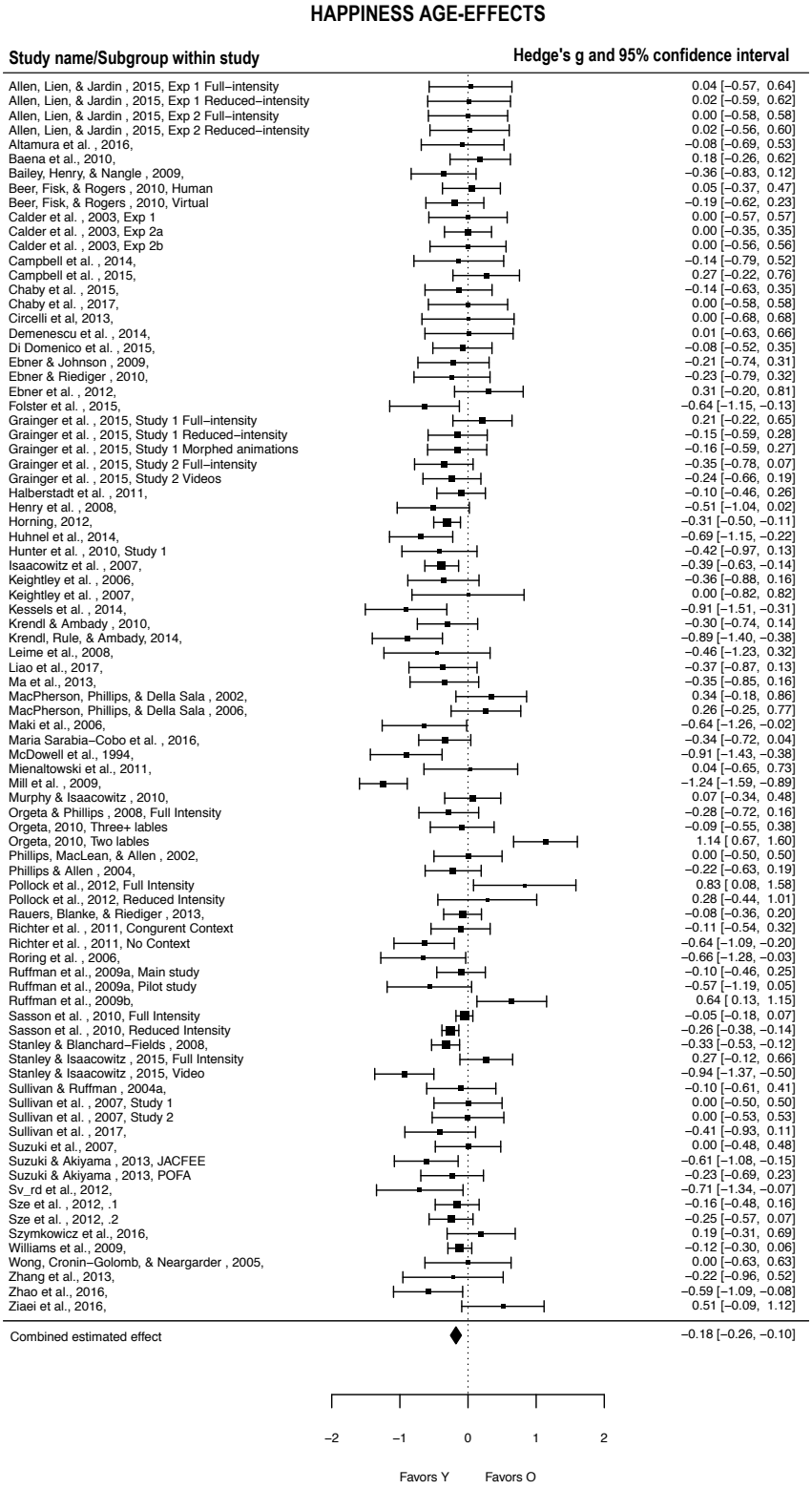
Age-effects for fear recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

Supplementary Figure 3.4

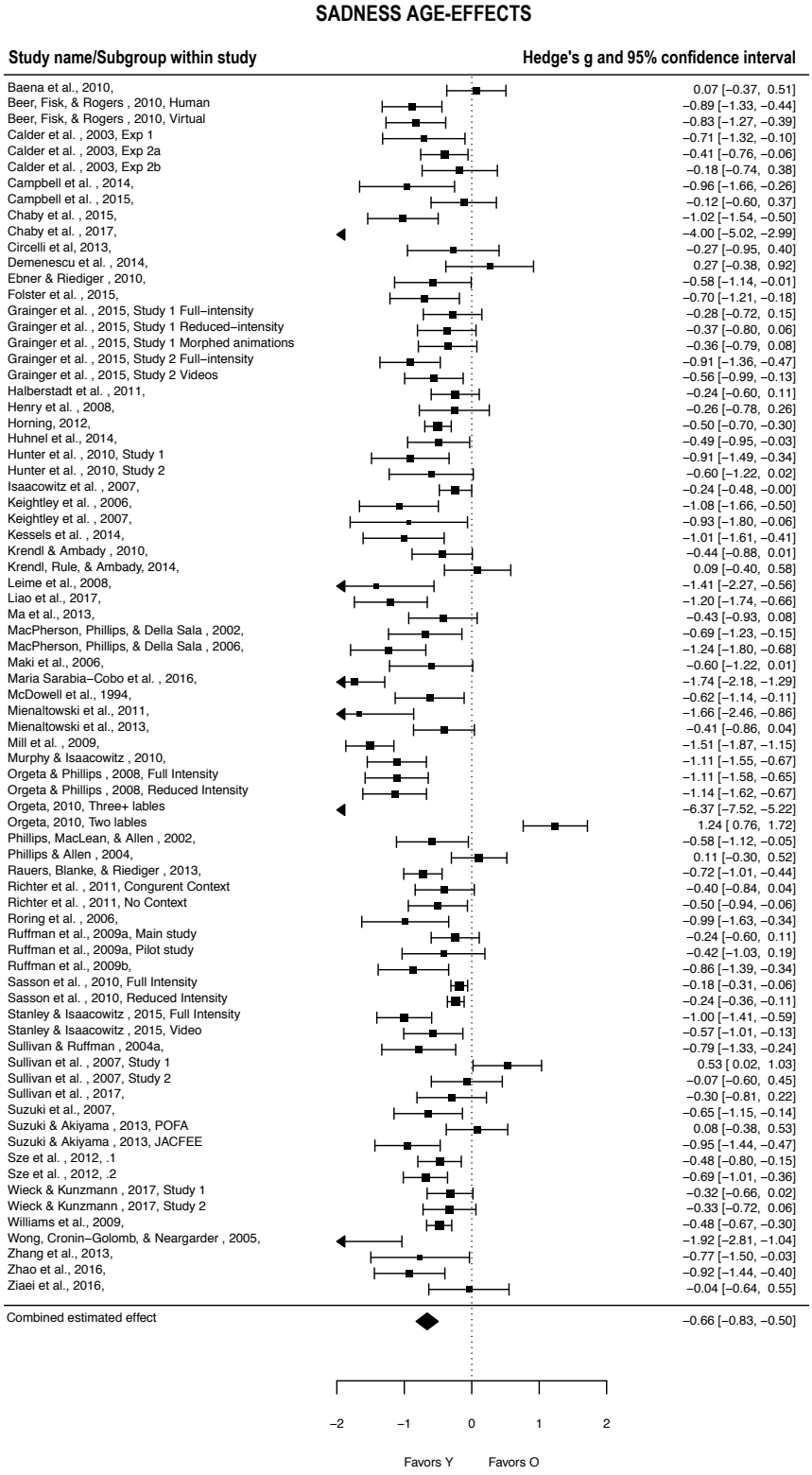
Age-effects for happiness recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

Supplementary Figure 3.5

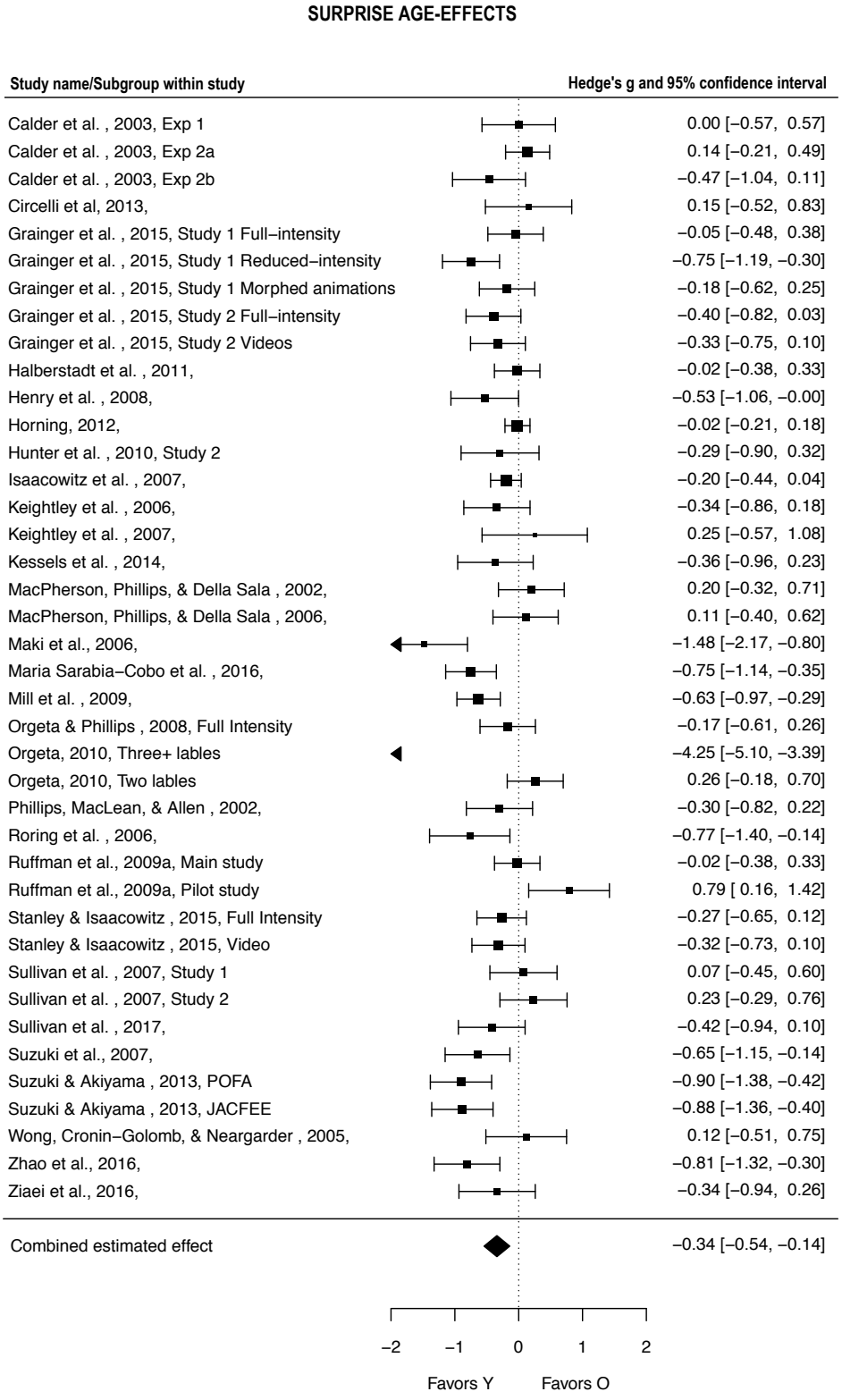
Age-effects for sadness recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

Supplementary Figure 3.6

Age-effects for surprise recognition for each study.



Note. Favours Y = young adults show superior performance. Favours O = older adults show superior performance.

CHAPTER 4: GENERAL INTRODUCTION TO OXYTOCIN AND SOCIAL COGNITION

4.1 Chapter Guide

One of the overarching aims of this thesis was to advance the current understanding of the *mechanisms* underlying older adults' emotion recognition difficulties. In particular, this thesis focused on exploring the oxytocin system as a potential biological mechanism. The current chapter describes the background, physiology, and pharmacology of the neuropeptide oxytocin, and provides an overview of the relationship between oxytocin in the central nervous system and the periphery. The practice of using nasal sprays to artificially modulate central oxytocin is also described. This is followed by a review of the current knowledge about the role of oxytocin in emotion recognition, including details about the effects of intranasal oxytocin on emotion recognition in young healthy controls and young clinical populations. The lack of research investigating the relationship between oxytocin and healthy ageing is then highlighted, demonstrating the need for research investigating the relationship between oxytocin and emotion recognition in healthy older adults. Note, that due to the empirical studies presented in this thesis focusing on behavioural outcomes, this review of the literature also focuses on behavioural studies. While imaging studies are touched on, a comprehensive overview of oxytocin imaging studies is not provided.

4.2 The Neuropeptide Oxytocin

4.2.1 Background

Oxytocin is a neuropeptide that is unique to mammals. It was the first reproduction hormone comprised of peptides to be discovered. Almost 50 years later, oxytocin's chemical structure of nine amino acids was uncovered and oxytocin was officially categorised as a nonapeptide ('nona' being the Latin derivative for nine); cysteine - tyrosine - isoleucine - glutamine - asparagine - cysteine - proline - leucine – glycine. The chemical structure of oxytocin is similar to that of vasopressin, another nonapeptide. While oxytocin and

vasopressin share seven of their nine amino acids, isoleucine and leucine are unique to oxytocin.

4.2.2 Oxytocin receptor system

The oxytocin system is complex and comprises of two distinct pathways. The hypothalamus releases some oxytocin into the bloodstream, via the pituitary, where it circulates throughout the periphery (Gimpl & Fahrenholz, 2001). However, being a peptide molecule, oxytocin cannot cross the blood-brain barrier (Horn & Swanson, 2013). Thus, for oxytocin to interact with neurons, some oxytocin produced by the hypothalamus is released directly into brain tissue and influences the central nervous system (Baribeau & Anagnostou, 2015). Figure 4.1 depicts the oxytocin receptor system and distinguishes the peripheral and central pathways.

In the peripheral pathway, oxytocin is secreted into the posterior pituitary via axonal release from the magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) (Ludwig & Leng, 2006). Oxytocin is stored in vesicles (Ludwig & Leng, 2006) until it is released into the bloodstream and circulated to various peripheral regions such as the liver, kidneys, and pancreas (Gimpl & Fahrenholz, 2001). The PVN also contains smaller parvocellular neurons, segregated to the magnocellular neurons. Some of these parvocellular neurons project to the median eminence and influence oxytocin release from the anterior pituitary (Campbell, 2016), thus forming part of the peripheral pathway. Additional regions in the peripheral oxytocin receptor system, such as the ovaries, testes, and prostate, are thought to not only be targets for peripheral oxytocin but also sites of regional oxytocin production (Gimpl & Fahrenholz, 2001).

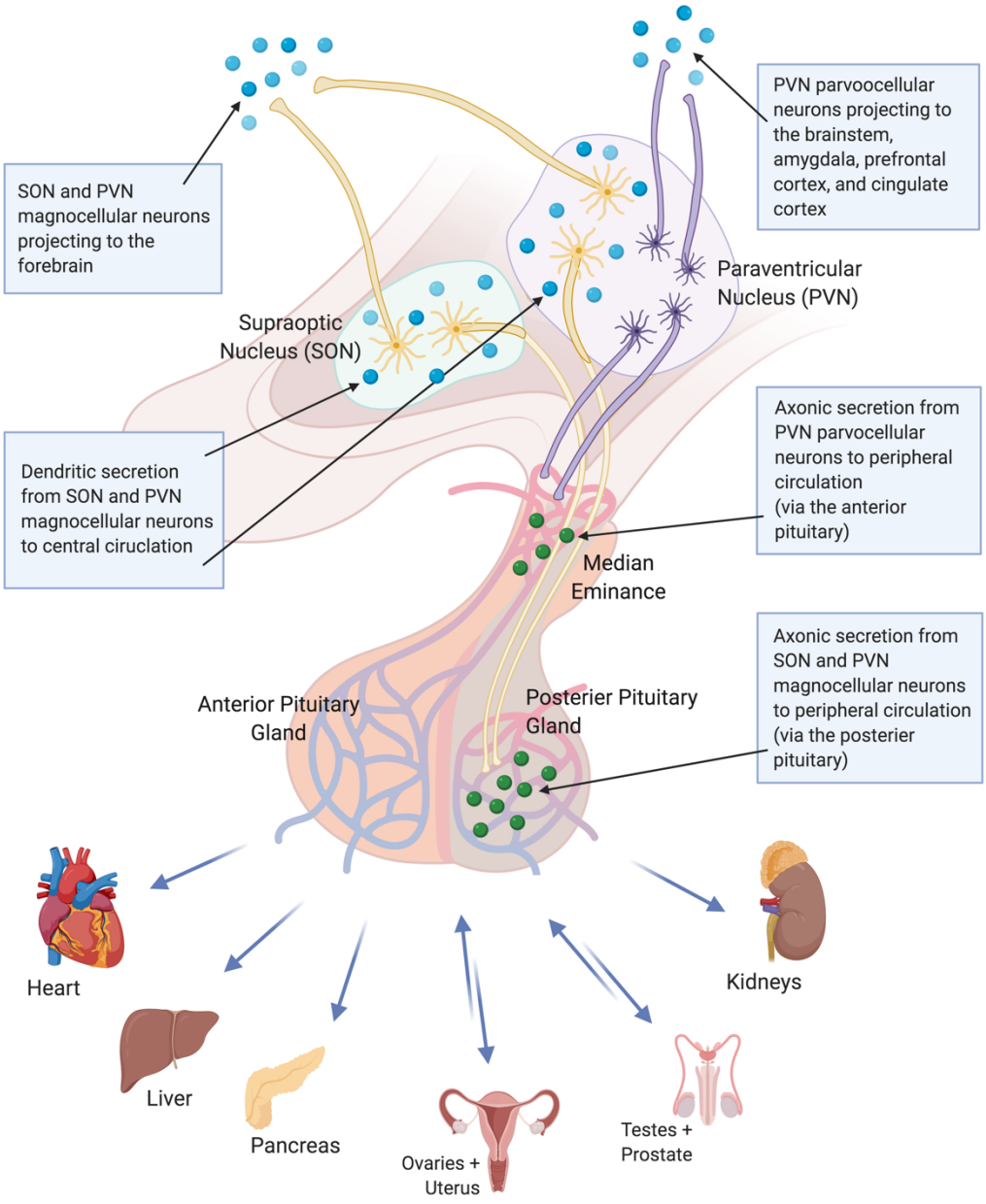
In the central pathway, oxytocin is released via dendritic excretion of the SON and PVN magnocellular neurons (Ludwig & Leng, 2006). Lentivirus-based axonal tracing has

also revealed the SON and PVN magnocellular neurons also project to forebrain regions (Knobloch et al., 2012), releasing oxytocin directly to these regions. Although some of the PVN parvocellular neurons project to the median eminence and form part of the periphery pathway, most of these neurons project to brain regions such as the brainstem, amygdala, prefrontal cortex, and cingulate cortex pathway (Baribeau & Anagnostou, 2015) and are therefore part of the central

Oxytocin not only has two distinct biological pathways, but also has two different actions (Burbach, 1986), and each serves different functions. Oxytocin acts as a hormone throughout the peripheral system where it is associated with water balance, bone density, metabolism, muscle tissue regeneration, and homeostasis (Colaianni et al., 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019). In contrast, oxytocin acts as a neurotransmitter in the central nervous system is linked to sexual behaviour, social behaviours and social memory (Heinrichs, von Dawans, & Domes, 2009; Lee, Macbeth, Pagani, & Scott Young, 2009). Thus, oxytocin plays a vital role in functions relating to both brain and physical health.

Figure 4.1

Visual depiction of the central and peripheral oxytocin pathways.



Note. Centrally excreted oxytocin is coloured blue, and peripherally excreted oxytocin is coloured green. The purple neurons represent the larger magnocellular neurons, and the yellow neurons represent the smaller parvocellular neurons. The blue arrows point to some of the peripheral targets that form part of the peripheral oxytocin system, such as the heart, liver and pancreas. However, these are examples, and as such, not all targets are pictures (e.g., fat cells, adrenal gland, mammary tissue etc.). The bi-directional arrows leading to and coming from the ovaries, testes, and prostate, represent the fact that these sex organs are both targets of peripheral oxytocin, but also regional sites of oxytocin excretion. Figure created with BioRender.com.

4.2.3 The relationship between oxytocin in central and peripheral systems

The relationship between central and peripheral oxytocin excretion is unclear. Using peripheral oxytocin concentration levels to estimate overall or central endogenous oxytocin, through sampling blood (plasma), saliva, and urine is popular because it is relatively non-invasive. However, there is evidence that peripheral oxytocin measures are not a viable method for inferring central oxytocin levels (Kagerbauer et al., 2013; Lefevre et al., 2017). For hormones that cross the blood-brain barrier, such as testosterone and estrogen, peripheral measures of concentration levels do correlate with central levels, and thus this inference can be made. In contrast, oxytocin is prevented from crossing the blood-brain barrier (Baribeau & Anagnostou, 2015), with animal research showing that only trace amounts (.002%) of intravenously administered oxytocin enters cerebral spinal fluid (Mens, Witter, & Van Wimersma Greidanus, 1983). Although the central and peripheral oxytocin pathways share neurons, sampling from the dendritic sites (central pathway) and axonal sites (peripheral pathway) of the SON and PVN magnocellular neurons indicates that the oxytocin output from these two points is not parallel (Ludwig & Leng, 2006). Furthermore, systemic osmotic stimulation elicits independent patterns of oxytocin release in blood and brain, with the central release being slower and more enduring than the relatively fast onset of peripheral release (Ludwig, Callahan, Neumann, Landgraf, & Morris, 1994; Neumann, Ludwig, Engelmann, Pittman, & Landgraf, 1993). Thus, oxytocin secretion from the central and peripheral pathways appears to be independent, and oxytocin samples taken from blood or saliva would only reflect the levels of oxytocin released into the periphery and not oxytocin output within the brain (Jurek & Neumann, 2018).

However, when cerebrospinal fluid (CSF), which is thought to reflect central oxytocin concentrations, and plasma samples from the same subjects are compared, the baseline oxytocin concentration levels are on occasion correlated; see (Carson et al., 2015). Most

commonly though, the oxytocin concentration levels from these central and peripheral samples are not correlated (Jokinen et al., 2012; Kagerbauer et al., 2013; Takagi et al., 1985; Takeda, Kuwabara, & Mizuno, 1985). Furthermore, a meta-analysis revealed that when all studies were considered together, overall there was no correlation of baseline peripheral and central oxytocin (Valstad et al., 2017). A more recent study identified that whether oxytocin concentrations in plasma and CSF correlated was dependent on the assay procedure used with Enzyme Immunoassay (EIA) showing a correlation but Radioimmunoassay (RIA) showing no association (Lefevre et al., 2017). Given these inconsistencies across assay procedures, and RIA being considered the gold standard procedure (Lefevre et al., 2017; Leng & Sabatier, 2016), this finding provides further evidence that peripheral oxytocin levels do not reliably indicate central oxytocin levels. Thus, the most parsimonious account of the findings is that baseline central and peripheral oxytocin levels show no association.

Despite a lack of correlation between baseline peripheral and central oxytocin levels, there is evidence to suggests that oxytocin secretion into the periphery and the central system is coordinated under some circumstances. Valstad et al. (2017) observed that there was a positive association between peripheral and central oxytocin levels *after* stress induction in rats. Furthermore, some neuroactive substances, angiotensin IV and CCK-8, trigger a simultaneous peripheral and central oxytocin release (Jurek & Neumann, 2018). It should be noted that when different methods are used for the blood sampling versus brain sampling that the outcomes cannot be compared (Jurek & Neumann, 2018). However, even when microdialysis systems that are designed for either blood or brain are used, the peripheral and central systems are shown to have a coordinated response to some stimuli (i.e., Neumann et al., 1993). Thus, although central and peripheral oxytocin secretion is typically independent and not parallel (Ludwig & Leng, 2006), it also appears that under certain circumstances central and peripheral oxytocin secretion can be activated simultaneously. Notably, even

when release is coordinated, the duration of the stimuli effects on oxytocin output in rats can vary for central versus peripheral systems (Ludwig et al., 1994; Neumann et al., 1993; Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013).

A coordinated release of oxytocin by the peripheral and central pathways in response to various stimuli is likely due to shared neural networks. Although the concentration and frequency of central and peripheral oxytocin secretion are not parallel, and thus baseline levels show no association, the communication and coordination between the two systems have biological merit. In contrast to the SON, the PVN has a complex network of neurons that includes both magnocellular and paraventricular neurons, and also a third 'integrative' neuron called interneurons (Ferguson, Latchford, & Samson, 2008). These interneurons are thought to have excitatory and inhibitory effects on the PVN magnocellular neurons (which are implicated in both central and peripheral pathways) and the PVN paraventricular neurons (which a largely implicated in the central pathway), and thus may coordinate their functions (Ferguson et al., 2008). Inducing stress is therefore thought to cause a coordinated central and peripheral release of oxytocin because the PVN is not only involved in central and peripheral oxytocin release but is also a key component of the hypothalamic-pituitary-adrenal (HPA) stress axis. (Jurek & Neumann, 2018; Valstad et al., 2017).

In summary, peripheral and central oxytocin release appears to be coordinated under certain conditions (e.g., stress activation). However, the central and peripheral pathways are distinct and the release quantity and timing of oxytocin release are not parallel. As such, baseline levels of peripheral oxytocin do not correlate with central oxytocin levels and thus peripheral oxytocin levels are not a reliable indicator of the central oxytocin levels, at least not until more research is done to convince us otherwise.

4.2.4 Oxytocin nasal spray

To investigate the role of central oxytocin in human behaviour, rather than using invasive brain sampling measures, researchers have administered oxytocin intranasally and tested the behavioural effects. Since the sixties, oxytocin has been administered in the form of a nasal spray to influence peripheral functions such as lactation (e.g., Luhman, 1963; Jurek & Neumann, 2018). However, it is only more recently that an intranasally administered nonapeptide, in particularly vasopressin, was shown to cross the blood-brain barrier and become detectable in the CSF (Born et al., 2002). This discovery was a breakthrough for the therapeutic use of oxytocin in humans, as alternative forms of oxytocin administration (e.g., intravenous) oxytocin had significant peripheral effects but were restricted from crossing the blood-brain barrier (Born et al., 2002; Pardridge, 1999). The evidence that nonapeptides administered intranasally may influence central functions led to an explosion of research investigating the effects of intranasal oxytocin on various cognitive functions, from increasing trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005) to improving emotion recognition accuracy (Guastella et al., 2010) and even restoring brain functions to emotional cues in clinical populations such as social anxiety disorder (Labuschagne et al., 2010, 2011) and Huntington's disease (Labuschagne et al., 2018).

However, whether or not intranasal oxytocin crosses the blood-brain barrier and has a direct effect on central functions is debated. As highlighted by Quintana, Smerud, Andreassen, and Djupesland (2018), the lack of a radiotracer for oxytocin makes it difficult to prove that intranasal oxytocin crosses the blood-brain barrier in humans. Research is advancing with developments of tracers used in Positron Emission Tomography (PET) to trace oxytocin's behaviour in the brain (Beard, Singh, Grundschober, Gee, & Tate, 2018a; Smith, Freeman, Voll, Young, & Goodman, 2012; Smith, Freeman, Voll, Young, & Goodman, 2013), but these are still under development. The growing body of neuroimaging

research shows that intranasal oxytocin does modulate brain activity (Horta et al., 2019; Quintana et al., 2015b) but does not prove that this effect is specifically due to oxytocin crossing the blood-brain barrier via the nose-to-brain pathway. However, some support for intranasal oxytocin having direct central effects comes from the growing evidence that CSF levels increase after intranasal oxytocin administration (Striepens et al., 2013; Valstad et al., 2017). Yet, even this evidence can be critiqued due to CSF sampling being from a spinal tap rather than a direct sampling from the brain regions where the central oxytocin receptors of interest reside (Churchland & Winkielman, 2012; Ruigrok & de Lange, 2015). As such, sampling brain extracellular fluid (ECF) would provide a more accurate test of the nose-to-brain delivery pathway of intranasal oxytocin (Ruigrok & de Lange, 2015). However, brain microdialysis is considered to be unethical for people without medical need, thus increased oxytocin in ECF post intranasal administration has only been shown in rats and mice (Neumann et al., 2013; Smith, Korgan, & Young, 2019). Therefore, until non-invasive means of measuring central oxytocin concentrations become available for humans, the evidence of intranasal oxytocin influencing central functions via the nose-to-brain pathway remains somewhat speculative.

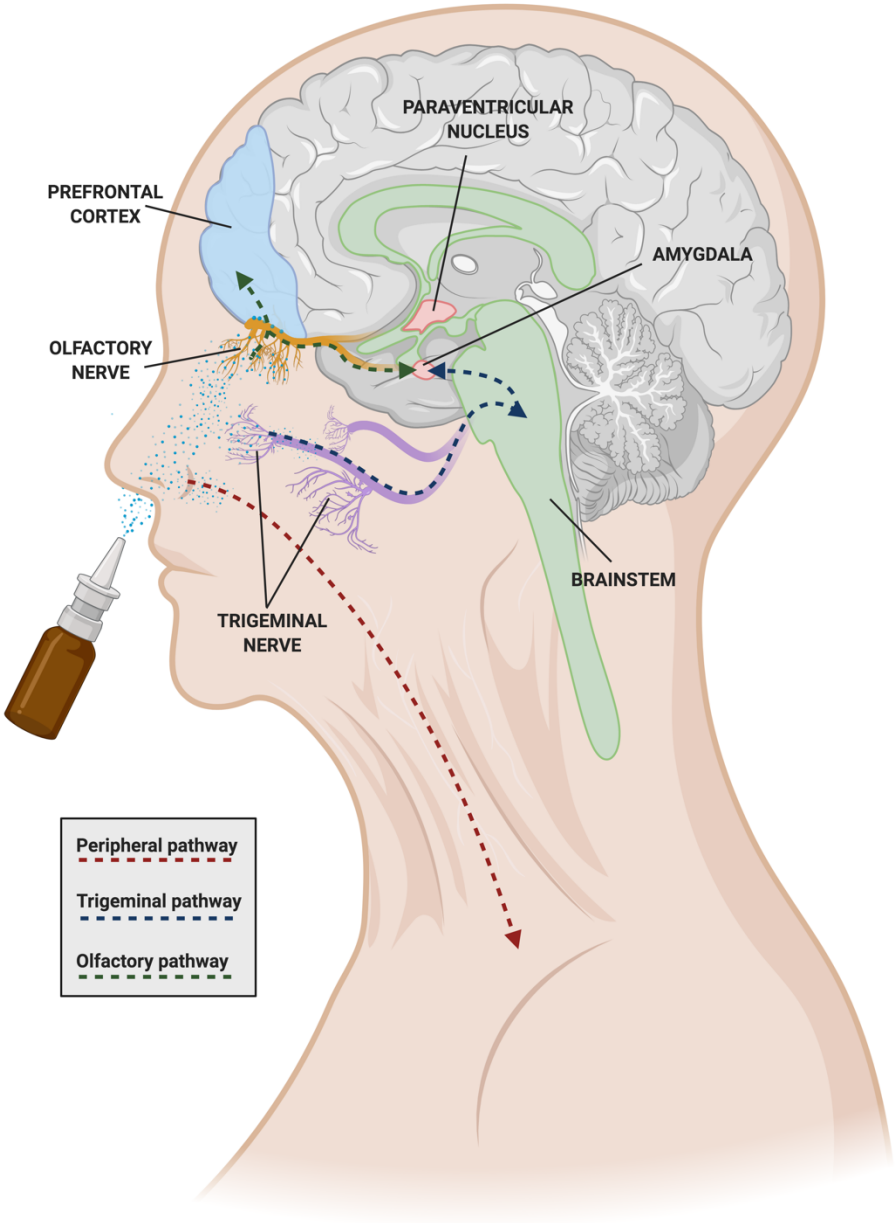
One school of thought is that since the majority of oxytocin nasal spray enters the periphery, then it may be a peripheral effect of intranasal oxytocin that indirectly modulates central processes (Evans, Dal Monte, Noble, & Averbeck, 2014; Leng & Ludwig, 2016; Martins, Paloyelis, & Prata, 2016). As detailed by, Quintana, Alvares, Hickie, and Guastella (2015a), intranasally administered oxytocin can enter the periphery via the olfactory nerve, the trigeminal nerve, and blood capillaries within the nasal cavity; see Figure 4.2. Leng and Ludwig (2016) evidence the known effects of intranasal oxytocin on peripheral targets by citing its historic use in childbirth. In current practice, intranasal oxytocin is used to increase lactation. Quintana (2018) asserted that if the central modulatory effects of intranasal

oxytocin are indeed attributable to an indirect peripheral effect, then the same outcomes would be observed for intravenously administered oxytocin as than administered intranasally. Quintana et al. (2018) tested this theory and reported that when participants were administered oxytocin intranasally and intravenously (on separate occasions), both forms of administration raised peripheral concentration levels, but only intranasal administration modulated central functions. However, see also Hollander et al. (2007) who reported that oxytocin administered intravenously improved social memory in adults with autism, though no direct comparison was made with intranasal administration.

In summary, how intranasal oxytocin exerts effects on central processes remains unclear. It may be a direct effect of oxytocin crossing the blood-brain barrier via the nose-to-brain pathway, or it may be an indirect effect of oxytocin entering the peripheral system. Regardless, the evidence remains that oxytocin administered intranasally does modulate brain activation and certain central process, such as social cognition (Quintana & Woolley, 2016)

Figure 4.2

Visual depiction of oxytocin pathways via the nasal cavity to both the central nervous system and peripheral after intranasal oxytocin administration.



Note. Figure created with BioRender.com.

4.3 The Role of Oxytocin in Emotion Recognition

4.3.1 Oxytocin and social cognition

Oxytocin is a key modulator of social behaviour (Donaldson & Young, 2008; Goodson & Thompson, 2010; MacDonald & MacDonald, 2010). Initial evidence of a link between oxytocin and social processing came from animal research, in particular the study of prairie voles. In voles, oxytocin has been shown to contribute to pair bond formation (Williams, Insel, Harbaugh, & Carter, 1994; Young & Wang, 2004), prevent the biological impacts of social isolation (Stevenson, McMahon, Boner, & Haussmann, 2019), reduce fear during social encounters (Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008), and modulate consoling behaviours (Burkett et al., 2016). In other rodents, oxytocin has also been shown to play a role in several social behaviours, such as social recognition and social memory (Dumais & Veenema, 2016; Lukas & de Jong, 2017; Lukas & Neumann, 2013). In mice, oxytocin knockout results in almost complete loss of social recognition, with this function restored via oxytocin infusion (Ferguson, Aldag, Insel, & Young, 2001).

Due to methodological restraints of testing/measuring oxytocin in the human brain, the role of oxytocin in social cognitive processing in humans has primarily been investigated by comparing behavioural outcomes or neuroimages whilst participants undertook social cognitive tasks after intranasal oxytocin versus placebo administration. These human studies have demonstrated links between the oxytocin system and various social cognitive domains including theory of mind (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Keech, Crowe, & Hocking, 2018), trust (Kosfeld et al., 2005; Van IJzendoorn & Bakermans-Kranenburg, 2012), emotion recognition (Leppanen, Ng, Tchanturia, & Treasure, 2017; Van IJzendoorn & Bakermans-Kranenburg, 2012); and social memory (Guastella, Mitchell, & Mathews, 2008; Rimmele, Hediger, Heinrichs, & Klaver, 2009). For the purpose of this thesis, the focus will be predominantly on oxytocin's role in emotion recognition.

4.3.2 Oxytocin and emotion recognition

Emotion recognition is a higher cognitive process and has therefore only been researched in humans. Initial meta-analyses in studies of healthy adult cohorts reported that oxytocin nasal spray improved emotion recognition accuracy across all emotions (Shahrestani et al., 2013; Van IJzendoorn & Bakermans-Kranenburg, 2012), suggesting an involvement of the oxytocin system in the recognition of emotions. However, Shahrestani et al. (2013) identified that this oxytocin effect was moderated by whether emotion recognition was implicit (e.g., mimicry) or explicit (e.g., labelling). For explicit emotion recognition, which aligns with the emotion recognition definition within the context of this thesis, nasal spray only improved recognition for fearful faces (Shahrestani et al., 2013). Notably, both of these initial meta-analyses only detected small effects (n = 7, Hedges' g = 0.29; Shahrestani et al. (2013) and n = 13, Cohen 0.21; Van IJzendoorn and Bakermans-Kranenburg (2012)), and lacked sufficient power to detect for which participant characteristics (e.g., sex) or conditions (e.g., task types) oxytocin had an effect on emotion recognition.

A more recent meta-analysis has focused specifically on explicit emotion recognition in healthy adult and clinical cohorts (Leppanen et al., 2017). Leppanen et al. (2017) reported that oxytocin improved fear recognition for healthy controls but not clinical groups (e.g., schizophrenia, anorexia nervosa, post-traumatic stress disorder), and marginally improved disgust recognition across healthy controls and clinical groups (there was insufficient data for a disgust subgroup analysis). Given the recency and comprehensiveness of that meta-analytic review (Leppanen et al., 2017), this thesis does not present a systematic review of the emotion recognition and oxytocin nasal spray literature. Rather, Table 4.1 presents the findings from Leppanen et al. (2017) and includes 11 additional studies, most of which have been published since that review was conducted.

From the data presented in Table 4.1, it is apparent that this additional literature does not consistently emulate the pattern of effects across emotions that were presented in Leppanen et al. (2017). In contrast to Leppanen et al. (2017), none of the additional studies reported an oxytocin effect for healthy controls' accuracy recognising fear (Grainger, Henry, Steinvik, & Vanman, 2018a; Horta de Macedo, Zuardi, Machado-de-Sousa, Chagas, & Hallak, 2014; Horta et al., 2019; Hubble et al., 2017; Schwaiger, Heinrichs, & Kumsta, 2019; Shin, Park, Jung, & Kwon, 2018; Timmermann et al., 2017). Conversely, Rutter, Norton, Brown, and Brown (2019) reported that oxytocin reduced fear recognition for participants with anxiety disorders, Timmermann et al. (2017) reported that it improved fear recognition for participants with Antisocial Personality Disorder, and Schwaiger et al. (2019) reported that it also improved fear recognition for healthy participants with a history of childhood abuse. In regards to disgust, only one study examined disgust, and the authors reported no oxytocin effect for either healthy controls or people with schizophrenia (Horta de Macedo et al., 2014). Thus, there is still not enough data to confirm if oxytocin has an effect on disgust recognition. Further effects incongruent with the findings of Leppanen et al. (2017) were, oxytocin decreasing sadness accuracy for young healthy adults (Grainger et al., 2018b), oxytocin increasing happiness recognition for healthy adults when given a higher dose (40 International Units (IU)) (Shin et al., 2018), oxytocin increasing happiness recognition for adults with Antisocial Personality Disorder (Timmermann et al., 2017), and oxytocin decreasing anger, sadness, and happiness recognition for adults with anxiety (Rutter et al., 2019).

In summary, there is evidence that intranasal oxytocin has an effect on emotion recognition accuracy. However, it is unclear for which emotions this effect is present, or what factors may moderate this effect. While the most evidence is present for intranasal oxytocin improving fear recognition, whether or not an oxytocin effect is detected seem to vary

depending on the type of individual studied (i.e., is present for some clinical groups and not others, and present for some healthy controls and not others). Meta-analyses on the effects of intranasal oxytocin on explicit emotion recognition effects have failed to detect effects for anger, sadness, and happiness. While there still appears to be very little evidence of an oxytocin effect for anger recognition for either healthy or clinical groups, there is evidence of an oxytocin effect for happiness and possibly sadness, but further meta-analysis would be required to confirm the magnitude and direction of such effects. The most recent meta-analysis detected a trend towards a disgust recognition effect, however, there is still too few studies that have included disgust for any conclusions to be made. Notably, the influence of sex on oxytocin effects is relatively undetermined, with many studies excluded females from their cohort (50% of studies in Table 4.1 were male only). Furthermore, given the findings of the meta-analytic review on emotion recognition in older adults presented in Chapter 3 of this thesis, the design of the emotion recognition tasks may also be a potential moderating factor. While oxytocin administration therefore has limited evidence as a clinical intervention, the data to date provides evidence that oxytocin is involved in emotion recognition, at least for some emotions. This relationship is further supported by neuroimaging research, which has shown that intranasal oxytocin modulates neural activation during facial emotion recognition tasks (for review see Tully, Gabay, Brown, Murphy, & Blackwood, 2018), in particular, for the amygdala and fusiform gyrus, two regions that are known to be involved in facial emotion regulation (Adolphs, 2010; Parvizi et al., 2012). Quintana and Woolley (2016) recommend that oxytocin research moves beyond simply testing if effects are present, but to examine, for which types of individuals (e.g., different personality traits, different experiences of trauma) and under what conditions (e.g., difficult tasks vs. easy tasks, single dose of oxytocin vs. chronic doses) oxytocin has an effect.

Table 4.1

Outcomes across emotion types for studies testing the effects of intranasal oxytocin (vs. placebo) on emotion recognition separated by healthy participants and clinical groups.

Study	Population	Design	*n*	Sex	IU	Task	Emotion						
							All	A	D	F	Sa	H	Su
Healthy Controls													
Bertsch et al., 2013		B	41	Females	26 IU	Labelling (eyes)	×	×	-	×	-	×	-
						Labelling (mouth)	×	×	-	×	-	×	-
Campbell et al., 2014		B	34	Males	20 IU	Labelling	×	-	-	-	-	-	-
			34	Females	20 IU		×	-	-	-	-	-	-
Chen et al., 2015		W	203	Males	24 IU	Labelling (morphing animations)	×	×	-	×	×	×	-
Domes, Steiner, Porges, and Heinrichs, 2013*		B	62	Males	24IU	Labelling (morphing animations)	×	×	-	-	-	×	-
Domes, Kumbier, Heinrichs, & Herpertz, 2014		W	14	Males	24 IU	Labelling (eyes)	×	-	-	-	-	-	-
						Labelling (mouth)	×	-	-	-	-	-	-
Fang, Lawson, and Wilhelm, 2019*		W	16	Mixed	24 IU	Labelling	×	-	-	-	-	-	-
Feeser et al., 2014		B	82	Males	24 IU	Labelling	×	×	×	↑	×	×	×
Fischer-Shofty, Shamay-Tsoory, Harari, and Levkovitz, 2010		W	27	Males	24 IU	Labelling	×	×	×	↑	×	×	×
Fragkaki and Cima, 2019*	Youth	W	100	Males	24 IU	Labelling (morphing animations)	×	×	×	↑	×	×	×

Gamer, Zurowski, and Büchel, 2010		B	46	Males	24 IU	Labelling (eyes)	×	-	-	×	-	×	-
				Males		Labelling (mouth)	×	-	-	×	-	×	-
Grainger et al., 2018b*		W	61	Mixed	24 IU	Labelling (videos)	×	×	–	×	↓	×	×
Horta et al., 2019*		B	48	Mixed	24 IU	Labelling (morphing animations)	×	×	-	×	×	×	-
Horta de Macedo et al., 2014*		W	20	Males	48 IU	Emotion matching (reduced intensity)	×	×	×	×	×	×	-
Hubble et al., 2017*		W	40	Males	24 IU	Labelling (reduced intensity)	×	×	-	×	×	×	-
Kirkpatrick, Lee, Wardle, Jacob, and de Wit, 2014		W	28	Males	20 IU	Labelling (reduced intensity)	×	×	-	×	×	×	-
			12		40 IU		×	×	-	×	×	×	-
			15	Females	20 IU		×	×	-	×	×	×	-
			10		40 IU		×	×	-	×	↑	×	-
Kirsch et al., 2005		W	15	Males	27 IU	Emotion matching	×	-	-	-	-	-	-
Koch et al., 2016	Trauma exposed healthy adults	W	40	Mixed	40 IU	Emotion matching	×	-	-	-	-	-	-
Lischke et al., 2012		B	47	Males	24 IU	Labelling (morphing animations)	×	×	-	↑	×	×	-
Marsh, Henry, Pine, & Blair, 2010		B	50	Mixed	24 IU	Labelling	×	×	×	×	×	↑	-
Schulze et al., 2011		B	56	Mixed	24 IU	Emotion discrimination	×	×	-	-	-	↑	-
Shin et al., 2018*		W	19	Males	32 IU	Labelling (reduced intensity)	×	×	-	×	×	×	×
			18	Males	40 IU		×	×	-	×	×	↑	×
Schwaiger et al., 2019*		B	40	Mixed	24 IU	Labelling (morphing animations)	×	×	-	×	×	×	–

Timmermann et al., 2017*		W	29	Mixed	24 IU	Labelling	-	×	-	×	-	×	-
Clinical Groups													
Averbeck, Bobin, Evans, and Shergill, 2012	Schizophrenia	W	21	Mixed	24 IU	Emotion discrimination (30% intensity)	↑	×	×	×	×	×	×
						Emotion discrimination (70% intensity)	↑	×	×	×	×	×	×
Bloch et al., 2019*	Schizophrenia	W	34	Mixed	-	Labelling	×	-	-	-	-	-	-
Bertsch et al., 2013	BPD	B	38	Females		Labelling (eyes)	×	×	-	×	-	↑	-
						Labelling (mouth)	×	×	-	×	-	×	-
Campbell et al., 2014	Older adults	B	34	Males	20 IU	Labelling	↑	-	-	-	-	-	-
			34	Females	20 IU	Labelling	×	-	-	-	-	-	-
Domes, Kumbier, Heinrichs, and Herpertz, 2014	ASD	W	14	Males	24 IU	Labelling (eyes)	↑	-	-	-	-	-	-
						Labelling (mouth)	×	-	-	-	-	-	-
Fang et al., 2019*	BDD	W	18	Mixed	24 IU	Labelling	×	-	-	-	-	-	-
Grainger et al., 2018b*	Older adults	W	59	Mixed	24 IU	Labelling (videos)	×	×	-	×	×	×	×
Guastella et al., 2015b	Schizophrenia	W	21	Males	24 IU	Labelling	×	-	-	-	-	-	-
						Labelling	×	-	-	-	-	-	-
Horta et al., 2019*	Older adults	B	48	Mixed	24 IU	Labelling (morphing animations)	×	×	-	×	×	×	-
Horta de Macedo et al., 2014*	Schizophrenia	W	20	Males	48 IU	Emotion matching (reduced intensity)	×	×	×	×	×	×	-

Koch et al., 2016	PTSD	W	36	Mixed	40 IU	Emotion matching	↑	-	-	-	-	-	-
Rutter et al., 2019*	Depression	B	30	Mixed	24 IU	Level of intensity at correct recognition (morphing animations)	-	×	-	×	×	×	-
	Anxiety		30	Mixed			-	↓	-	↓	↓	↓	-
Schwaiger et al., 2019*	History of childhood abuse	B	40	Mixed	24 IU	Labelling (morphing animations)	↑	↑	-	↑	×	×	-
Timmermann et al., 2017*	APD	W	22	Mixed	24IU	Labelling	-	×	-	↑	-	↑	-

Note. * = Not included in Leppanen et al. (2017); IU = International Units (of oxytocin in the nasal spray); PTSD = Post-Traumatic Stress Disorder; BDD = Body Dysmorphic Disorder; BPD = Borderline Personality Disorder; APD = Antisocial Personality Disorder; ADSD = Autism Spectrum Disorder; B = between group design; W = within group design; X = no effects found; ↑ = oxytocin increased emotion recognition; ↓ = oxytocin decreased emotion recognition - = not examined.

4.4 Oxytocin and Healthy Ageing

Since oxytocin is involved in emotion recognition, and emotion recognition accuracy declines in healthy ageing (see Chapters 2 and 3), it may be that the oxytocin system plays a role in the decline of this skill with age. However, despite oxytocin being the first peptide reproductive hormone to be discovered, there is still relatively little known about the oxytocin system in ageing.

There is reason to expect an age-related decline in bodily oxytocin concentration levels because of the known interplay between the sex hormones, testosterone and estrogen, and the oxytocin systems, and evidence of sex hormone declines in old age. Reduced testosterone is linked to a loss of neural oxytocin receptors in rats (Arsenijevic & Tribollet, 1998), but not central oxytocin innervation in rats (Goudsmit, Fliers, & Swaab, 1988) or peripheral concentration levels in human adults (Gordon, Pratt, Bergunde, Zagoory-Sharon, & Feldman, 2017). In contrast, reduced estrogen is linked to diminished peripheral and central oxytocin concentration, and loss of neural oxytocin receptors in rats (e.g., de Kloet, Voorhuis, Boschma, & Elands, 1986), with estrogen administration then increasing oxytocin levels centrally in rats (Caldwell et al., 1989), and peripherally in humans (Boos, Stock, & Schoultz, 1994; Chibbar, Wong, Miller, & Mitchell, 1995). In younger adults, there are mixed sex-effects reported for plasma oxytocin levels. There is evidence for no sex difference in oxytocin production (Jokinen 2012) but there is also evidence of males producing more oxytocin than females (Weisman 2013). Testosterone and estrogen concentration levels decline with age, but the trajectories differ between sexes. Males experience a more pronounced age-related decline of testosterone (Elmlinger, Kühnel, Wormstall, & Döller, 2005), whereas females experience a marked age-related decline of estrogen post-menopause (Eskes & Haanen, 2007). Given the link between sex hormones and oxytocin, not only is an

age-related decline in oxytocin likely, but the trajectory may also differ across older men compare to older women.

4.4.1 Peripheral oxytocin and ageing

The trajectory of peripheral oxytocin concentration levels in healthy ageing remains relatively undetermined, due to limited research and conflicting outcomes. Peripheral oxytocin sampling is popular because it is non-invasive to obtain; through blood, saliva or urine sampling. However, despite the ease of sampling, few studies have investigated the peripheral oxytocin system in older adult humans. A recent meta-analysis of endogenous oxytocin across the lifespan identified seven studies that included older adult samples (i.e., mean age of 65+), six of which used peripheral measures and found a small age-effect in the direction of peripheral oxytocin concentration levels increasing with age (Engel et al., 2019). However, the meta-analysis also revealed significant variation in the oxytocin concentrations derived from different sample types (e.g., un-extracted blood vs. extracted blood), which confounded the age-effect (Engel et al., 2019). Un-extracted samples are often used for peptide analysis under the premise that this type of sampling yields a more sensitive measure of peptide concentrations (Carter et al., 2007; MacLean et al., 2019; Plasencia, Luedicke, Nazarloo, Carter, & Ebner, 2019). However, this technique has been critiqued for producing inflated and erroneous peptide concentrations that are highly influenced by protein interference (Leng & Sabatier, 2016). It is therefore difficult to compare oxytocin concentrations from samples with different assay procedures, so meta-analyses contrasting peripheral oxytocin levels in young and older adults from different studies should be interpreted with caution. Thus, from these studies that have measured older adults' peripheral concentration levels but not compared them to a younger adult sample, it is unclear how ageing influences peripheral oxytocin concentration.

To date, only two[1] studies have directly compared older adults' peripheral oxytocin to a young adult sample, thus using the same sampling type and assay procedure for both groups. Both studies used plasma samples, but Forsling, Montgomery, Halpin, Windle, and Treacher (1998) used extracted plasma and RIA whereas Plasencia et al. (2019) used non-extracted plasma and EIA. Of these two studies, Forsling et al. (1998) used the more accurate assay procedure (Leng & Sabatier, 2016), but Plasencia et al. (2019) reported on a much larger sample (n = 101 vs. n = 24) and included both sexes rather than only males. Both studies reported that no age-effect was present for oxytocin concentration (Forsling et al., 1998; Plasencia et al., 2019). For vasopressin, the two studies produced conflicting results. Plasencia et al. (2019) reported that older adults had significantly higher vasopressin concentration, whereas Forsling et al. (1998) found that older adults had significantly lower vasopressin concentration when compared to young adults. Plasencia et al. (2019) also examined the moderating effect of sex and reported that no age by sex interaction emerged for either oxytocin or vasopressin, but that overall females showed higher oxytocin concentration than males (Plasencia et al., 2019).

4.4.2 Central oxytocin and ageing

To understand the oxytocin system in healthy ageing in full, both central and peripheral concentration levels need to be assessed, yet older adults' central oxytocin levels also remain underdetermined. Measuring central oxytocin in humans is invasive, as sampling requires the collection of CSF (Lefevre et al., 2017). Despite oxytocin research being a rapidly expanding field, the rarity of opportunities to collect CSF likely accounts for why,

[1] Note that a third publication (Ebner et al., 2019) reported on a subset of the same sample as Plasencia et al. (2019)

only one published measure of older adults' CSF oxytocin concentration comparative to young adults could be located (Raskind et al., 1986). The outcome of that study was that there were no age differences for central oxytocin or vasopressin concentrations (Raskind et al., 1986). Yet, even CSF sampling has been critiqued for not be a true measure of baseline oxytocin levels in the brain (Lefevre et al., 2017). Lefevre et al. (2017) highlighted that CSF samples are often extracted using a lumbar puncture, and given the distance of the sampling site from the brain, the sample is likely degraded. Moreover, the process of a lumbar puncture can influence oxytocin production because of the acute stress associated with the procedure itself.

For central oxytocin levels to truly be analysed, CSF sourced directly from the brain, especially from its ventricles, would be required. Several studies have examined immunocytochemically identified vasopressin and oxytocin neurons post-mortem in humans. Three studies reported no age-related changes for oxytocin (Fliers, Swaab, W. Pool, & Verwer, 1985; Swaab, 1995; Wierda et al., 1991), but a fourth reported an age-related decline in oxytocin (Calzà, Pozza, Coraddu, Farci, & Giardino, 1997). Two examines vasopressin levels and reported a slight decrease in these levels (Calzà et al., 1997; Fliers et al., 1985), but these decreases may have been mediated by the time of death with age affecting the circadian patterns of vasopressin production (Forsling et al., 1998; Hofman & Swaab, 1994). In contrast, animal studies, which allow for sampling directly from the brain, have identified age-related increases in central oxytocin (e.g., Parker, Hoffman, Hyde, Cummings, & Maestripieri, 2010; Zbuzek, Fuchs, Zbuzek, & Wu, 1988). However, there is also evidence from animal research suggesting age-related decreases of central oxytocin (e.g., Melis, Stancampiano, Fratta, & Argiolas, 1992), or no change (e.g., Fliers & Swaab, 1983). Non-invasive methods of assessing central oxytocin would be required to resolve these conflicting findings and determine the true trajectory of central oxytocin in healthy ageing.

4.4.3 Oxytocin manipulation in older adults

Despite age being a potential moderator of oxytocin effects, the research investigating oxytocin effects in ageing is limited and has produced conflicting outcomes. Five studies were located that had investigated the effects of intranasal oxytocin on social behaviours in an older adult sample, and three of these studies had examined emotion recognition. Across this research, oxytocin improved older adults' ability to postulate what others' are thinking (theory of mind) (Grainger et al., 2018b), increased older males' attention to feelings (Ebner et al., 2015), and improved older males' emotion recognition accuracy on a static facial emotion recognition task (Campbell et al., 2014). In contrast, oxytocin decreased older females' attention to and clarity of feelings (Ebner et al., 2015), had no impact on older adults' trust (Grainger et al., 2018a), and had no impact on older adults' emotion recognition on dynamic facial emotion recognition tasks (Grainger et al., 2018b; Horta et al., 2019). Note, however, that although Horta et al. (2019) reported no emotion recognition behavioural effects, they did report that oxytocin modulated brain activation during emotion recognition.

There are two key differences within the methods of the studies that investigated the effect of oxytocin for older adults' emotion recognition, which might account for the conflicting results. First, Campbell et al. (2014) and Horta et al. (2019) both used independent groups, which does not guarantee the groups are matched on emotion recognition ability, whereas, Grainger et al. (2018b) used a repeated-measures design to test within groups. Second, Campbell et al. (2014) used an emotion recognition task with stimuli that were static, whereas Horta et al. (2019) and Grainger et al. (2018b) used dynamic stimuli (morphed animations and videos, respectively). Since Campbell et al. (2014) was the only one of these three studies to report an oxytocin effect, and was also the only study to have used static stimuli, it may be that task type moderated oxytocin's effects in these ageing studies. Indeed, the meta-analysis presented in Chapter 3 of this thesis revealed that task characteristics (i.e.,

number of included emotions, stimulus format, and image set used) accounted for a significant amount of variance in the facial emotion recognition age-effects produced for most emotions.

It is plausible that the presence or absence of oxytocin effects could also be accounted for by task difficulty, since variation in oxytocin effects can be accounted for by item difficulty (Domes et al., 2007; Guastella et al., 2010). Moreover, Horta et al. (2019) showed that for dynamic emotion recognition stimuli, older and younger adults had similar amygdalar connectivity, yet for static emotion recognition stimuli there are differences in amygdalar connectivity older adults compared to young adults (Keightley et al., 2007; St Jacques et al., 2010). These findings provide evidence that older adults process static and dynamic emotional faces differently and that oxytocin's effects could therefore be dependent on the format of the stimuli.

Further research would be necessary to confirm the finding that oxytocin improves older males' accuracy in recognising emotions from static facial expressions (Campbell et al., 2014). This is particularly important given the common critique of intranasal oxytocin research lacking reproducibility (Leng & Ludwig, 2016; Walum, Waldman, & Young, 2016). It is also important for future research in this field to test enough participants to have sufficient statistical power, as intranasal oxytocin research often lacks statistical power (Leppanen et al., 2017). Leppanen et al. (2017) recommend, on the basis of their power analysis, that intranasal trials include at least 64 participants per group for between-group designs, and 34 participants in total for within-group designs. This would suggest that two of the three studies on the effects of intranasal oxytocin on older adults' emotion recognition did not test enough participants (Campbell et al., 2014; Horta et al., 2019). Another consideration when it comes to statistical power, is ensuring that there is sufficient data to test moderating

effects, since oxytocin effects can be context-dependent (Bartz, Zaki, Bolger, & Ochsner, 2011) and also vary by sex (Seeley, Chou, & O'Connor, 2018).

4.5 Chapter Summary

The limited studies and also the limitations within these few studies examining the effects of intranasal oxytocin on emotion recognition in ageing, provided a strong motivation for the current thesis to examine the intranasal oxytocin effects in healthy older adults in more detail in an experimental chapter (see Chapter 6). Moreover, given the limited evidence on peripheral oxytocin levels in human ageing, we also explored this in the current thesis (Chapter 7). Next, however, a detailed chapter will follow on the methodologies involved in these experimental chapters.

CHAPTER 5: GENERAL EXPERIMENTAL METHODS

5.1 Chapter Guide

In this chapter, the methodology for Study 2 (Chapter 6) and Study 3 (Chapter 7) are detailed. While overviews of the experimental methods can also be found in those chapters, these papers were written with publication restrictions (such as strict word limits) in mind. Thus, the method sections in those experimental chapters lack some details that may aid the reader's understanding of the research conducted. The first section of the current chapter outlines the participant information (e.g., an overview of the participant sample, recruiting methods, and inclusion criteria). Then the study procedures are detailed, including saliva sampling, drug dosage/administration, and the counterbalancing and blinding procedures. Finally, the facial emotion recognition task is described in full, including details about the manipulations made to the stimuli, and each questionnaire is outlined and referenced.

5.2 Participant Information

5.2.1 Participants

A total of 120 participants completed the overall study, 60 healthy older adults (60-80 years) and 60 young adults (18-33 years), with equal numbers of males and females in each age group. A power analysis conducted by the most recent meta-analytic review of oxytocin effect on emotion recognition reported that within-subject designs should have at least 34 participants (Leppanen et al., 2017). We tested almost four times this amount to ensure that age and sex differences could be analysed. Extreme age group comparison was used (i.e., older adults compared to younger adults rather than testing multiple age groups across the lifespan) because: i) it replicates the methodology of the majority of healthy ageing the research, ii) the novelty of the research warranted extreme age analyses as a first step to inform future lifespan analyses; and iii) the expensive nature of the neurosciences techniques; a lifespan approach requires testing a much greater number of participants.

5.2.2 Recruitment

Participants were recruited from Australian Catholic University in Melbourne, a registry of volunteers who had previously participated in research with the Cognition and Emotion Research Centre, an aged-care organisation (Villa Maria Catholic Homes), and via social media and personal networks. Participants were screened over the phone prior to participating.

5.2.3 General exclusion criteria

Participants were excluded if they had any neurological or psychiatric illnesses or were taking anti-psychotic or anti-depressant medications. Screening was also conducted for substance use, with participants excluded if they had a history of substance abuse or dependence or were a current smoker. Participants were also excluded if they had a history of heart disease, were currently pregnant or breastfeeding, or were taking hormone supplements. However, young females using hormone-based contraception were not excluded because of findings that oxytocin levels are comparable between women with and without contraceptive intake (Weisman et al. 2013), and reports that oxytocin effects do not differ when women on oral contraception are included versus excluded (Campbell et al., 2014). Crystallised intelligence, as measured by the National Adult Reading Test (NART) (Nelson & Willison, 1991), and years of education were recorded to characterise the age groups, and to match the men and women within each group. Older adults were screened for intact cognitive ability using the Telephone Interview for Cognitive Status (TICS) (Brandt, Spencer, & Folstein, 1988), and excluded if they scored in the mildly, moderately, or severely impaired ranges (i.e., score of 25 or less). If participants had cold or flu symptoms on the day of testing, the session was rescheduled. All participants spoke English fluently.

5.3 Study Procedures

5.3.1 Study design

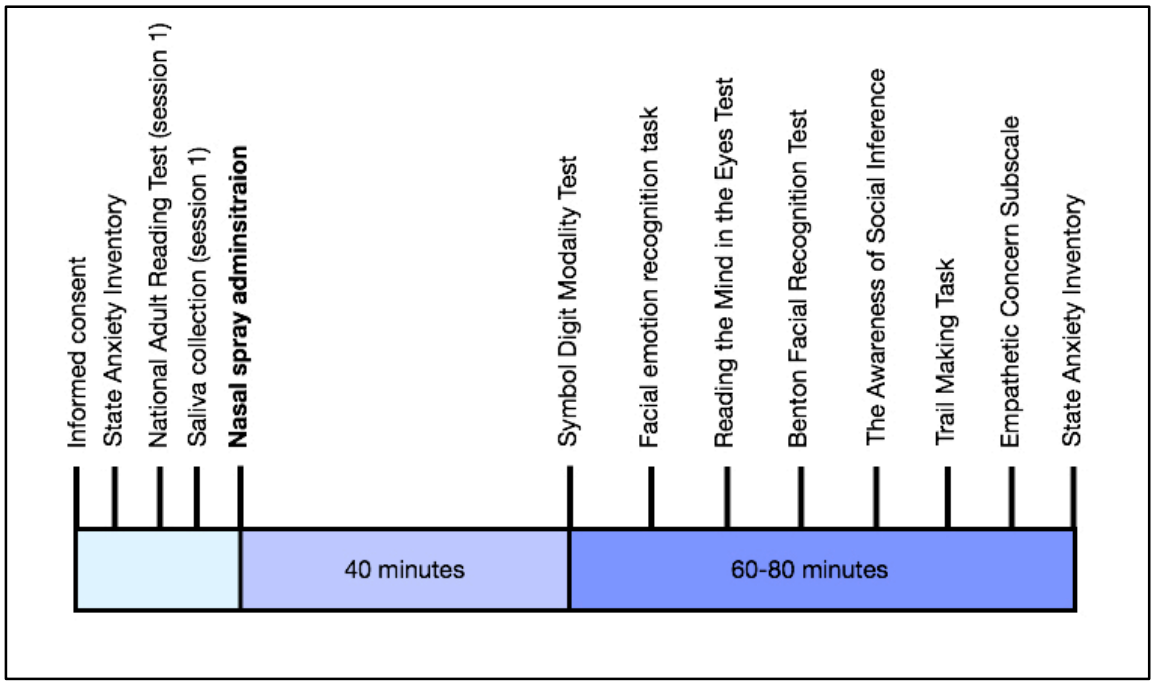
The research project involved a placebo-controlled cross-over randomised study (Chapter 6), in which the oxytocin system was manipulated by administering intranasal oxytocin relative to placebo. In addition, saliva samples taken from the same participants at the initial testing session, to measure endogenous peripheral oxytocin, vasopressin, estradiol, and testosterone, formed a second study (Chapter 7).

5.3.2 Study procedures overview

All participants attended two separate 2-3 hour testing sessions at Australian Catholic University Melbourne campus (active condition and placebo condition), see **Appendix A** for study materials. The sessions were conducted four weeks apart for all participants to control for young females' menstrual cycle (all tested during their mid-luteal phase) and to reduce practice effects. Upon arrival to the first testing sessions, participants provided informed consent then complete the NART. They then provided a saliva sample and self-administered the assigned nasal spray (placebo or active) before the test battery was administered. Participants also took a questionnaire home to complete and return at the second session. The questionnaire included demographic questions as well as additional measures of loneliness, social functioning, personality, trait anxiety, and autistic traits. The second session followed the same protocol as the first, but without the collection of a saliva sample. The study protocol was approved by the Human Research Ethics Committee at the Australian Catholic University and registered with the Australian New Zealand Clinical Trials Registry (**Appendix B**). Figure 5.1 provides a visual depiction of the procedural sequence.

Figure 5.1

Protocol timeline showing the approximate number of minutes post nasal spray administration that tasks were conducted.



Note. There were several tasks included in the test battery, such as the Symbol Digit Modality Test, that due to the scope of the current thesis, are not detailed or analysed in the manuscript.

5.4 Saliva Collection

5.4.1 Saliva collection procedure

Two x 1 ml samples of saliva were collected from each participant, prior to administration of the oxytocin, to measure their endogenous hormone concentration levels. To avoid the possibility of food substances contaminating the saliva, participants were advised to, where possible: not eat a major meal within 60 minutes of sample collection. Since caffeine may compromise the assay by lowering saliva pH and increasing bacterial growth, participants were also instructed to restrict caffeinated drinks on the day of testing to one cup, and to drink this no sooner than three hours before sample collection. The samples

were collected via the 'passive drool' method (see the manufacture's instructional video (https://www.youtube.com/watch?v=8-AxSDHLbBE), which involves four main steps:

1. Participants open the saliva collection aid packet and remove the saliva collection aid.
2. Participants insert the saliva collection aid into the cryovial.
3. Participants allow saliva to pool in their mouth, tilt their head forwards, insert the saliva collection aid into their mouth, and allow the saliva to gently flow through the saliva collection aid into the cryovial.
4. Participants remove the saliva collection aid from the cryovial and tightly screw the lid onto the cryovial to enclose the sample.

Saliva collection took between five and 45 minutes to complete. When participants appeared to be having difficulty producing saliva, they were instructed to focus on a photograph of a person eating a lemon, as this visual input has been shown to increase the salivatory response (Hagenmuller, Rossler, Wittwer, & Haker, 2014). As dry mouth is also a common response to stress or anxiety (Ying Joanna & Thomson, 2015), participants were encouraged to take their time and the researcher avoided watching them by doing other tasks or leaving the room. For further detail on saliva collection and storage see **Appendix A – 10.**

5.4.2 Saliva storage and analysis

Any samples that contained visible blood were immediately discarded. Saliva samples were kept cool on ice and frozen within two hours of collection. Saliva samples were stored long term at -80°C in a freezer located in the purpose-built psychopharmacology lab within the School of Psychology, until assay. One 1 ml sample per participant was sent via courier, packaged with dry ice to prevent thawing, to two specialised labs. Oxytocin and vasopressin were extracted according to the method of Landgraf's lab (RIAgnosis, Munich, Germany)

using highly sensitive and specific RIA (Kagerbauer et al., 2013). Testosterone and estradiol concentration levels were extracted at Stratech Scientific (http://www.stratechscientific.com.au), according to the manufacturer's instruction using commercially available kits (Salimetrics, USA).

5.5 Drug Administration

5.5.1 Drug overview

A compounding pharmacy was enlisted to formulate the nasal sprays (Dartnell's Compounding Pharmacy) to the required specifications. The oxytocin sprays were made from oxytocin powder to the strength of 40 IU/ml. As each puff releases 0.1ml of the solution, this concentration resulted in 4 IU being administered per puff of the nasal spray. The sprays also contained sorbitol trituration, glycerine and preserved water. The placebo sprays were prepared in a similar manner without the inclusion of oxytocin powder. The inclusion of glycerine in the placebo sprays ensured that both sprays had a similar taste, as the spray can run down the back of the throat during administration. To prevent degradation of the active oxytocin, the nasal sprays were refrigerated and if unused were disposed of after 5 weeks.

5.5.2 Drug dosage

A dose of 24 IU (6 puffs) was selected as this is the most commonly used dosage (Leppanen et al., 2017; Shahrestani et al., 2013). This dose is more effective compared to 40 IU (Cardoso & Ellenbogen, 2013; Spengler et al., 2017) and has been demonstrated to influence amygdala function (Spengler et al., 2017), cortisol levels (e.g., Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Quintana et al., 2015b), and social cognitive behaviours (e.g., Kosfeld et al., 2005; Schulze et al., 2011).

5.5.3 Drug delivery

In both sessions, participants self-administered a single dose of either placebo or oxytocin nasal spray; depending on their assigned condition order. This involved administering a total of six puffs in an alternating nostril sequence resulting in a total of three puffs per nostril (12 IU per nostril). Splitting administration across nostrils improves absorption (Guastella et al., 2013). Between each puff, participants were instructed to wait for 45-seconds to avoid flooding the nose. The researcher timed the nasal spray administration with a stopwatch and informed the participants when it was time for the next spray and which nostril to spray into. Before commencing the puff sequence, the researcher shook the nasal spray and instructed the participants to prime the pumping mechanism by doing four pre-puffs in the air. By having the participants conduct the nasal spray priming it allowed them to become familiar with the process and to work out how to best hold the bottle. Priming the spray was necessary to ensure even distribution of the oxytocin per puff. Participants were instructed to insert the tip of the nasal spray into their nostril, to hold the bottle vertical and parallel to their face, to exhale and then inhale while simultaneously pressing down firmly on the nasal spray. Participants could block their other nostril and/or tilt their head back if they found it helpful. Participants were asked to blow their nose before administrating the spray if they felt at all congested. During and after administration participants could dab at their nose if spray began to drip but were instructed not to blow their nose for at least 20 minutes after the final puff.

5.5.4 Drug timing

Before administering the nasal spray participants provided a saliva sample and completed any baseline or demographic measures; see Figure 5.1 for a visual depiction of the session timeline. Following the last puff, participants waited for 40-minutes before commencing the cognitive test battery. The 40-minute waiting period allows for oxytocin to

reach peak pharmacodynamics effects in the brain (Spengler et al., 2017) and is consistent with previous protocols (Labuschagne et al., 2010, 2011).

Spengler et al. (2017) identified that pharmacodynamic effects of oxytocin are at their peak from 45 to 70 minutes post-administration. As this research was part of a larger study, the test battery included other measures not included in this methodological overview and thus took participants between 60 minutes and 80 minutes to complete. Participants therefore completed the last questionnaire between 100 and 120 minutes after administering the nasal spray. However, salivary levels of oxytocin are observed to remain consistently elevated up to 135 minutes post oxytocin nasal spray administration (Huffmeijer et al., 2012), and Weisman, Zagoory-Sharon, and Feldman (2012) report a peak increase in oxytocin salivary levels lasting from 45 to 120 minutes post-administration.

5.6 Counterbalancing and Blinding Procedures

5.6.1 Blinding procedures

The nasal sprays were ordered and collected from the pharmacy by a researcher not involved in testing. The bottles came unmarked and the same researcher labelled them with an A or B to denote whether they were oxytocin or placebo; see Figure 5.2 for an example. None of the researchers conducting the testing sessions were privy to which letter represented which condition. Participants were asked at the end of the second session which spray they thought was the oxytocin to see if participants were able to distinguish between the active and placebo condition.

Figure 5.2

Photograph of one of the nasal sprays (A) used in the clinical trial.

5.6.2 Counterbalancing

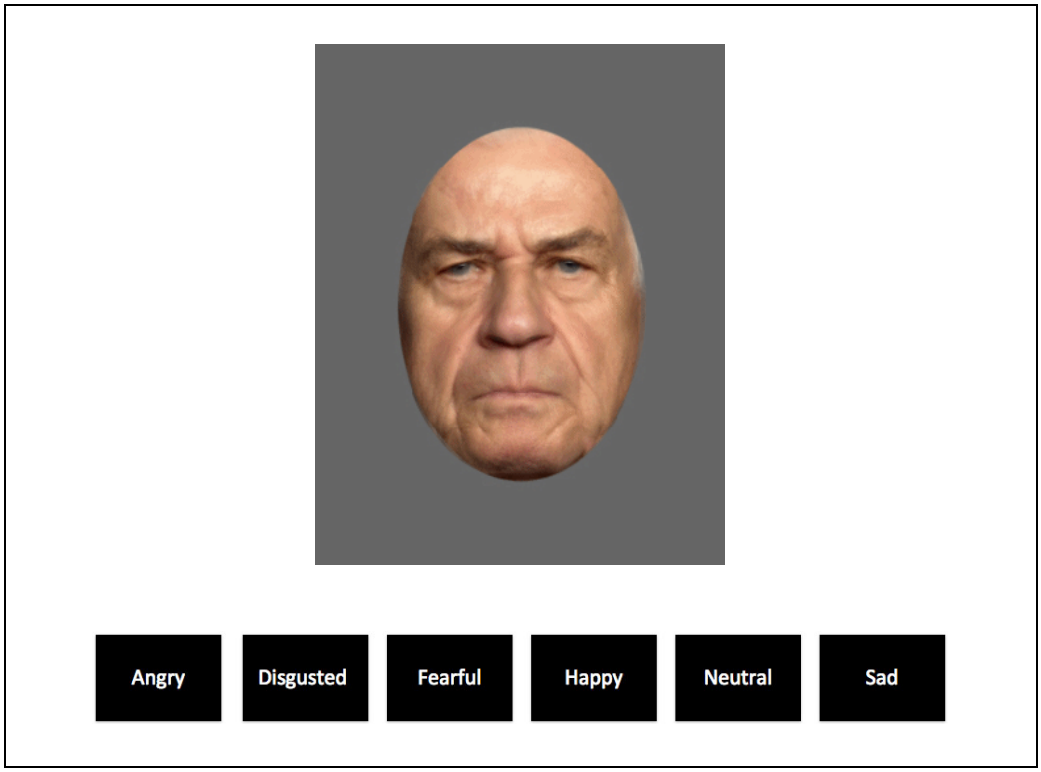
Participants were randomly allocated to one of two condition orders; active oxytocin spray in the first session with placebo spray in the second session, or vice versa. The allocation was done by the same researcher who ordered and labelled the nasal sprays. Initially, block randomisation was used, with separate blocks used for younger and older participants, to balance condition order across age groups. Then, so that gender differences could also be examined, for the last 20 participants, dynamic randomisation was used to ensure that condition order was perfectly counterbalanced across gender groups within each age group. Thus, exactly 15 participants of each age and gender group (e.g., older adult females) had the active oxytocin spray in the first session and 15 had the oxytocin spray in the second session. Tasks with alternate forms were not counterbalanced, so the precise counterbalancing of the condition order also ensured that equal numbers of each age and gender group completed task version A under the active condition as task version B.

5.7 Facial Emotion Recognition Task

A facial expression-labelling task was used to measure the key outcome variable of emotion recognition. Participants were shown neutral expressions and reduced-intensity facial expressions of anger, fear, disgust, sadness and happiness, of twelve different posers (three young females, three young males, three older females and three older males). The faces were displayed on the laptop screen, with the six labels continually displayed beneath each image (i.e., 'angry', 'disgusted', 'fearful', 'happy', 'neutral', and 'sad'); see Figure 5.3 for an example item. Participants were asked to select the emotion label that best categorised the facial expression and instructed to respond as quickly and accurately as possible. This procedure mimics that of Ruffman, Murray, Halberstadt, and Taumoepeau (2010).

Figure 5.3

An example item from the facial expression labelling task depicting an older adult male poser displaying an anger expression at 50% intensity.



5.7.1 Reducing the emotional intensity of facial expression stimuli

From the meta-analytic review presented in this thesis (see Chapter 3), it is apparent that the most commonly used stimuli for facial emotion recognition task in healthy ageing research are photographs of human actors displaying facial expressions of emotions sourced from published image sets. The benefit of using stimuli from published image sets is that the stimuli have already undergone a validation process. This allows researchers to be confident that the angry faces, for example, do exhibit anger.

However, as highlighted in Chapter 2 of this thesis, the main concern with these published image sets is that they often only contain photographs depicting *full-intensity* expressions of emotion. Full-intensity expressions are relatively easy to recognise, and researchers in the ageing field that use them frequently report ceiling effects for facial emotion recognition accuracy of one or both age-groups (i.e., younger v. older adults). As ceiling effects prevent the true maximum accuracy of one or both groups from being detected, tasks involving these stimuli have limited sensitivity to age differences in performance.

Reducing the emotional intensity of the facial expressions is one way to mitigate these ceiling effects as it makes the task more difficult. Across emotion recognition research more broadly, reduced-intensity photographs of facial expressions of emotion are already used in facial emotion recognition tasks when greater task sensitivity is required (e.g., Fenske et al., 2015; Smith, Montagne, Perrett, Gill, & Gallagher, 2010). The current study therefore adopted this approach.

For the experimental study, the FACES image set (Ebner et al., 2010) was selected as the stimulus for the facial emotion recognition task. It was selected because it is produced in colour and includes older adult posers, which likely mitigates any own-age recognition

biases. The FACES image set is comprised only of full-intensity expressions, and no reduced-intensity versions were available at the time. Permission was therefore acquired from the creators of the FACES image set to use morphing software to create reduced-intensity versions of their photographs of full-intensity expressions.

FantaMorph software was used to create the reduced-intensity stimuli. Twelve posers, three males and three females from the two age groups, were selected from the FACES database. The faces were first cropped to be a consistently sized oval shape and centralised within a grey rectangle so that the target face and neutral face were aligned. Next, the 'face locator' tool was used to apply 100 anchor points to both the photograph of the actor depicting the target expression and the photograph of the actor's neutral expression in corresponding locations. The target points were placed around key facial regions such as the eye, nose and mouth; see Figure 5.4 for an example. For each face (e.g., young female number 1 depicting anger) two images were created of differing emotional intensity; one that was 50% target expression and 50% neutral expression (see Figure 5.5 for an example), and one that was 75% target expression and 25% neutral expression. There was therefore a total of 120 reduced-intensity images made; two levels of intensity, for each of the 12 posers, for each of the five target emotions included in the FACES database (i.e., anger, disgust, fear, happiness and sadness).

Figure 5.4

Example of the 100 target point locations that were applied to the neutral faces and corresponding target faces to create the facial emotion recognition task stimuli.

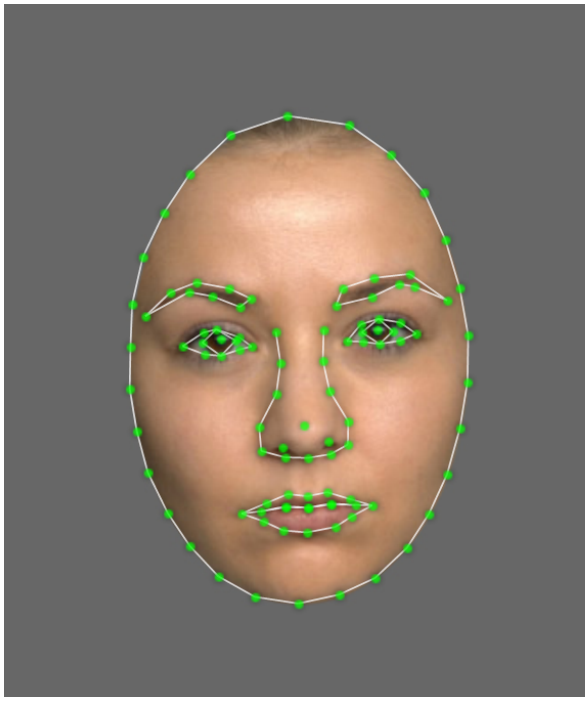
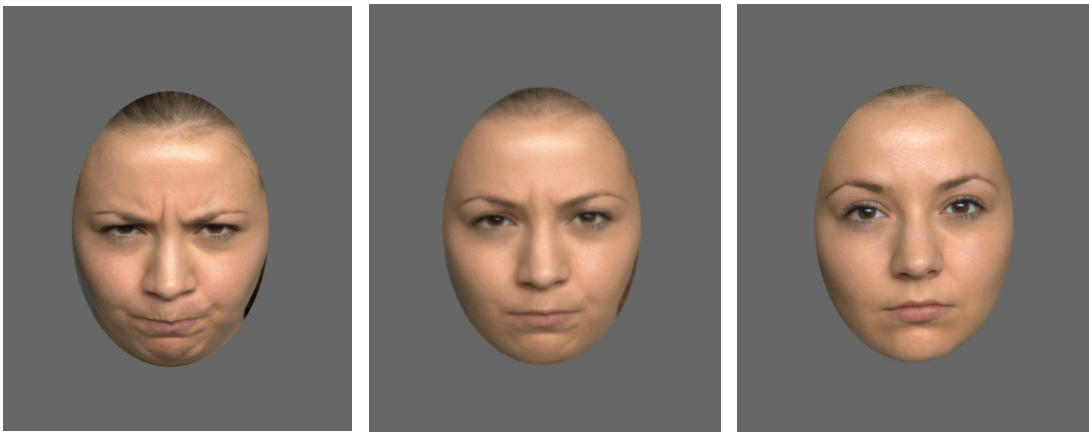


Figure 5.5

Example of the target and neutral faces, sourced from the FACES database, before and after being digital morphed into one image to create the facial emotion recognition task stimuli.



100% Angry **50% Angry** **100% Neutral**

Note. From left to right the faces depict an original 100% angry facial expression, a morphed photograph that is 50% angry expression and 50% neutral expression, and an original 100% neutral expression.

5.7.2 Facial emotion recognition task versions

There were two different versions of the task created, with each version containing one photograph (either the 50% or 75% intensity) of each actor expressing each of the 5 target emotions and a neutral expression; a total of 72 images. If version A contained the 50% intensity photograph of the actor expressing anger, version B would contain the 75% intensity photograph of that actor's expression of anger. Across both versions of the task there was a mix of intensities. Though the intensities were not perfectly balanced across the two versions, and thus one version was plausibly easier than the other, any differences in task difficulty were accounted for by counterbalancing condition order within and between age and gender groups. Within each version of the task, participants viewed the items in a randomised order.

5.8 Questionnaires

The following questionnaires were included in the questionnaire packet that participants completed between session. These questionnaires measure variables, or obtain information about demographics, that are known to modulate intranasal oxytocin effects (e.g., Bartz et al., 2011; Radke & de Bruijn, 2015) or be influenced by oxytocin administration (e.g., Heinrichs et al., 2003).

5.8.1 Trait anxiety

The trait anxiety inventory from the State and Trait Anxiety Inventories (STAI) was used to measure of participant's typical anxiety levels (Spielberger, Gorsuch, & Lushene, 1970). For this questionnaire, participants were required to rate each of the 20 items (e.g., "I feel secure") on a scale from one to four (with one being 'almost never', and four being 'almost always'), how they generally feel.

5.8.2 Social functioning

The Social Functioning Scale (SFS) was used to identify the number and frequency of prosocial activities participants currently engage in (Birchwood, Smith, Cochrane, Wetton, & Copestake, 1990). For this questionnaire, participants were required to indicate on a scale from zero to three (with zero being 'never', and three being 'often'), how frequently they engaged in each of the 20 listed activities (e.g., visiting relatives in their home, parties, going to the cinema).

5.8.3 Loneliness

The UCLA Loneliness Scale was included in the questionnaire packet to measure the extent to which participants subjectively feel lonely and have a sense of isolation (Russell, Peplau, & Ferguson, 1978) (Russell, Peplau, & Cutrona, 1980). Participants were instructed to indicate how often each of the 20 statements described them (e.g., "I lack companionship"), on a 4-point scale (with 'O' being "I often feel this way", and 'N' being "I never feel this way".

5.8.4 Personality traits

The Big Five Inventory (BFI; John & Srivastava, 1999) was included in the questionnaire packet to assess participants' personality type on five continuums: **extraversion** vs. introversion, **agreeableness** vs. antagonism, **conscientiousness** vs. lack of direction, **neuroticism** vs. emotional stability, and **openness** vs. closed to experience. Participants were instructed to indicate how much each of the 44 characteristics applied to them (e.g., "Can be cold and aloof"), on a scale from one to five (with one being 'strongly disagree', and five being 'agree strongly'.

5.8.5 Autistic traits

The Adult Autism Spectrum Quotient (AQ) was used to assess the degree that participants had traits associated with the autism spectrum. Noting, however, that none of the participants reported having being diagnosed with Autism Spectrum Disorder (ASD). For this questionnaire, participants were asked to indicate on a four-point scale (from 'strongly disagree' to 'strongly agree'), how strongly they agreed with each of the 50 statements (e.g., "I prefer to do things with other, rather than on my own").

5.9 Data Analysis

Each of the three studies included in this thesis (Chapters 3, 4, and 5) involved different statistical analyses that were conducted in a variety of software data analysis packages. The statistical analyses are detailed in full in their respective chapters, however, a brief overview follows.

For the meta-analysis (Chapter 3), the study effects (Hedge's g) were calculated using Comprehensive Meta-Analysis (Borenstein, Hedges, Higgins, & Rothstein, 2003). This software was selected due to the ease in which it can calculate effect sizes from various data types (e.g., group means and standard deviations, chi-square, independent t-test), while also being able to collapse subgroups (e.g., males and females, or different conditions). The moderators (e.g., stimulus format) were then assessed using multi-level meta-analysis models. These models were conducted with the 'metafor' package (Viechtbauer, 2010), in R (R Core Team, 2018), using the RStudio interface (RStudio Team, 2019). Multi-level models were used to enable multiple effects from the same study to be included (e.g., the same participants completed a static facial emotion recognition task and a dynamic facial emotion recognition task), and their dependence accounted for.

For the study examining the effects of oxytocin on young and older adults' facial emotion recognition (Chapter 5), Tobit regression models were fitted to the data using Stata (StataCorp, 2019). Tobit regression models (also called censored regression models) were used because they account for dependent variables that are either left or right censored. For the emotion recognition task, participants could only obtain a recognition rate between zero and one for each emotion. Thus, the dependent variables had an upper limit of one, and were negatively skewed in distribution. Variables of interest (e.g., oxytocin condition, personality traits) were included as fixed effects, but participant ID was included as a random effect to account for the repeated measures design. The follow up Bayesian analyses of variance (ANOVA) that were run to assess the strength of evidence for the null models versus the alternate models were conducted in JASP (JASP Team, 2020), using the default settings.

For the study comparing young and older adults' peripheral peptide and hormone concentration levels (Chapter 6), a multivariate linear model (MANOVA) was run in SPSS (IBM Corp., 2017); with the concentration of oxytocin, vasopressin, testosterone, and estradiol as the dependent factors, and age (young and old) and sex (female and male) as the fixed effects. Once again, the follow up Bayesian ANOVAs that were run to assess the strength of evidence for the null models versus the alternate models were conducted in JASP (JASP Team, 2020).

CHAPTER 6: STUDY 2 - INTRANASAL OXYTOCIN DID NOT MODULATE OLDER ADULTS' EMOTION RECOGNITION ACCURACY

6.1 Chapter Guide

The following chapter comprises a clinical trial of the effects of oxytocin nasal spray (24 IU) on young and older adults' facial emotion recognition. The aims of this study were; to (i) determine whether intranasally administered oxytocin modulates young and older adults' facial emotion recognition accuracy, thus exploring whether oxytocin within the central nervous system is involved in older adults' facial emotion recognition difficulties, and (ii) to identify participant characteristics (e.g., sex, personality traits) that moderate any oxytocin effects. This paper was prepared with publication in mind, and thus, to maintain a publishable manuscript length, the unbiased hit rates are presented in the main manuscript and the raw hit rates are included as supplementary materials that are presented directly following the manuscript; see '6.7 Supplementary Material'.

Preliminary data from this study was presented at the Aikenhead Centre of Medical Centre Research Week conference, 5th August 2019 (see 'Research Outputs', pp. 15). In addition to my supervisors, and the staff and students that assisted in data collection, we are also grateful to Dr Natalie Ebner and colleagues for allowing us to use their FACES stimuli. We also thank Prof. Markus Heinrich for contributing his expertises to the design and development of this study.

6.2 Abstract

An inability to recognise what other people are feeling is detrimental for effective interpersonal functioning and has been linked to reduced social functioning and diminished mental health. Healthy older adults are one population who demonstrate difficulty recognising emotions from facial expressions. To date, however, the mechanisms behind this difficulty remain unclear. The neuropeptide oxytocin is one possible mechanism that may contribute to older adults' poor emotion recognition ability. This study tested the role of oxytocin, when administered intranasally, in modulating young and older adults' facial emotion recognition accuracy. Sixty young adults (aged 18–33) and 60 older adults (aged 65–80), balanced for sex, completed a facial emotion recognition task of six basic emotions (anger, fear, sadness, disgust, happiness and neutral) in a placebo-controlled cross-over randomised trial of 24 IU of oxytocin nasal spray versus placebo nasal spray. Unbiased hit rates were calculated for each emotion separately, due to differences in neuroanatomical representations of the individual emotions. Mixed-effects models revealed that oxytocin had no impact on older adults' facial emotion recognition accuracy. However, oxytocin did improve young females' sadness recognition. The findings from this study suggest that intranasal oxytocin had no age-related benefit on recognising facial expressions of emotion in older adults.

6.3 Introduction

In everyday social interactions, people observe and interpret facial expressions to postulate what others may be feeling. This non-verbal form of communication is a vital aspect of interpersonal functioning, with facial emotion recognition difficulties associated with decreased social functioning (Hooker & Park, 2002; Trevisan & Birmingham, 2016). Difficulty recognising facial expressions of emotion is a marker of several neurodegenerative diseases, such as Alzheimer's disease and Huntington's disease (Kordsachia et al., 2017; Kordsachia et al., 2018a; 2018b; Phillips et al., 2010) However, healthy older adults also demonstrate difficulty recognising facial expressions of emotions (Hayes et al., 2020); Chapter 3.

Despite social cognitive abnormalities being critical predictors of mental health and well-being in old age (Phillips et al., 2010; Szanto et al., 2012), the causes of age-related declines in facial emotion recognition accuracy remain undetermined. The competing theories largely comprise two overarching mechanisms; motivation and biology. Motivational perspectives include the positivity effect (Reed & Carstensen, 2012), the selective engagement hypothesis (Hess, Germain, Swaim, & Osowski, 2009; Hess et al., 2001), and the Dynamic Integration Theory (Labouvie-Vief, 2009). Generally, these theories suggest that older adults retain the ability to recognise emotion from facial expressions, but are not motivated to engage their limited cognitive resources to do so. Indeed, when the stimuli presented in the emotion recognition task improve older adults' current experience (i.e., images of happiness) (Reed & Carstensen, 2012), or when the task is meaningful or rewarding (i.e., they believe the poser to share similar attributes to them) (Zhang et al., 2013) older adults exhibit less difficulty recognising the emotions. Conversely, from biological perspectives, older adults are thought to have trouble recognising facial displays of emotion because of age-related neurobiological decline. Neuropsychological models link age-related

declines in facial emotion recognition accuracy to structural and functional changes in key brain regions that underlie emotion recognition processing (Phillips et al., 2002). Some of the brain regions involved in the processing of emotions include the temporal regions, the amygdala, the insula, and parts of the prefrontal cortex such as the orbitofrontal cortex, inferior frontal gyrus and anterior cingulate cortex (Dricu & Frühholz, 2016; Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012; Vytal & Hamann, 2010). Neuropsychological models, therefore, predict a direct correspondence between the degree of age-related brain changes and the subsequent functionality in the emotion recognition processes supported by these brain regions (Ruffman et al., 2008).

Although the dominant neuropsychological theory focuses on structural degeneration of neural regions, a possible alternative neuropsychological theory is that the production of key neurotransmitters involved in social cognitive processes diminishes with age. Oxytocin is a neuropeptide known to play a role in social cognition (Heinrichs et al., 2009), and therefore could be a biological mechanism involved in older adults' facial emotion recognition difficulties. Yet, relatively little is known about the oxytocin system in ageing (Huffmeijer et al., 2013).

In humans, the study of oxytocin's modulatory role on behaviour has been done via nasal spray administration of oxytocin that is thought to provide a direct path to the brain (Quintana et al., 2018). Although oxytocin can be measured in peripheral circulation via blood, saliva or urine samples, and despite central and peripheral oxytocin production being coordinated under certain conditions (Kagerbauer et al., 2013; Valstad et al., 2017), there is very limited support that baseline peripheral oxytocin levels approximate baseline oxytocin levels in the central nervous system (Valstad et al., 2017). A truly accurate measurement of older adults' central oxytocin, to help explain some of the age-related changes in a psychological function (e.g., emotion recognition), would require sampling of CSF directly

from the brain (Lefevre et al., 2017). Oxytocin administered in the form of a nasal spray is thought to influence oxytocin centrally, rather than just peripherally, as the nasal passage is a conduit for oxytocin to cross the blood-brain barrier (Quintana et al., 2018). Oxytocin nasal spray, therefore, provides a non-invasive means of testing oxytocin's role on central functions, such as emotion recognition.

To our knowledge, only three studies have tested the effects of intranasal oxytocin on older adults' facial emotion recognition, and these had conflicting results (Campbell et al., 2014; Grainger et al., 2018b; Horta et al., 2019). Campbell et al. (2014) reported that 24 IU of intranasal oxytocin (vs. placebo) improved overall facial emotion recognition for older adults, specifically for older men (vs. older females and younger adults) that was not specific to any emotion. This is in contrast to Grainger et al. (2018b) and Horta et al. (2019) reporting that no oxytocin effect emerged for either young or older adults at a similar dose of 24 IU of intranasal oxytocin (vs. placebo). Campbell et al. (2014) provided two explanations for the age by sex effect of oxytocin. First, older men have greater difficulty recognising facial expressions of emotion compared to older women and are therefore more in need of an oxytocin boost. Indeed, according to Bartz's (2011) interactionist account of the mixed oxytocin findings within the literature, oxytocin only improves performance for individuals who find the task more demanding. Second, oxytocin administration has been shown to influence brain activity to emotional stimuli differently for men compared to women (Rilling et al., 2014).

One methodological difference between these three studies that may account for the conflicting outcomes, is the different facial emotion recognition measures used. From Chapter 3, a meta-analytic review of facial emotion recognition difficulties in healthy ageing showed that the combined influence of several task characteristics (i.e., the number of emotions included in the task, the format of the stimuli, and the image set from which the

stimuli were sourced) was a significant moderator of the age effects produced (Hayes et al., 2020). This meta-analytic finding has implications for research investigating the effects of oxytocin nasal spray on facial emotion recognition in healthy ageing, as it suggests that older adults' ability to recognise emotions from facial stimuli depends on the task characteristics and therefore oxytocin's effects may vary also by the type of task. As such, it is worth noting that Campbell et al. (2014) used static photographs of full-intensity emotional depictions, whereas both the two studies that reported null oxytocin effects used dynamic facial emotion recognition stimuli that are inherently subtler (Grainger et al., 2018b; Horta et al., 2019).

Here we emulated the Campbell et al. (2014) study and tested the effects of 24 IU of intranasal oxytocin, compared to placebo, on young and older adults' facial emotion recognition accuracy on a static task. However, we also built on that study by using a repeated measures design, rather than independent groups, to truly account for differences in participants' baseline performance. Moreover, we used a large sample (N = 120), because both the Campbell et al. (2014) and Horta et al. (2019) studies were underpowered based on the recommendations provided in the most recent review of intranasal oxytocin studies looking at emotion recognition (Leppanen et al., 2017).

Despite Campbell et al. (2014) reporting an oxytocin effect that was not emotion-specific, it is likely that the effects of oxytocin on older adults' recognition of different emotions would vary in magnitude. Meta-analyses of the effects of oxytocin on young/middle-aged adults' facial emotion recognition accuracy provide evidence of emotion-specific effects (Leppanen et al., 2017; Shahrestani et al., 2013). Specifically, both meta-analyses indicated that for explicit facial emotion recognition, an oxytocin effect is most evident for the recognition of fear. It is unclear for which emotions older adults would most likely demonstrate oxytocin benefits. Next, we provide an account of factors that may help determine how oxytocin is likely to influence emotion recognition abilities in older adults.

Firstly, older adults have greater difficulty recognising some emotions than others, and given the Bartz's interactionist account, oxytocin would be expected to show the greatest improvement for recognition of the emotions that they are most impaired for. For the type of facial emotion recognition task used in this study, older adults typically have the most difficulty recognising sadness and surprise, followed by fear, anger, and disgust, and the least difficulty for happiness (Hayes et al., 2020). Thus, the strongest oxytocin effects would be expected for recognition of surprise and sadness, and the least for recognition of happiness.

Secondly, given the complex neurobiological relationship between ageing and emotion recognition, oxytocin is likely to influence each emotion differently. Although still unclear, different brain regions are likely utilised in the recognition of different emotions. For example, it is thought that happiness recognition predominantly resides in the temporal regions, fear in the amygdala, and anger and disgust in the inferior frontal gyrus (Lindquist et al., 2012; Vytal & Hamann, 2010). In addition, oxytocin has varying effects on different brain regions. Oxytocin administration typically inhibits amygdala output by shifting output towards prefrontal regions (Bos, Panksepp, Bluthé, & Honk, 2012), which would be expected to have the greatest influence on fear recognition which reside predominantly in the amygdala. However, the influence of oxytocin on amygdala output is also emotion-specific, with evidence that intranasal oxytocin decreases amygdala response to fearful faces and increases amygdala response to happy faces (Gamer et al., 2010). Finally, age-related decline in brain regions is not uniform. The amygdala remains relatively intact in healthy ageing, with evidence of robust amygdala activation during emotion processing in older and young adults (Campbell, Grady, Ng, & Hasher, 2012; Hampson et al., 2012; Raz et al., 1997; St Jacques et al., 2010), which would suggest a pattern of results similar to that observed in younger adults. However, the frontal and temporal regions experience age-related volumetric

degeneration (Bartzokis et al., 2001), which could result in oxytocin having a differing effect on anger, disgust, and happiness recognition for older adults compared to young adults.

Thirdly, emotional expressions elicit different responses in the receiver. Happiness, sadness, and anger all elicit approach urges (Carver & Harmon-Jones, 2009; Labroo & Rucker, 2010). However, of these three emotions, happiness and sadness are non-threatening and highly affiliative (i.e., bonding) orientated. Conversely, fear is typically considered to elicit avoidance, and disgust is ambiguous in terms of whether it can be categorised as an approach or avoidance orientated emotion, but in most instances, disgust elicits avoidance motivation (Lerner & Keltner, 2001; McNally, 2002). Oxytocin increases empathy for pain and sickness (Riem, Voorthuis, Bakermans-Kranenburg, & van Ijzendoorn, 2014; Shamay-Tsoory et al., 2013). Oxytocin is also thought to increase approach motivation (Kemp & Guastella, 2011), but this effect is thought to be mediated by the perceived safety of the cue (i.e., approach behaviours increase for safe cues but not unsafe cues) (Olff et al., 2013). From this perspective, recognition of non-threatening affiliative or "bonding" emotions (i.e., sadness and happiness), might benefit the most from oxytocin administration (Pavarini et al., 2019).

The aim of this exploratory, placebo-controlled cross-over randomised trial was to assess in detail the impact of a single dose of 24 IU of intranasal oxytocin on the accuracy of emotion recognition in a healthy population of young and older adults to identify any age, sex, and emotion-specific effects of oxytocin. We also explored several participant characteristics as potential moderators of oxytocin's effects, given evidence of context and person dependent oxytocin effects (Bartz et al., 2011). These were personality traits, autistic traits, and trait anxiety. Although the oxytocin effect reported by Campbell et al.'s (2014) was not emotion-specific, the current study explored the possibility of oxytocin influencing emotion recognition differently for each emotion given (i) the differing difficulty older adults

demonstrate in recognising different emotions, (ii) the differing pattern of age-related declines in neural regions involved in emotion processing, and (iii) the differing approach/avoid and threatening/non-threatening orientations of each emotion.

6.4 Method

6.4.1 Participants

A total of 120 healthy participants completed the study, 60 healthy older adults (65-80 years old) and 60 young adults (18-33 years old), with equal numbers of males and females in each age group. Participants were excluded if they had any neurological or psychiatric illnesses, or were taking anti-psychotic or anti-depressant medications. Screening was also conducted for substance use, with participants excluded if they had a history of substance abuse or dependence, or were a current smoker. Participants were also excluded if they had a history of heart disease, were currently pregnant or breastfeeding, or were taking hormone supplements. Females on hormone-based contraception were not excluded as Campbell et al. (2014) reported no difference in results when these participants were included versus when they were omitted. All young females were tested in the luteal phase of their menstrual cycle for both testing sessions. Crystallised intelligence, as measured by the NART (Nelson & Willison, 1991), and years of education were recorded to characterise the study population (see Table 6.1). Older adults were screened for intact cognitive ability using the TICS (Brandt, Spencer, & Folstein, 1988) and excluded if they scored in the mildly, moderately, or severely impaired ranges (i.e., score of 25 or less). All participants spoke English fluently. If participants had cold or flu symptoms on the day of testing, the session was rescheduled. Participants were asked to abstain from food, for at least 1 hour before the testing session, and to restrict caffeine intake on the day of testing.

6.4.2 Questionnaires

Participants were sent a questionnaire pack to complete outside of the testing session in their own time. The SFS (Birchwood et al., 1990) and the UCLA Loneliness Scale (Russell et al., 1980) were included in the questionnaire to provide a demographic profile of the study cohort. The SFS indicated the number and frequency of prosocial activities and relationships participants currently engage in, and the UCLA Loneliness Scale showed the extent to which participants felt lonely and their ability to cope with loneliness. The BFI (John & Srivastava, 1999), the trait anxiety inventory from the STAI (Spielberger et al., 1970), and the Autism Spectrum Quotient Test (AQ) (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001), were included as potential moderators of oxytocin effects for the following reasons. One of the dominant accounts for oxytocin effects is that it has anxiolytic properties (MacDonald & Feifel, 2014; Shamay-Tsoory & Abu-Akel, 2016), i.e., that it reduced participant anxiety thereby contributing to improvements in task performance. Thus, participants with higher trait levels of anxiety may benefit more from intranasal oxytocin administration. There is also evidence that more extroverted people have higher levels of oxytocin (Andari, Schneider, Mottolese, Vindras, & Sirigu, 2012), and those that are more openness to experience demonstrate lower oxytocin DNA methylation, which potentially results in greater oxytocin expression (Haas, Smith, & Nishitani, 2018). Thus, participants that are lower in extraversion and openness may benefit more from oxytocin administration. Lastly, there is evidence that intranasal oxytocin effects are stronger for individuals with higher autistic traits (Bethlehem et al., 2017; Xu et al., 2015).

6.4.3 Procedure

Participants were randomly allocated to one of two condition orders; active oxytocin administration in the first session and placebo in the second session, or vice versa. In the active condition, participants self-administered 24 IU of oxytocin via a nasal spray. A dose of

24 IU (6 puffs) was selected as this is the most commonly used dosage (Leppanen et al., 2017; Shahrestani et al., 2013). This dose is more effective compared to 40 IU (Cardoso & Ellenbogen, 2013; Spengler et al., 2017) and has been demonstrated to influence amygdala function (Spengler et al., 2017), cortisol levels (e.g., Heinrichs et al., 2003; Quintana et al., 2015b), and social-cognitive behaviours (e.g., Kosfeld et al., 2005; Schulze et al., 2011). The six puffs were administered as per the recommendations of Graustella and MacLeod (2012) in an alternating nostril sequence to increase absorption (Guastella et al., 2013). Participants were instructed to wait for 45-seconds between puffs to avoid flooding the nose. In the placebo condition, participants self-administered 6 puffs of a saline nasal spray using the same procedures as in the active condition. Forty minutes after drug/placebo administration, participants began a battery of tasks. To allow young females to be tested in the luteal phase of their menstrual cycle, and to reduce practice effects, the two testing sessions were held approximately 4 weeks apart for all participants.

6.4.4 Emotion recognition task

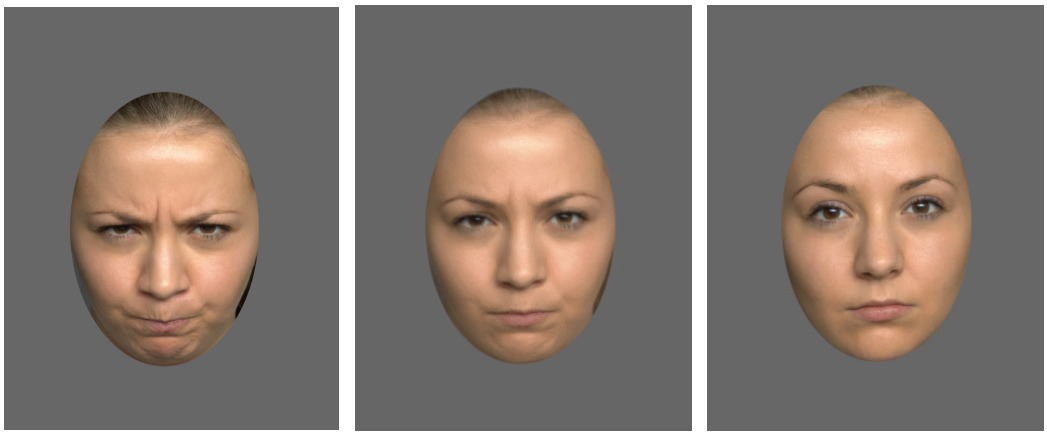
The emotion recognition task was given approximately 55 minutes (M = 53.90, SD = 5.22) post nasal spray. The timing was based on the peak pharmacodynamics effect window for oxytocin, which is from 45 to 70 minutes post-administration (Spengler et al., 2017). Participants were shown 72 photographs of people depicting anger, disgust, fear, happiness, sadness, and a neutral expression from the FACES database (Ebner et al., 2010). The posers comprised six older adults (three females, three males), and six young adults (three females, three males). Given the ceiling effects frequently encountered when full-intensity photographs are used in emotion recognition tasks, we chose to reduce the intensity of the emotions depicted in the stimuli. Oxytocin effects have also been shown to be most present for healthy adults for more difficulty social cognition task items, but not easy social cognition task items (Domes et al., 2007). Reducing the intensity of the photographs was expected to

increase task difficulty (Orgeta & Phillips, 2008) and in turn increase sensitivity to oxytocin effects.

To reduce the intensity of the emotions, each facial expression was morphed with a neutral expression of the same poser using FantaMorph software (Abrosoft); for an illustration, see Figure 6.1. Before each image was morphed, it was first cropped to be a consistently sized oval shape and centralised within a grey rectangle so that the target face and neutral face were aligned. For each image, two images were created of differing emotional intensity; one that was 50% target expression and 50% neutral expression, and one that was 75% target expression and 25% neutral expression. Two versions of the task were created, with one of the two images assigned to each version. Each version contained a mix of intensities and the images were presented in a randomised order. For each of the 72 faces, participants were asked to label the emotion in the expression by selective one of the six unchanging labels below the image (i.e., angry, sad, fearful, disgusted, happy, or neutral). The next image was displayed after the participants had made their choice, no time limit was given.

Figure 6.1

Example of the target and neutral faces, sourced from the FACES database, before and after being digital morphed into one image to create the facial emotion recognition task stimuli.



100% Angry **50% Angry** **100% Neutral**

Note. From left to right the faces depict an original 100% angry facial expression, a morphed photograph that is 50% angry expression and 50% neutral expression, and an original 100% neutral expression.

6.4.5 Data analysis

To observe trends in participants' responding for our interpretations, confusion matrices were created for females and males in each age group depicting the average emotion classifications across oxytocin conditions; see Table 6.2. We then calculated two different accuracy scores for each participant. The first was raw hit rates, the most commonly used accuracy score used in emotion recognition research, which is simply the proportion of correctly labelled items for each emotion. However, due to criticisms that raw hit rates do not take into account biased responding, we also calculated unbiased hit rates (Wagner, 1993). Unbiased hit rates factor in both the number of correct responses, but also the number of times that emotion was incorrectly used to label a different emotion (Table 6.2 of the Results depicts the average misattributions). The formula used to calculate unbiased hit rates for each emotion was: $Hu = a^2/(b \times (a + c))$, where a = number of correct trials for that emotion, b = total number of trials for that emotion (in our task this was always 12), and c = number of

times that emotion was incorrectly selected for another emotion. For example, if a participant selected the label ‘sadness’ for every item in the facial emotion recognition task, they would receive an unbiased hit rate of $12^2/(12 \times (12 + 60)) = .17$, because they correctly labelled all 12 sad faces as sadness, but also incorrectly labelled the 60 alternate faces as sadness. In contrast, they would receive a perfect score for raw rate hit rate of H = 1.00, because they correctly labelled each sad face as sadness. Thus, the unbiased hit rate more accurately reflects their inability to differentiate sadness from other emotions. Unbiased hit rates are reported within the main manuscript, however, for ease of comparison to other research, raw hit rates (and the associated analyses) are provided as supplementary material; see ‘6.7 Supplementary Material’.

A multivariate analysis of variance was used to assess baseline differences between the age groups’ recognition of each emotion. The first mixed-effect regression models were then fitted to each emotion with oxytocin condition (oxytocin vs. placebo), condition order (oxytocin first vs. oxytocin second), time of testing (morning vs. afternoon), age (young adults vs. older adults), sex (males vs. females), openness, conscientiousness, extraversion, agreeableness, and trait anxiety were included as fixed effects and participant ID was included as a random effect. Neuroticism was not included due to collinearity with trait anxiety. Note also, that as only half of the participants completed the questionnaire assessing autistic traits (the AQ), this moderator was analysed in a separate model as to not reduce the power of the main model. Due to the nature (proportion of correct response) and distribution (negatively skewed with ceiling effects) of outcome variables, Tobit regression models were used. Condition order and time of testing were included to control for any influence these factors may have had on performance and oxytocin effects across sessions. Condition order has been shown to interact with oxytocin effects, whereby participants who receive oxytocin in the first session demonstrate greater retention of the task when tested days later than those

that had placebo first (Hollander et al., 2007), or an effect emerges for the first session but not the combined sessions (Schwaiger et al., 2019).

Given our interest in age and sex differences, we then ran the second models within the age-group with sex by oxytocin included as an interaction, controlling for any other factors that had been significant in the first model. Due to the possibility of null effects (Grainger et al., 2018b; Horta et al., 2019), we also conducted follow up testing using Bayesian ANOVAs. Bayesian ANOVAs test the level of evidence in the data for a null model compared to an alternate model (Quintana & Williams, 2018). These ANOVAs were run within age-groups, with sex, oxytocin condition, and their interaction as fixed factors.

6.5 Results

6.5.1 Demographics

The demographic information for each age group is presented in Table 6.1. The age groups did not differ on years of education, social functioning, loneliness, or autistic traits. On average, the young adults had higher trait anxiety than the older adults, but the older adults had higher IQ, as measured by the NART. The young and older adults also differed in terms of personality, with the older adult group being on average more open, agreeable, and conscientious, but less neurotic than the young adult group. The two groups were matched on estrogen levels, but the older adults had significantly lower testosterone levels.

Table 6.1

Demographic information for young and older adults.

	Young Adults			Older Adults			
	Female	Male	p^a	Female	Male	p^b	p^c
n	30	30		30	30		
Age	22.67 (3.69)	24.07 (4.11)	.17	70.77 (4.82)	74.17 (5.86)	.02	
Years education	15.90 (1.80)	16.26 (2.46)	.52	16.17 (3.83)	15.18 (3.72)	.32	.48
IQ	101.28 (7.17)	105.30 (7.42)	.04	115.59 (8.21)	113.78 (8.37)	.40	<.001
Trait anxiety	40.53 (10.21)	40.79 (8.93)	.92	30.52 (6.52)	34.30 (10.28)	.11	<.001
Social functioning	29.64 (8.04)	27.21 (9.43)	.32	28.79 (7.04)	26.97 (9.48)	.42	.76
Loneliness	14.60 (14.20)	13.41 (13.07)	.74	10.37 (11.58)	12.33 (11.16)	.51	.25
Autistic traits[d]	14.58 (8.36)	19.48 (6.60)	.07	13.50 (4.31)	17.60 (6.10)	.02	.18
Personality							
Openness	3.28 (0.75)	3.54 (0.64)	.17	3.81 (0.46)	3.72 (0.65)	.53	.003
Extraversion	3.26 (0.81)	3.31 (0.71)	.81	3.39 (0.86)	3.20 (0.92)	.42	.96
Agreeableness	4.13 (0.54)	3.46 (0.70)	<.001	4.07 (0.49)	4.01 (0.49)	.62	.03
Conscientiousness	3.90 (0.71)	3.29 (0.68)	.001	4.16 (0.43)	3.86 (0.57)	.03	.001
Neuroticism	3.10 (0.78)	2.77 (0.59)	.07	2.40 (0.67)	2.54 (0.74)	.47	<.001
Testosterone	68.80 (35.83)	164.18 (60.81)	<.001	59.58 (27.03)	117.95 (39.13)	<.001	.01
Estrogen	1.04 (0.64)	1.51 (0.94)	.03	1.10 (0.59)	1.56 (1.25)	.08	.72

Note. Independent t-tests were used to compare groups. [a] = sex differences within the young adult sample, [b] = sex differences within the older adult sample, [c] = difference between age-groups with sex combined, [d] = only a subset of the sample completed the Autism Quotient, 13 young females, 23 young males, 20 older females, and 25 older males.

Within the age groups, males and females were matched for years of education, trait anxiety, social functioning, loneliness, openness, agreeableness, and neuroticism. Young males had higher IQ than young females, were less agreeable and conscientious, had higher testosterone and estrogen, but were matched for age. Older males had a higher mean age than older females, by four years on average, had more autistic traits, were less conscientious, had higher testosterone levels, but were matched for IQ, agreeableness, and estrogen.

6.5.2 Facial emotion recognition performance

In Table 6.2, confusion matrices present the average responses for each emotion by each group across oxytocin conditions. All participants commonly mislabelled disgusted faces as angry, and older adults additionally also commonly mislabelled angry faces as disgusted. All participants commonly mislabelled sad faces as neutral. Older men also commonly mislabelled fearful faces as neutral.

Box plots depicting the unbiased hit rate of each group under the oxytocin condition versus placebo condition are presented in Figure 6.2. To test for baseline differences between the age groups, a multivariate analysis of variance comparing young and older adults (collapsed across sex) during the placebo session indicated that older adults were significantly less accurate than young adults for all emotions (*p*s ranged from <.001 to .002). Similar findings were obtained for raw hit rates (see '6.7 Supplementary Material'), though no age-effect emerged for happiness or neutral.

Table 6.2

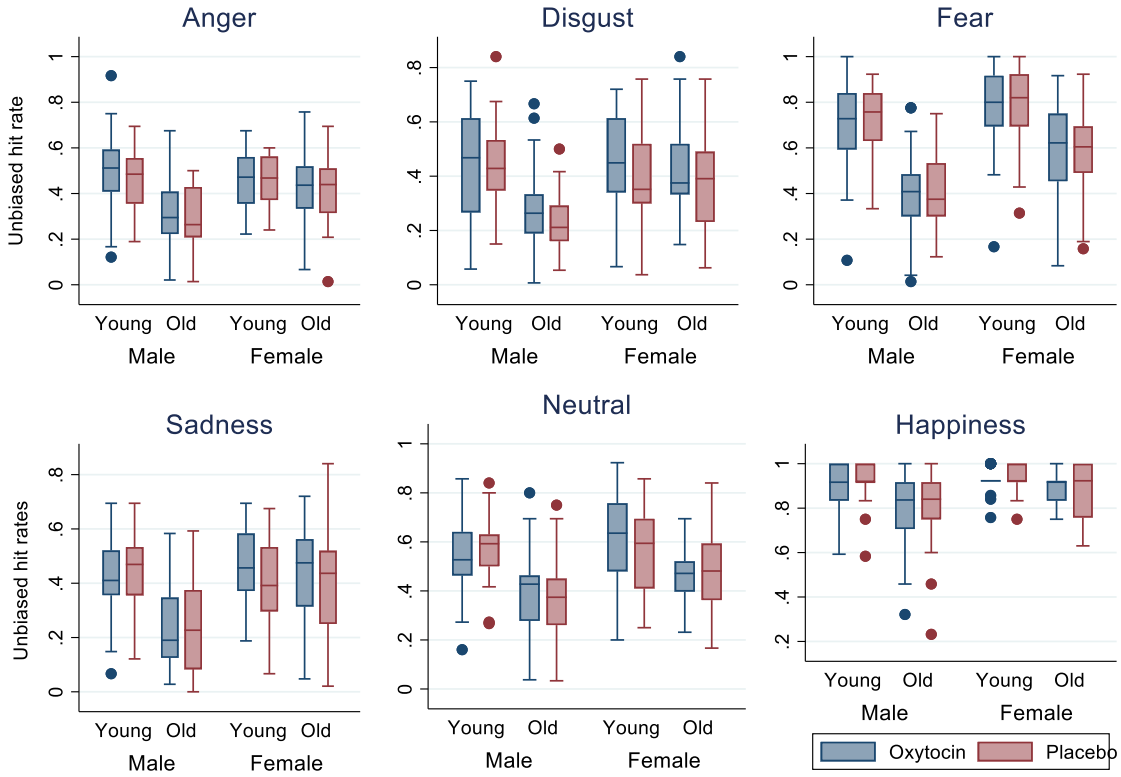
Confusions matrices presenting average responses for each emotion by each age and sex group across placebo and oxytocin conditions.

	YOUNG FEMALES												**OLDER FEMALES**											
	PLACEBO						OXYTOCIN						PLACEBO						OXYTOCIN					
	A	D	F	H	S	N	A	D	F	H	S	N	A	D	F	H	S	N	A	D	F	H	S	N
A	**.69**	.28	.01	.00	.04	.06	**.69**	.27	.02	.01	.04	.05	**.59**	.18	.04	.00	.03	.04	**.60**	.18	.05	.00	.02	.05
D	.09	**.56**	.05	.00	.11	.01	.05	**.58**	.05	.00	.07	.01	.17	**.57**	.04	.01	.07	.04	.14	**.57**	.03	.00	.04	.04
F	.04	.02	**.90**	.01	.08	.02	.05	.01	**.90**	.00	.10	.02	.06	.04	**.75**	.01	.14	.02	.06	.02	**.75**	.01	.13	.03
H	.00	.00	.00	**.96**	.02	.00	.01	.01	.00	**.96**	.02	.01	.01	.02	.01	**.93**	.01	.09	.00	.02	.01	**.95**	.02	.01
S	.10	.07	.01	.00	**.58**	.10	.11	.07	.02	.00	**.64**	.08	.05	.07	.03	.00	**.55**	.07	.06	.11	.01	.01	**.62**	.08
N	.08	.07	.03	.03	.17	**.80**	.09	.06	.01	.02	.14	**.84**	.11	.12	.13	.05	.20	**.73**	.13	.11	.14	.04	.17	**.79**
	YOUNG MALES												**OLDER MALES**											
	PLACEBO						OXYTOCIN						PLACEBO						OXYTOCIN					
	A	D	F	H	S	N	A	D	F	H	S	N	A	D	F	H	S	N	A	D	F	H	S	N
A	**.68**	.26	.01	.01	.01	.05	**.69**	.21	.01	.01	.02	.06	**.46**	.21	.04	.00	.05	.05	**.46**	.17	.04	.00	.04	.03
D	.06	**.58**	.04	.00	.08	.03	.09	**.60**	.06	.01	.08	.04	.19	**.42**	.09	.00	.10	.05	.18	**.46**	.07	.01	.09	.06
F	.04	.01	**.88**	.02	.10	.01	.04	.02	**.86**	.02	.11	.02	.09	.07	**.62**	.01	.17	.04	.11	.09	**.60**	.02	.17	.04
H	.00	.00	.00	**.93**	.00	.00	.00	.00	.00	**.92**	.01	.01	.02	.04	.05	**.94**	.05	.05	.01	.04	.05	**.92**	.04	.05
S	.09	.08	.03	.02	**.64**	.09	.09	.09	.03	.02	**.63**	.09	.06	.09	.03	.01	**.35**	.09	.07	.11	.05	.00	**.38**	.07
N	.13	.06	.04	.02	.16	**.82**	.09	.08	.05	.04	.15	**.79**	.18	.16	.18	.05	.28	**.72**	.16	.14	.19	.06	.28	**.75**

Note. A = anger, D = disgust, F = fear, H = happiness, S = sadness, N = neutral. The average proportion of correct responses are in bold. Columns indicate the target emotion, rows indicate the label selected.

Figure 6.2

Median unbiased hit rates for each emotion, age and sex across oxytocin conditions.



6.5.3 Oxytocin effects

Next, we used the first Tobit regression models to test the effects of age, sex, oxytocin condition, condition order, personality traits, and trait anxiety across the oxytocin and placebo conditions. In Table 6.3, these models revealed that when looking across age-groups, no oxytocin effects emerged (*p*'s ranged from .10 to .58). Openness and trait anxiety did not influence emotion recognition accuracy. A separate model revealed that autistic traits also did not influence emotion recognition accuracy. Condition order, extraversion, conscientiousness, and agreeableness only influenced recognition accuracy for one emotion each (e.g., more

extraverted participants were less accurate at recognising fear). However, age and sex effects were present across all emotions, even after accounting for the other variables (Table 6.3).

We then ran the models within age-groups (Table 6.4), to identify if there were sex, oxytocin, or sex by oxytocin effects that were specific to one age-group. We did not control for condition order since the initial models indicated that this variable did not influence performance across sessions. For each emotion, we initially included all moderators that had been significant in the initial models (e.g., for fear we included extraversion in addition to age and sex). However, as none of these variables, or their interaction with oxytocin effects, were significant for either age group, they were dropped from the models. In Table 6.4, the within age-group models show that for older adults there were no significant oxytocin effects (*p*'s ranged .19 to .79), or sex by oxytocin interactions (*p*'s ranged .24 to .70). However, sex was found to be a significant moderator of older adults' facial emotion recognition accuracy across all emotions (*p*'s ranged <.001 to .04).

In contrast to older adults, for younger adults, sex was not a significant moderator of (*p*'s ranged .06 to .75). However, for younger adults, there was an oxytocin effect for happiness, whereby they were more accurate in the placebo, than the oxytocin, condition (B = 0.04, *p* = .03); but an effect compounded by ceiling effects (see Figure 6.2). We highlight that when the same analyses were run on participants' raw hit rates, no oxytocin effect emerged for young adults' happiness recognition (*p* = .30) (see '6.7 Supplementary Material'). Thus, we emphasise caution in interpreting the happy findings given the effects of ceiling and inconsistency in outcomes reported from unbiased vs. raw hit rates. It seemed that young adults accurately identified a relatively equal number of happy faces in the oxytocin and placebo conditions, but made more happiness misattributions in the oxytocin condition.

For young adults, there was also an oxytocin by sex interaction for sadness, whereby females were significantly more accurate in the oxytocin (vs. placebo) condition (B = -0.06, *p*

= .02) but males demonstrated no difference between conditions (B = 0.02, p = .52). Again, the same analyses were run on the raw hit rates, and the oxytocin effect for sadness was present when all participants (young and older) were combined (p = .02), rather than a sex by oxytocin interaction specific to young adults (see '6.7 Supplementary Material'). A distinction needs to be made between the ability to recognise sad faces and the ability to distinguish sadness from other emotions. From the confusion matrices, it is clear that although older adults correctly identified a greater number of sad faces in the oxytocin condition, they also misattributed the sadness label a greater number of times, in particular for disgust; older men made 2% more errors in the oxytocin condition, and older women made 5% more sadness misattribution errors in the oxytocin condition. Thus, oxytocin did not necessarily make older adults more accurate at distinguishing sadness from other emotions, rather it made them more likely to select the sadness label more generally. We are therefore more confident in reporting an effect of oxytocin on sadness recognition in the young females (based on the unbiased hit rate analyses).

Table 6.3

The effects of oxytocin and each potential moderator on facial emotion recognition unbiased hit rates for all participants combined.

	Anger		Disgust		Fear		Happiness		Sadness		Neutral	
	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*
Oxytocin	-0.01 (-0.04 to 0.02)	.58	-0.03 (-0.07 to 0.01)	.10	0.01 (-0.03 to 0.05)	.55	0.01 (-0.01 to 0.03)	.30	-0.02 (-0.05 to 0.01)	.22	-0.01 (-0.04 to 0.02)	.49
Oxytocin order	-0.04 (-0.09 to 0.001)	.05	-0.01 (-0.06 to 0.05)	.83	-0.02 (-0.07 to 0.04)	.58	-0.02 (-0.06 to 0.02)	.29	-0.03 (-0.08 to 0.02)	.29	-0.02 (-0.06 to 0.03)	.51
Age	-0.12 (-0.18 to -0.07)	<.001	-0.12 (-0.18 to -0.06)	<.001	-0.29 (-0.37 to -0.24)	<.001	-0.09 (-0.14 to -0.05)	<.001	-0.10 (-0.17 to -0.04)	.001	-0.17 (-0.23 to -0.12)	<.001
Sex	0.05 (0.01 to 0.10)	.03	0.06 (0.01 to 0.12)	.03	0.11 (0.05 to 0.17)	<.001	0.04 (0.001 to 0.08)	.04	0.10 (0.05 to 0.16)	<.001	0.07 (0.02 to 0.12)	.01
Openness[a]	-0.01 (-0.05 to 0.02)	.45	0.01 (-0.03 to 0.05)	.50	0.03 (-0.01 to 0.07)	.17	0.01 (-0.02 to 0.04)	.60	-0.03 (-0.07 to 0.01)	.21	0.03 (-0.01 to 0.06)	.15
Conscientiousness[a]	0.01 (-0.03 to 0.05)	.67	0.02 (-0.02 to 0.07)	.34	0.01 (-0.03 to 0.06)	.58	0.03 (0.001 to 0.07)	.05	0.002 (-0.04 to 0.05)	.93	0.04 (-0.01 to 0.08)	.08
Extraversion[a]	0.01 (-0.02 to 0.04)	.48	0.02 (-0.02 to 0.05)	.38	-0.04 (-0.08 to -0.004)	.03	0.01 (-0.01 to 0.03)	.45	0.02 (-0.02 to 0.05)	.40	0.02 (-0.01 to 0.06)	.12

Agreeableness[a]	-0.03 (-0.07 to 0.01)	.14	-0.02 (-0.07 to 0.03)	.42	0.02 (-0.03 to 0.08)	.40	-0.001 (-0.03 to 0.03)	.99	-0.06 (-0.11 to -0.01)	.02	-0.03 (-0.07 to 0.02)	.26
Trait anxiety	-0.002 (-0.01 to 0.001)	.14	0.001 (-0.003 to 0.004)	.71	-0.003 (-0.01 to -0.001)	.07	-0.001 (-0.003 to 0.002)	.75	-0.003 (-.05 to .001)	.08	0.001 (-0.003 to 0.003)	.90

Note. B = beta, CI = 95% confidence interval. [a] = domains from the BFI. Reference variables were oxytocin (negative coefficient represents worse performance in placebo), oxytocin first (negative coefficient represents worse performance when oxytocin is given second), AM (negative coefficient represents worse performance in PM), young adults (negative coefficient represents worse performance by older adults), male (negative coefficient represents worse performance by females). Results highlighted in blue represent significant findings of $p \leq .05$.

Table 6.4

The effects of oxytocin, sex, and their interaction on unbiased hit rates for each age group.

	Anger		Disgust		Fear		Happiness		Sadness		Neutral	
	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*	B(95% CI)	*p*
Young adults												
Oxytocin	-0.03 (-0.10 to 0.03)	.32	0.002 (-0.07 to 0.08)	.97	0.02 (-0.06 to 0.09)	.65	0.04 (0.003 to 0.07)	.03	0.02 (-0.04 to 0.08)	.49	0.02 (-0.03 to 0.08)	.43
Sex	-0.04 (-0.10 to 0.03)	.30	0.01 (-0.07 to 0.10)	.75	0.05 (-0.03 to 0.14)	.24	0.03 (-0.01 to 0.08)	.11	0.03 (-0.04 to 0.10)	.46	0.08 (-0.002 to 0.15)	.06
Oxytocin by sex	0.03 (-0.07 to 0.12)	.55	-0.06 (-0.16 to 0.05)	.28	-0.002 (-0.10 to 0.10)	.98	-0.02 (-0.07 to 0.02)	.32	-0.08 (-0.16 to -0.003)	.04	-0.08 (-0.16 to 0.002)	.06
Older adults												
Oxytocin	-0.02 (-0.08 to 0.04)	.52	-0.04 (-0.11 to 0.02)	.19	0.03 (-0.70 to 0.10)	.47	0.006 (-0.04 to 0.05)	.79	-0.008 (-0.06 to 0.05)	.79	-0.03 (-0.09 to 0.03)	.29
Sex	.11 (0.04 to 0.20)	.003	0.14 (0.06 to 0.21)	<.001	0.21 (0.11 to 0.30)	<.001	0.09 (0.03 to 0.16)	.01	0.21 (0.12 to 0.29)	<.001	0.08 (0.003 to 0.15)	.04
Oxytocin by sex	.02 (-0.06 to 0.10)	.61	0.02 (-0.07 to 0.11)	.70	-0.04 (-0.15 to 0.07)	.46	-0.01 (-0.08 to 0.05)	.64	-0.03 (-0.11 to 0.05)	.53	0.05 (-0.03 to 0.13)	.24

Note. B = beta, CI = 95% confidence interval. Reference variables were oxytocin (negative coefficient represents worse performance in placebo) and male (negative coefficient represents worse performance by females). Results highlighted in blue represent significant findings of $p \leq .05$.

6.5.4 Bayesian evidence

To test the level of evidence in support of null models in comparison to alternate models, we then ran Bayesian ANOVAs for each emotion within each age-group. For older adults, across the emotions, Bayesian ANOVAs revealed anecdotal to moderate evidence for null models, relative to alternative models, for the main effect of oxytocin. This evidence was anecdotal for disgust and sadness (bf_{10} = 0.49 and 0.33), and moderate for all other emotions (bf_{10}'s ranged from 0.19 to 0.21). For older adults, across the emotions, Bayesian ANOVAs revealed anecdotal to extreme evidence in favour of main sex effects (e.g., in favour of older females vs. males for all emotions; see Table 6.4). This evidence was anecdotal for anger (bf_{10} = 1.58), moderate for happiness (bf_{10} = 6.47), strong for neutral (bf_{10} = 14.94), and extreme for disgust, fear, and sadness (bf_{10}'s ranged from 632.29 to 1190.46). For all emotions, the combined main effect model was preferred 2.3 to 4 times more to the interaction model, which represents anecdotal to moderate evidence for null interaction models across the emotions.

For young adults, across emotions, Bayesian ANOVAs revealed moderate evidence for null models, relative to alternative models for the main effect of oxytocin (bf_{10}'s ranged from 0.23 to 0.31) for all emotion expect happiness. For happiness, there was anecdotal evidence in favour of an oxytocin effect (bf_{10} = 1.18) that contrasted previous results showing a slightly better performance in the placebo condition (vs. oxytocin). For young adults, across emotions, Bayesian ANOVAs revealed anecdotal to moderate evidence for null models, relative to alternative models, for the main effect of sex (e.g., in favour of young males vs. females for anger recognition, and in favour of young females for all other emotions; see Table 6.4). This evidence was anecdotal for fear, happiness, and neutral (bf_{10}'s ranged from 0.45 to 0.59), and moderate for anger, disgust, and sadness (bf_{10}'s ranged from 0.28 to 0.30). For all emotions except sadness and neutral, the combined main effect model was preferred

2.3 to 4 times more to the interaction model, which represents anecdotal to moderate evidence for null interaction models across these emotions. For sadness and neutral, the interaction model was preferred to the main effect model by a factor of 1.53 for sadness and 1.22 for neutral, providing anecdotal evidence for interaction models for these emotions.

6.6 Discussion

In this study, we aimed to test the effects of 24 IU of intranasal oxytocin in older adults' ability to recognise emotion from facial expressions. Our exploratory analyses revealed that intranasal oxytocin had no main effect on older adults' recognition accuracy, with no differences from placebo spray across the emotions (with 2.1-5 times more evidence for null models than alternative main effect models). This was also independent of sex, with no sex by oxytocin interactions in older adults (with null sex by oxytocin interactions preferred to combined main effects models by 2.3-4 times), despite evidence for a main effect of sex for the recognition of each emotion (with 0.6 to more than 1,000 times the evidence across emotions for the alternative main effect models than the null models), with older men demonstrating inferior accuracy to older women (in particular for disgust, fear, and sadness). For younger adults, there was evidence of intranasal oxytocin effects for happiness and sadness, but the evidence was not convincing. Specifically, in young adults, there was some evidence of intranasal oxytocin affecting happiness recognition with inferior performance in the oxytocin (vs. placebo) condition, but this effect was confounded by ceiling effects and this alternative main effect model was only preferred by 1.2 times more evidence over the null model. There was also evidence that intranasal oxytocin (vs. placebo) improved sadness recognition for young females. However, a sex by oxytocin interaction model was only preferred by 1.53 times to the combined main effects model, and the null models were preferred to the main oxytocin effect model by 3.33 times, and the main sex effect model by 3.45 times.

Our results are in line with previously reported null oxytocin effects for facial emotion recognition in older adults (Grainger et al., 2018b; Horta et al., 2019), but they contrast with the finding that oxytocin improves older males' facial emotion recognition (Campbell et al., 2014). There are notable differences and similarities in the task designs from these studies compared to our study that are worth highlighting given evidence that task designs moderate performance on emotion recognition in older adults (Hayes et al., 2020). Although our results are consistent with two of the previous studies, our stimuli differed to these studies in that we used static stimuli instead of the dynamic stimuli used by Grainger et al. (2018b) and Horta et al. (2019). In this way, our stimuli were more similar to that of Campbell et al. (2014), who also used static stimuli, yet our results contrast with that study. It may, therefore, be that rather than the format of the stimuli (i.e., static vs. dynamic) influencing oxytocin effects, that it is the intensity of the stimuli (i.e., overt vs. subtle) that influences oxytocin effects. That is, the contrast in findings from Campbell et al. (2014) and our study, could be because we used reduced-intensity stimuli, unlike their full-intensity stimuli. Notably, the animations used by Horta et al. (2019) started at a reduced intensity, and the naturalistic videos used by Grainger et al. (2018b) were likely subtler than the full-intensity emotion depictions used in the Campbell et al. (2014) study. Thus, there appears to be a pattern whereby no oxytocin effects may only emerge for older adults' when subtler, or more naturalistic, stimuli are used.

An additional difference between the current study and the Campbell et al. (2014) study is that Campbell et al. (2014) used independent groups whereas we used a repeated measures design. Thus, it is possible that the older men in Campbell et al. (2014) oxytocin and placebo conditions were not matched in emotion recognition accuracy. Emotion recognition can decline sharply from ages 60 and 70 onwards (Mill et al., 2009; Williams et al., 2009) and it is, therefore, plausible to have two groups of older adults that significantly differ in facial emotion recognition performance, unrelated to drug administration. Notably,

Campbell et al. (2014) do not report having matched their independent groups on age. However, even with demographically matched groups, counterbalanced repeated measure designs are the only way to ensure the oxytocin and placebo groups are matched. Given the evidence of oxytocin effects being context-dependent (Bartz et al., 2011), it is hard to directly compare the studies. Nonetheless, we used a large sample of participants, a repeated measures design with a well-developed task (that included young and older adult stimuli with more naturalistic, i.e., reduced-intensity, expressions), and reported on unbiased hit rates as a more accurate representation of task performance.

Despite the difficulty comparing studies, the pattern that emerged whereby oxytocin did not affect older adults' facial emotion recognition accuracy for subtler stimuli, does fit with Bartz's (2011) interactionist account of the varying oxytocin effects in the literature. Bartz et al. (2011) suggest that it is the interaction between item difficulty and group ability that moderates the oxytocin effects. For example, in studies on the effects of oxytocin on theory of mind, oxytocin seems to only improve performance for items that the group found to be at a certain level of difficulty (Bartz et al., 2011). For a healthy adult sample, intranasal oxytocin had a stronger effect for 'difficult' items (Domes et al., 2014), whereas for a sample of youth with ASD, intranasal oxytocin had a stronger effect for 'easy' items (Guastella et al., 2010). Thus, it appears that the ASD group may have found the 'difficult' items too difficult to benefit from a single dose of oxytocin. It might therefore be that for subtle stimuli, which are presumably harder to recognising than overt facial expressions, older males are too impaired to benefit from a single dose of oxytocin. In contrast, there is evidence to suggests that oxytocin effects would instead be stronger for subtle stimuli. Specifically, there is evidence that the prefrontal cortex is more involved in recognition of subtle than overt emotional expressions (Willis et al., 2014), which would suggest greater oxytocin effects for subtler stimuli given oxytocin typically shifts output to prefrontal regions (Bos et al., 2012).

With regards to the intranasal oxytocin findings in young adults, we were cautious with not over-interpreting the happiness finding. Although younger adults were marginally more accurate at recognising happiness in the placebo condition, the ceiling effects we encountered for happiness, despite reducing the intensity of the facial expressions, limit the validity of this finding. However, we highlight some prior evidence in the context of our sadness finding whereby there was some evidence of oxytocin improving young females' sadness accuracy. A sexually dysmorphic facial emotion recognition oxytocin effect that favours females has previously been reported for emotion recognition accuracy (Yue, Yue, Liu, & Huang, 2018). However, it is unclear why sadness was the only emotion to be modulated by oxytocin. Meta-analytic reviews report that oxytocin typically improves young adults' recognition of fear rather than sadness (Leppanen et al., 2017; Shahrestani et al., 2013), but these meta-analyses did not examine sex-specific effects. There were several factors that we recognised in our introduction as having the potential to contribute to oxytocin influencing older adults' recognition of one emotion more than others. Two of these would also apply to young adults, (i) variation in the difficulty participants had recognising each emotion, and (ii) variation in the approach/avoid and threatening/non-threatening orientations of each emotion. An oxytocin effect specific to sadness recognition fits with both of these factors. First, previous research has identified that oxytocin effects emerge for healthy adults for difficult social cognitive items but not easy items (Domes et al., 2007), and sadness was the emotion that young females found the most difficult to recognise. Second, a sadness-specific oxytocin effect fits with the theory that oxytocin increases approach motivation (Kemp & Guastella, 2011). Oxytocin has also been linked to being others orientated and found to increase empathy to pain and distress (Riem et al., 2014; Shamay-Tsoory et al., 2013). It may therefore be predicted that oxytocin would have the strongest impact on sadness and happiness recognition (i.e., the two non-threatening approach motivating

emotions), which is consistent with oxytocin increasing facial mimicry for sadness and happiness but not for anger and fear (Pavarini et al., 2019).

Overall, although there was some evidence of oxytocin modulating emotion recognition in young adults, our results provide no convincing support for intranasal oxytocin modulating older adults' emotion recognition difficulties and therefore does not provide evidence for the oxytocin system being implicated in healthy older adults' social cognition. It is possible that oxytocin may even adapt with age to protect against decline of this skill, but this remains to be further examined. Future research could explore the effects of multiple doses of oxytocin to clarify if the lack of effect was specific to a single dose and that perhaps older adults needed more to see a change in behaviour. We emphasise the strength of the study's design involving repeated measures, a large sample of participants, and the fact that we accounted for potential trait moderators (anxiety, personality, and autistic traits) but highlight that future research could investigate other potential moderators, such as childhood adversity (Schwaiger et al., 2019) and socioeconomic status (Sun, Vuillier, Deakin, & Kogan, 2020) in an attempt to move beyond simply reporting whether an effect is present and instead also investigate for who and under what conditions oxytocin may have an effect (Quintana & Woolley, 2016).

6.7 Supplementary Material

6.7.1 Results

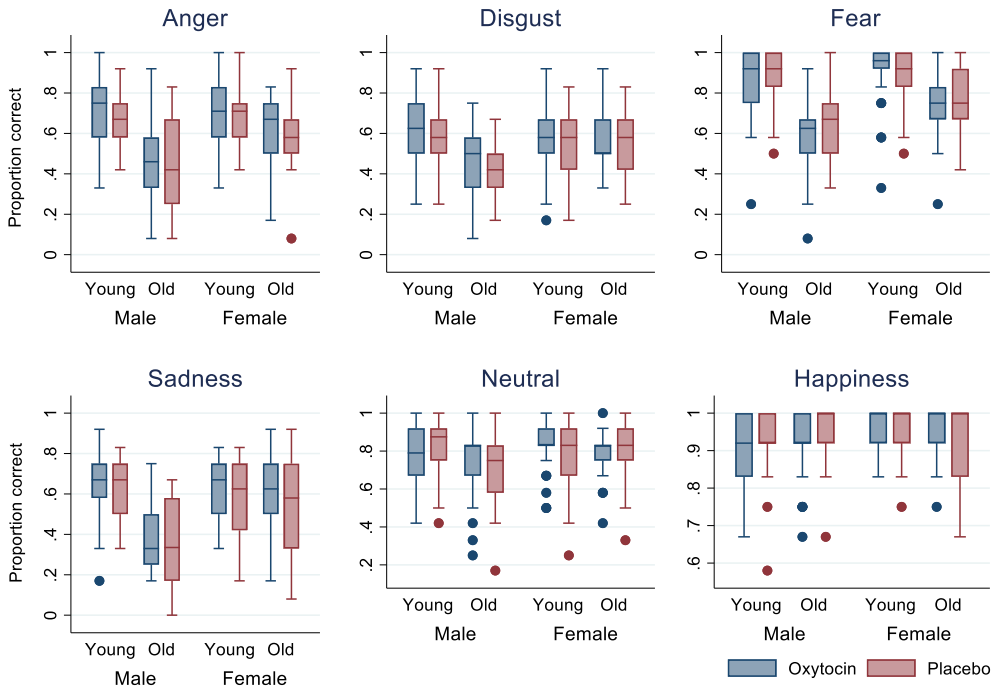
In these supplementary materials, we present the results on the raw hit rates, with the main manuscript reporting the results on the unbiased hit rates, from the facial emotion recognition task.

6.7.1.1 Facial emotion recognition performance

Box plots depicting the raw hit rate accuracy (proportion correct) of each group under the oxytocin condition versus placebo condition are presented in Supplementary Figure 6.1. To test for baseline differences between the age groups, a multivariate analysis of variance young and older adults (collapsed across sex) during the placebo session indicated that older adults were significantly less accurate than young adults for anger, fear, disgust, and sadness (*p*s ranged from <.001 to .02), but not happiness ($p = .44$) and neutral ($p = .10$).

Supplementary Figure 6.1

Median raw hit rates (proportion correct) for each emotion of each age and sex group separated by oxytocin condition.



6.7.1.2 Oxytocin effects

Next, we used the first Tobit regression models to test the effects of age, sex, oxytocin condition, condition order, personality traits, and trait anxiety across the oxytocin and placebo conditions. In Supplementary Table 6.1, these models revealed that when looking across age-groups combined, a significant oxytocin effect emerged for sadness (p = .02), with participants demonstrating superior sadness hit rates in the oxytocin condition compared to placebo. However, no oxytocin effects were present for any of the other emotions (ps ranged from .37 to .99). A separate model revealed that autistic traits also did not influence emotion recognition accuracy. Condition order influenced accuracy for anger and sadness, with superior accuracy being associated with oxytocin administered in the second session compared to the first. In terms of main moderating variables, age moderated emotion recognition accuracy for all emotions except happiness, with young adults demonstrating superior accuracy to older adults. Sex only moderated accuracy for anger, fear, and sadness, with females demonstrating superior accuracy to males. In terms of the additional potential moderators, trait anxiety, openness, and conscientiousness did not influence performance. Extraversion was associated with increased disgust recognition but decreased fear recognition, and agreeableness was associated with decreased anger recognition.

We then ran the second models within each age-groups (Supplementary Table 6.2), to identify if there were sex, oxytocin, or sex by oxytocin effects that were specific to one age-group. In Supplementary Table 6.2, these models show that within each age group there were no significant oxytocin effects or sex by oxytocin interactions for any of the emotions. There was, however, evidence of an oxytocin by sex interaction for young adults' recognition of neutral faces, but the effect failed to reach significance for both females (B = -0.04, p = .06) and males (B = 0.03, p = .21).

For each emotion, we also ran an additional model that included any other variables that had been significant in the main models (e.g., for anger we ran a model that also included condition order, agreeableness, and their interactions with oxytocin condition). For anger, this additional analysis revealed that agreeableness only decrease anger recognition accuracy for older adults (b = -0.18, 95% CI -0.35 to -0.01, p = .04) and not young adults (b = -0.10, 95% CI -0.21 to 0.02, p = .11). For sadness there was a significant oxytocin condition by condition order interaction for young adults (b = 0.12, 95% CI 0.02 to 0.23, p = .02). However, the oxytocin effect failed to reach significance for both participants that had oxytocin first (b = -0.05, 95% CI -0.12 to 0.02, p = .16) and participants that had oxytocin second (b = 0.07, 95% CI -0.01 to 0.15, p = .07). There were no other significant findings from these additional models and thus, condition order, agreeableness, and extraversion were deemed not to be moderators of oxytocin effects.

Supplementary Table 6.1

The effects of oxytocin and each potential moderator on facial emotion recognition raw hit rates for all participants combined.

	Anger		Disgust		Fear		Happiness		Sadness		Neutral	
	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
Oxytocin	-0.001 (-0.04 to 0.04)	.99	-0.02 (-0.05 to 0.02)	.37	0.01 (-0.02 to 0.05)	.55	0.01 (-0.01 to 0.02)	.45	-0.04 (-0.07 to -0.01)	.02	-0.01 (-0.03 to 0.02)	.62
Oxytocin order	-0.05 (-0.12 to -0.004)	.03	0.01 (-0.04 to 0.06)	.74	0.001 (-0.05 to 0.05)	.99	-0.004 (-0.03 to 0.02)	.74	-0.07 (-0.13 to -0.01)	.03	-0.01 (-0.06 to 0.05)	.84
Age	-0.18 (-0.24 to -0.12)	<.001	-0.07 (-0.13 to -0.01)	.02	-0.24 (-0.30 to -0.18)	<.001	-0.02 (-0.05 to 0.01)	.13	-0.14 (-0.21 to -0.07)	<.001	-0.08 (-0.14 to -0.02)	.01
Sex	0.08 (0.02 to 0.13)	.004	0.05 (-0.01 to 0.10)	.10	0.07 (0.02 to 0.13)	.01	0.02 (-0.01 to 0.04)	.24	0.12 (0.05 to 0.18)	.001	0.02 (-0.04 to 0.08)	.45
Openness[a]	-0.02 (-0.06 to 0.02)	.38	0.02 (-0.02 to 0.06)	.39	0.03 (-0.01 to 0.07)	.15	0.01 (-0.01 to 0.03)	.43	-0.02 (-0.07 to 0.02)	.34	0.02 (-0.02 to 0.08)	.35
Conscientiousness[a]	0.03 (-0.02 to 0.07)	.22	0.01 (-0.03 to 0.06)	.55	0.04 (0.002 to 0.09)	.06	0.02 (-0.003 to 0.04)	.09	-0.04 (-0.09 to 0.02)	.20	0.03 (-0.02 to 0.07)	.30
Extraversion[a]	-0.001 (-0.03 to 0.03)	.93	0.04 (0.002 to 0.07)	.04	-0.03 (-0.07 to -0.001)	.05	0.01 (-0.01 to 0.02)	.47	0.001 (-0.04 to 0.04)	.96	0.02 (-0.01 to 0.06)	.24

Agreeableness[a]	-0.06 (-0.11 to -0.02)	.01	-0.01 (-0.06 to 0.03)	.59	0.002 (-0.04 to 0.05)	.94	0.02 (-0.002 to 0.04)	.08	-0.04 (-0.09 to 0.02)	.23	0.003 (-0.05 to 0.05)	.92
Trait anxiety	-0.003 (-0.01 to 0.001)	.08	0.002 (-0.001 to 0.01)	.18	-0.001 (-0.004 to 0.002)	.56	0.001 (-0.001 to 0.002)	.90	-0.003 (-0.01 to 0.001)	.12	-0.002 (-0.01 to 0.001)	.29

Note. B = beta, CI = 95% confidence interval. [a] = domains from the BFI. Reference variables were oxytocin (negative coefficient represents worse performance in placebo), oxytocin first (negative coefficient represents worse performance when oxytocin given second), AM (negative coefficient represents worse performance in PM), young adults (negative coefficient represents worse performance by older adults), male (negative coefficient represents worse performance by females). Results highlighted in blue represent significant findings of $p \leq .05$.

Supplementary Table 6.2

The effects of oxytocin, sex, and their interaction, on raw hit rates for each age group.

	Anger		Disgust		Fear		Happiness		Sadness		Neutral	
	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
Young adults												
Oxytocin	-0.01 (-0.09 to 0.07)	.78	-0.03 (-0.10 to 0.05)	.50	0.03 (-0.04 to 0.09)	.43	0.01 (-0.01 to 0.04)	.30	0.01 (-0.04 to 0.07)	.68	0.03 (-0.01 to 0.07)	.16
Sex	0.01 (-0.07 to 0.08)	.89	-0.03 (-0.11 to 0.06)	.52	0.0 (-0.03 to 0.12)	.25	0.05 (0.01 to 0.09)	.02	0.01 (-0.07 to 0.08)	.89	0.05 (-0.03 to 0.12)	.23
Oxytocin by sex	0.01 (-0.10 to 0.12)	.86	0.003 (-0.10 to 0.11)	.96	-0.02 (-0.11 to 0.06)	.58	-0.01 (-0.05 to 0.02)	.46	-0.07 (-0.15 to 0.01)	.07	-0.07 (-0.03 to -0.01)	.03
	Anger		Disgust		Fear		Happiness		Sadness		Neutral	
	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*	B (95% CI)	*p*
Older adults												
Oxytocin	0.001 (-0.06 to 0.06)	.98	-0.04 (-0.11 to 0.04)	.33	0.02 (-0.05 to 0.10)	.54	0.02 (-0.1 to 0.05)	.21	-0.03 (-0.08 to 0.03)	.41	-0.04 (-0.09 to 0.02)	.25
Sex	0.13 (0.04 to 0.23)	.01	0.11 (0.03 to 0.19)	.01	0.16 (0.07 to 0.24)	.001	0.03 (-0.01 to 0.07)	.16	0.24 (0.15 to 0.34)	<.001	0.03 (-0.05 to 0.12)	.44
Oxytocin by sex	-0.003 (-0.09 to 0.08)	.94	0.04 (-0.07 to 0.14)	.49	-0.02 (-0.13 to 0.08)	.67	-0.04 (-0.08 to 0.01)	.14	-0.04 (-0.13 to 0.0)	.29	0.05 (-0.03 to 0.14)	.22

Note. B = beta, CI = 95% confidence interval. Reference variables were oxytocin (negative coefficient represents worse performance in placebo) and male (negative coefficient represents worse performance by females). Results highlighted in blue represent significant findings of $p \leq .05$.

CHAPTER 7: STUDY 3 – TRAJECTORY OF PERIPHERAL OXYTOCIN IN AGEING: AS MEASURED IN SALIVA

7.1 Chapter Guide

The following chapter comprises the results of a comparison between young and older adults' peripheral oxytocin, vasopressin, testosterone, and estradiol concentration levels, as measured from saliva samples. Given the current lack of knowledge about the oxytocin system in healthy ageing (Ebner et al., 2013; Huffmeijer et al., 2013), this study aimed to identify whether older adults demonstrate a decline in peripheral oxytocin concentration, and to investigate if the ageing pattern differed between sexes and/or mirrored the patterns of age-related decline observed for other hormones.

Notably, there are many examples of peripheral oxytocin levels predicting emotion recognition ability in clinical populations (Goldman et al., 2008; Rubin et al., 2011; Strauss et al., 2015; Taurines et al., 2014), and social cognition more broadly (Hayashi et al., 2019; Mutu Pek et al., 2019; Unti et al., 2018). However, we were cautious about making direct correlations between participants' peripheral oxytocin levels and their facial emotion recognition accuracy because of growing evidence that peripheral oxytocin concentration levels do not predict oxytocin concentrations in the central nervous system, which is the part of the oxytocin system that is associated with emotion recognition. Peripheral oxytocin is linked to physiological functions such water balance, bone density, metabolism, muscle tissue regeneration, and homeostasis (Colaianni et al., 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019), and has not been proposed to play a role in social cognition. As such, participants' peripheral oxytocin was analysed and presented in a separate study to the study presented in Chapter 6.

However, peripheral oxytocin concentration levels in healthy ageing, were deemed to warrant investigation as part of this thesis due to the evidence that there may be an *indirect* relationship between peripheral concentration and social skills. This evidence includes the many studies that have observed correlations between the two (see above citations for

examples), but also the theory that intranasal oxytocin may modulates social cognitive skills via an indirect peripheral effects (Leng & Ludwig, 2016; Martins et al., 2016). An age-related decline in peripheral oxytocin concentration may, therefore, suggest that peripheral oxytocin is indirectly connected to age-related social cognitive deficits in some way.

In addition to my supervisors, and the staff and students that assisted in saliva sampling, we are also grateful to Prof. Rainer Landgraf for extracting oxytocin and vasopressin concentration levels from the saliva samples using his highly sensitive radioimmunoassay. Once again, we also thank Prof. Markus Heinrich for contributing his expertises to the design and development of this study.

7.2 Abstract

There is growing interest in the role of oxytocin in psychological and physical health functions in various healthy and clinical groups. In healthy ageing, much of the research to date rely on animal research as a source of information for inferring age-related changes in the oxytocin system. The few human ageing studies have examined central oxytocin actions through the administration of oxytocin nasal spray. In contrast, very little is known about the peripheral oxytocin system and possible age-related changes. The present study compared healthy young and older adults' peripheral oxytocin and vasopressin concentrations (N = 122), using extracted saliva samples and highly sensitive and specific radioimmunoassay. No age-effects, or age by sex interactions, emerged for either oxytocin or vasopressin. Bayesian hypothesis testing revealed that there was only anecdotal evidence for a null age-effect for oxytocin, consistent with previous research, but moderate evidence of a null age-effect for vasopressin that contrasted previous research. Stable salivary oxytocin and vasopressin levels in healthy old adults may have protective implications given the role of these peptides in mechanisms such as water balance, metabolism, and homeostasis. However, peripheral oxytocin is only a component of the oxytocin system, and future research needs to examine if reduced central oxytocin secretion or loss of central oxytocin receptors could underpin age-related cognitive and physical declines.

7.3 Introduction

To date, research investigating the oxytocin system in healthy ageing has focused on *central* oxytocin functions, especially through the use of intranasal oxytocin administration to modulate psychological functions and brain activity in a relatively easy and non-invasive approach (for review, see Chapter 4). There is mixed evidence of the effects of intranasal oxytocin, with some suggesting oxytocin increases meta-mood functions, theory of mind, and emotion recognition in older adults (Campbell et al., 2014; Ebner et al., 2015; Grainger et al., 2018b), whilst there is also evidence of no effects on trust and emotion recognition (Grainger 2018b; this thesis; Chapter 6). In contrast, there is little to no research on *peripheral* oxytocin functions in healthy ageing, such as concentration levels, to help us fully understand one of the most basic levels of function of the oxytocin system in human ageing. It is not known whether peripheral oxytocin levels change, possibly decline, with normal human ageing that may help explain some of the typical declines in certain psychological and physical health functions with age (Huffmeijer et al., 2013).

Though oxytocin is often associated with childbirth and lactation, it is important for various other health functions that are essential for older adults. Oxytocin released directly into the central nervous system acts as a neurotransmitter and is linked to sexual behaviour, social behaviours and social memory (Heinrichs et al., 2009; Lee et al., 2009). Oxytocin released into the bloodstream circulates throughout the periphery and is associated with water balance, bone density, metabolism, muscle tissue regeneration, and homeostasis (Colaianni et al., 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019). Most recently, it is thought that oxytocin has an allostatic role whereby it is not only important for regulatory functions such as thermoregulation and homeostasis, but it also makes physiological changes in anticipation of future environmental changes (Quintana & Guastella, 2020). Therefore, oxytocin may provide some protective function to our health. In this study, we will examine

peripheral salivary oxytocin levels as well as related hormones to help us better understand the nature of the peripheral oxytocin system in the context of healthy ageing.

7.3.1 Studying the oxytocin system

Next, we highlight three critical points to consider in the study of the oxytocin system: i) the relationship between central vs. peripheral oxytocin, ii) measuring central vs. peripheral oxytocin levels, and iii) the influence of sex and sex-related hormones. First, while endogenous oxytocin is released both centrally and peripherally, the relationship between central and peripheral oxytocin excretion is unclear. There is evidence that these processes are coordinated under some circumstances (Carson et al., 2015; Valstad et al., 2017), either by simultaneously releasing oxytocin, or interacting with each other in a cyclic pattern (i.e., a release of peripheral oxytocin triggers a release of central oxytocin). However, there is also evidence that peripheral and central oxytocin concentrations are not correlated and thus cannot be inferred from each other (Kagerbauer et al., 2013; Valstad et al., 2017). Thus, inferences made from central or peripheral oxytocin studies need to be made with caution, and it is important to study both systems to help understand the oxytocin system. Here, we will study age differences in peripheral oxytocin concentration levels as a first step.

Second, although central oxytocin levels are also relatively undetermined in human ageing, measuring central oxytocin in humans is invasive as sampling requires the collection of CSF (Lefevre et al., 2017). Despite oxytocin research being a rapidly expanding field, the rarity of opportunities to collect CSF likely accounts for why, to our knowledge, there is currently only one published measure of older adults' CSF oxytocin concentration compared to young adults (Raskind et al., 1986). Raskind et al. (1986) observed no age-effects for oxytocin or vasopressin concentrations, which is a similar neuropeptide with functions and systems that overlap with oxytocin, in young or older adults' CSF. Furthermore, three studies that have examined immunocytochemically identified vasopressin and oxytocin neurons post-

mortem also reported no age-related changes for central oxytocin (Fliers et al., 1985; Swaab, 1995; Wierda et al., 1991), but a fourth reported an age-related decline (Calzà et al., 1997). Slight decreases in central vasopressin were detected (Calzà et al., 1997; Fliers et al., 1985), but these may have been mediated by the time of death with age affecting the circadian patterns of vasopressin production (Hofman & Swaab, 1994). In contrast, animal studies, which are still the primary source of evidence for estimating central oxytocin levels in ageing, indicate that older rhesus macaques have increased concentrations of central oxytocin compared to younger ones (e.g., Parker et al., 2010). However, there is also evidence from animal research suggesting that older rats have decreased concentrations of central oxytocin compared to younger animals (e.g., Melis et al., 1992), or that older and younger rats do not differ in central concentrations levels (e.g., Fliers & Swaab, 1983). Non-invasive methods, such as an oxytocin receptor-specific radioligand (Gimpl, Reitz, Brauer, & Trossen, 2008) to assess central oxytocin, would be required to resolve these conflicting findings. However, to date, this has not been developed for human use (Beard et al., 2018b), though research is advancing with developments of tracers used in PET to trace oxytocin's behaviour in the brain (Smith et al., 2012; Smith et al., 2013).

Conversely, peripheral measures of oxytocin derived from blood, saliva, or urine provide a non-invasive way to obtain oxytocin concentrations. Yet, research investigating the peripheral oxytocin system in older adult humans is also limited. A meta-analysis of endogenous oxytocin across the lifespan identified seven studies that included older adult samples (i.e., mean age of 65+), six of which used peripheral measures and found a small age-effect in the direction of oxytocin increasing with age (Engel et al., 2019). However, the meta-analysis also revealed significant variation in the oxytocin concentrations derived from different sample types (e.g., unextracted blood vs. extracted blood), which confounded the age-effect (Engel et al., 2019). Measuring unextracted oxytocin from blood or saliva is often

reported under the premise that it is a more sensitive measure of oxytocin/vasopressin concentrations (Carter et al., 2007; MacLean et al., 2019; Plasencia et al., 2019). However, this technique has been critiqued for producing inflated and erroneous peptide concentrations that are highly influenced by protein interference (Leng & Sabatier, 2016). It is therefore difficult to compare oxytocin concentrations from samples with different assay procedures, so meta-analyses contrasting peripheral oxytocin levels in young and older adults from different studies should be interpreted with caution. Here we contrast young and older adults' peptide concentrations using identical assay procedures for both groups.

Lastly, sex is a factor of interest for oxytocin research because of the known interplay between the sex hormones testosterone and estrogen and the oxytocin system. Reduced testosterone is linked to a loss of neural oxytocin receptors in rats (Arsenijevic & Tribollet, 1998), but not central oxytocin innervation in rats (Goudsmit et al., 1988) or peripheral concentration levels in human adults (Gordon et al., 2017). In contrast, reduced estrogen is linked to diminished peripheral and central oxytocin concentration, and loss of neural oxytocin receptors (e.g., de Kloet et al., 1986), with estrogen administration increasing oxytocin levels both peripherally and centrally (e.g., Boos et al., 1994; Caldwell et al., 1989; Chibbar et al., 1995). In younger adults, there are mixed sex-effects reported for plasma oxytocin levels. For instance, there is evidence for no sex differences in oxytocin production (Jokinen et al., 2012) but there is also evidence of males producing more oxytocin than females (Weisman, Zagoory-Sharon, Schneiderman, Gordon, & Feldman, 2013). Moreover, testosterone and estrogen both decline with age, but the trajectories differ between sexes. Males experience a more pronounced age-related decline of testosterone (Elmlinger et al., 2005), whereas females experience a marked age-related decline of estrogen post-menopause (Eskes & Haanen, 2007). Therefore, given the link between sex hormones and oxytocin, an age by sex interaction for oxytocin production is likely. Here we contrast peptide levels of

both sexes within each age group, and also measure estradiol and testosterone levels to observe ageing patterns between these sex hormones and the peptides.

To our knowledge, only two previous studies[2] have directly compared older and younger adults' peripheral oxytocin concentrations using the same sampling type and assay procedure. Both studies used plasma samples, but Forsling et al. (1998) used extracted plasma and RIA and Plasencia et al. (2019) used non-extracted plasma and EIA. Of these two studies, Forsling et al. (1998) used the most valid assay procedure (Leng & Sabatier, 2016) in 24 male participants, but Plasencia et al. (2019) reported on a much larger sample ($N = 101$) and included both sexes rather than only males. Both studies reported that no significant age differences were present for oxytocin concentration (Forsling et al., 1998; Plasencia et al., 2019). For vasopressin concentrations, the two studies produced conflicting results. Plasencia et al. (2019) reported that older adults had significantly higher vasopressin concentration than young adults; whereas, Forsling et al. (1998) reported that older adults had significantly lower vasopressin concentrations than young adults. Plasencia et al. (2019) also examined the moderating effect of sex and reported that no age by sex interaction emerged for either oxytocin or vasopressin concentrations, but that overall, females showed higher oxytocin concentration than males (Plasencia et al., 2019).

7.3.2 The current study

The current study aimed to provide clarity and new knowledge about the peripheral oxytocin levels in healthy young and older adults to progress our understanding of the oxytocin system in human ageing. We did an extreme age-group comparison of oxytocin and

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[2] Note that a third publication (Ebner et al., 2019) reported on a subset of the same sample as Plasencia et al. (2019).

vasopressin concentration in saliva samples, to confirm if there are age-differences and/or age by sex interactions for these peptides. To increase the validity and reliability of the findings, we measured peripheral oxytocin and vasopressin concentrations in a large sample (N =122) of healthy young and older adults, matched on sex, using assay procedures that are less susceptible to protein interference (i.e., RIA with extracted samples). Given the limited evidence to date, this research was considered exploratory and therefore no specific hypotheses were made. We also sought to explore other sex-related factors by measuring participants' peripheral testosterone and estradiol levels to identify consistencies, or inconsistencies, between the patterns of age-related differences observed for the neuropeptides compared to the sex hormones.

7.4 Methods

7.4.1 Participants

Of the 123 participants involved in the primary study (Chapter 6), 122 elected to provide saliva samples. The final sample comprised of 60 older adults (60-80 years) and 62 young adults (18-33 years), with approximately equal numbers of males and females in each age group. All participants provided written consent and the research was approved by the Australian Catholic University's Human Research Ethics Committee. Participants were screened before participating. Exclusion criteria included: self-reported neurological or psychiatric illnesses, anti-psychotic or anti-depressant medication use, substance use, history of substance abuse, regular smoking, history of heart disease, pregnancy, breastfeeding, and hormone supplements. Crystallised intelligence, was measured by the NART (Nelson & Willison, 1991), and years of education were recorded to characterise the age and sex groups. As we were interested in typical ageing, rather than neurodegenerative diseases, older adults were screened for intact cognitive ability using the telephone interview for cognitive status

(Brandt, Spencer, & Folstein, 1988), and excluded if they scored with the mildly, moderately, or severely impaired ranges (i.e., score of 25 or less).

7.4.2 Saliva collection

Two 1 ml samples of saliva were collected from each participant using the passive drool method. To avoid the possibility of food substances contaminating the saliva, participants were advised to not eat within 60 minutes of sample collection. Since caffeine may compromise the assay by lowering saliva ph. and increasing bacterial growth, participants were also instructed to restrict caffeinated drinks on the day of testing (only one cup, no sooner than three hours before sample collection). Females provided samples during the luteal menstrual cycle phase to control for fluctuating levels of estradiol, and its interaction with oxytocin signalling (Jirikowski, Caldwell, Pedersen, & Stumpf, 1988; Shukovski, Healy, & Findlay, 1989).

7.4.3 Saliva storage and assay

Saliva samples were kept cool on ice and frozen within two hours of collection. Saliva samples were stored long term at -80°c, until assay. One 1 ml sample per participant was sent via courier, packaged with dry ice to prevent thawing, to two specialised labs. Oxytocin and vasopressin were extracted according to the method of Landgraf's lab (RIAgnosis, Munich, Germany) using highly sensitive and specific RIA (Kagerbauer et al., 2013). Testosterone and estradiol concentration levels were extracted at Stratech Scientific (http://www.stratechscientific.com.au), according to the manufacturer's instruction using commercially available kits (Salimetrics, USA).

7.4.4 Statistical analysis

The testosterone ELISA kit had an assay ranged of 6.1 pg./mL - 600 pg./mL (Salimetrics, 2020), however, three participants had testosterone levels reported outside the

valid range (>600pg/ml), and were thus excluded from further analyses. The final included sample size for testosterone was, therefore, n = 119. Two participants, one for oxytocin and one for estradiol, had concentrations levels greater than four standard deviations from the overall mean, so were categorised as extreme outliers. The extreme outlier values were replaced with the next highest score plus one unit, as described as a viable solution for reducing the impact of outliers by Tabachnick and Fidell (2013). Square root transformations were used to normalise the distribution for each dependent variable. The data in Table 7.2 and Figure 7.1 depicts non-transformed data to aid interpretation (i.e., a more meaningful measure of pg./ml).

A MANOVA was conducted on the transformed data; with the concentration of oxytocin, vasopressin, testosterone, and estradiol as the dependent factors, and age (young and old) and sex (female and male) as the fixed effects; see Table 7.2. Any interactions were further investigated using Bonferroni adjusted simple effect comparisons.

For any null effects, Bayesian hypothesis testing was used as a means of examining the relative evidence for an alternative and null hypothesis (Quintana & Williams, 2018; Wagenmakers et al., 2018). A Bayes factor value of >1 when comparing an alternative and null model (bf_{10}) is considered evidence favouring the alternative model, whereas a bf_{10} value <1 is considered evidence favouring the null model. For bf_{10} values in favour of the null model, the following classifications are made: <0.01 = extreme evidence, 0.01 – 0.03 = very strong evidence, 0.03 – 0.10 = strong evidence, 0.10 – 0.33 = moderate evidence, 0.33 – 1 = anecdotal evidence (Lee & Wagenmakers, 2014).

7.5 Results

7.5.1 Demographic analyses

Multivariate analysis of variance on the key demographic variables reported in Table 7.1 indicated that within each age group, males and females were matched for age and years education. Older males and females and their young adult counterparts were also matched for years of education. However, older adults, both male and females, had significantly higher IQ (as measured by the NART) than their young adult counterparts (p <.001). Within the age groups, young males had significantly higher IQ than the young females (p = .04), whereas the older males and females did not differ.

Table 7.1

Sample demographics and mean concentration (pg./ml) of oxytocin, vasopressin, testosterone and estradiol

	Young adults		Older adults	
	Female	Male	Female	Male
N	30	31	30	31
Age	22.67 (3.69)	24.10 (4.22)	71.00 (4.80)	73.94 (5.90)
Years education	15.90 (1.80)	16.12 (2.54)	16.14 (3.90)	15.2 (3.67)
IQ[c]	101.28 (7.17)	105.44 (7.33)	116.15 (7.80)	113.40 (8.49)
Oxytocin	1.47 (0.38)	1.58 (0.62)	1.53 (0.27)	1.69 (0.43)
Vasopressin	2.11 (0.63)	1.89 (0.46)	2.00 (0.50)	2.06 (0.66)
Testosterone	68.80 (35.83)	170.07 (61.17)	55.78 (20.61)	119.00 (38.89)
Estradiol	1.04 (0.64)	1.48 (0.9)	1.09 (0.57)	1.55 (1.23)

Note. [a] Represents WAIS full-scale IQ estimate as measured by the National Adult Reading Test (NART). Standard deviation reported in parentheses.

7.5.2 Main analyses

Table 7.1 presents the means and standard deviations for oxytocin, vasopressin, testosterone, and estradiol concentrations. Table 7.2 and Figure 7.1 present the statistical results. For oxytocin, there was neither an age or a sex main effect, or age by sex interaction, suggesting all groups had relatively consistent oxytocin levels. A Bayesian ANOVA revealed anecdotal evidence for a null model, relative to an alternative model, for both the main effect of age (bf_{10} = 0.58) and sex (bf_{10} = 0.40). The combined main effect model was preferred to the interaction model by a factor of 3.83, which represents moderate evidence for a null interaction model. Previous research has also reported non-significant main age effects (Forsling et al., 1998; Plasencia et al., 2019), as well as a non-significant age by sex interaction (Plasencia et al., 2019). Furthermore, the magnitudes of the main age-effect and age by sex interaction reported by (Plasencia et al., 2019) (d = .12 and d = .02 respectively) are similar to the null effects reported in the current study (d = .28 and d = .07 respectively). Together this may suggest greater support for null main effects as well as a null interaction model. Thus, our finding that there are no oxytocin differences across both age and sex is consistent with the most parsimonious account of the data to date.

The same pattern emerged for vasopressin, whereby there was neither an age or a sex main effect, or age by sex interaction. A Bayesian ANOVA revealed moderate evidence for a null effect of age (bf_{10} = 0.21) and moderate evidence for a main effect of sex (bf_{10} = 0.23). The combined main effect model was preferred to the interaction model by a factor of 1.48, which represents anecdotal evidence for a null interaction model. Previous research has also reported a null age by sex interaction (d = .10) (Plasencia et al., 2019). Together this may suggest greater support for null interaction models. Notably, our null main age-effect (d = .06), is inconsistent with the previously reported age-effects, whereby in one study older adults had significantly higher vasopressin than young adults (d = .83) (Plasencia et al.,

2019), and in the other study older adults had significantly lower vasopressin than young adults (d = .74) (Forsling et al., 1998). However, we combined these effects in a random-effects model and it produced an overall null effect: d = .11 [95% CI = -1.78; 1.20]. Thus, our finding that there are no vasopressin differences across both age and sex is consistent with the most parsimonious account of the data to date.

For testosterone, the age by sex interaction was non-significant, however, both the main effects of age and sex were significant. Older adults, had significantly lower levels of testosterone than young adults, and males, had significantly more testosterone than females. A Bayesian ANOVA revealed moderate evidence for a main effect of age (bf_{10} = 3.37) and overwhelming evidence for a main effect of sex (bf_{10} > 1000). The interaction model was preferred to the combined main effect model by a factor of 1.07, providing almost no evidence in favour of either model.

For estradiol, there was no main effect for age or age by sex interaction, but there was a main effect for sex, whereby males surprisingly had more estradiol than females. A Bayesian ANOVA revealed moderate evidence for a null effect of age (bf_{10} = 0.22) and moderate evidence for a main effect of sex (bf_{10} = 3.25). The main effect model was preferred to the interaction model by a factor of 1.14, which represents anecdotal evidence for a null interaction model.

Table 7.2

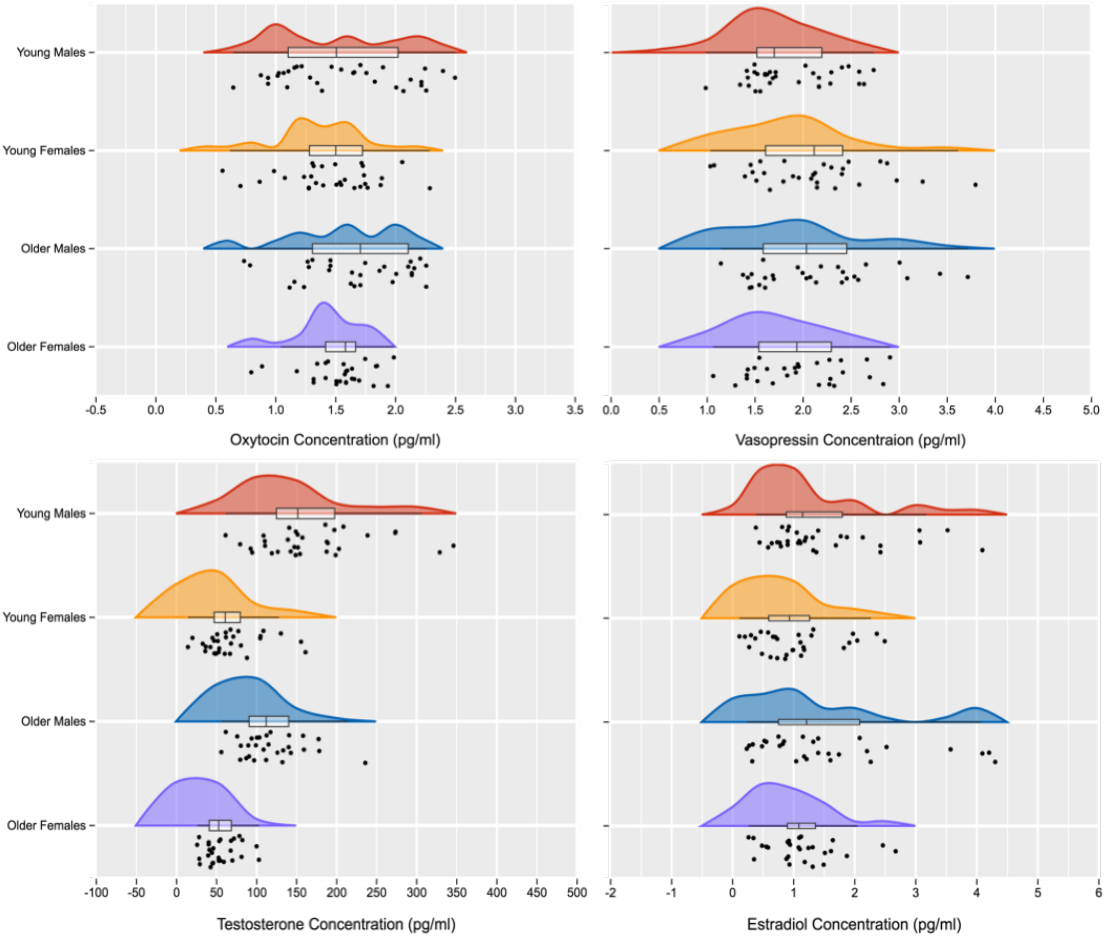
Multivariate linear model test of between subjects.

Dependent variable	Fixed effect	Type III SS[a]	*Df*	Mean square	*F*	*p*	η^2
Oxytocin	Age group	0.07	1	0.07	2.31	.13	0.02
(*n* = 122)	Sex	0.05	1	0.05	1.54	.22	0.01
	Age group x sex	0.01	1	0.01	0.16	.69	0.001
Vasopressin	Age group	0.004	1	0.004	0.10	.75	0.001
(*n* = 119)	Sex	0.01	1	0.01	0.28	.60	0.002
	Age group x sex	0.09	1	0.09	2.24	.14	0.05
Testosterone	Age group	54.72	1	54.72	13.82	<.001*	0.13
(*n* = 121)	Sex	497.75	1	497.75	125.68	<.001*	0.52
	Age group x sex	13.25	1	13.25	3.35	.07	0.03
Estradiol	Age group	0.03	1	0.03	0.23	.64	0.002
(*n* = 122)	Sex	0.78	1	0.78	6.17	.01*	0.05
	Age group x sex	0.04	1	0.04	0.29	.59	0.003

Note. [a] sum of squares, * statistically significant main effects or interaction effects $p < .05$.

Figure. 7.1

Mean concentration levels, distribution, and confidence intervals for oxytocin, vasopressin, testosterone, and estradiol split by age and sex.



7.6 Discussion

In a comprehensive study, we present the findings of the largest comparison of young and older adults' peripheral concentrations of oxytocin and vasopressin to date, and also include measures of testosterone and estradiol. Our findings suggest that both oxytocin and vasopressin remain relatively stable in healthy ageing, as older and younger adults had relatively equal levels of peripheral oxytocin and vasopressin. Relative to the alternative hypothesis, there was 1.7 times more evidence for a null age-difference for oxytocin, and 4.8 time more evidence of a null age-difference for vasopressin. In terms of sex-differences, our

results indicated that sex did not play a significant role in moderating neuropeptide age-differences. Relative to the alternative hypothesis, there was 2.5 times more evidence for a null sex-difference for oxytocin, and 4.3 times more evidence for a null sex-difference for vasopressin. There was also 3.8 times more evidence for a null age by sex interaction relative to the alternative hypothesis for oxytocin, and 1.5 times more evidence for a null age by sex interaction relative to the alternative hypothesis for vasopressin. To further explore the role of sex, we also measured concentration levels of sex hormones to establish if these had similar age differences to oxytocin and vasopressin. We found that relative to the alternative hypothesis, there was 4.5 times more evidence for a null age-difference for estradiol. In contrast, there was 3.4 times more evidence for age-difference for testosterone, whereby older adults had lower levels of testosterone than young adults. In terms of age by sex interactions for the sex hormones, there was 1.1 times more evidence relative to the alternative hypothesis for a null interaction for estradiol, and for testosterone there was 1.1 times more evidence in favour of an age by sex interaction. Thus, for both sex hormones, there was minimal difference in preference for a null interaction versus an alternative hypothesis. Thus, the trajectory of testosterone and estradiol in healthy ageing appears similar for both males and females. In summary, our results provide preliminary evidence that oxytocin, vasopressin, and estradiol remain stable in healthy ageing for both males and females, whereas testosterone declines for both males and females.

Older adults maintaining the peripheral concentration of oxytocin and vasopressin may protect them from the deterioration of peripheral functions. These peptides play a significant role in water balance, bone density, metabolism, muscle tissue regeneration, and homeostasis (Colaianni et al., 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019), which are functions that commonly decline with age. As such, an age-related decline of oxytocin and vasopressin would suggest that these peptides may contribute to

some of these physiological losses. For example, a decline of peripheral oxytocin is linked to poor muscle regeneration in aged rats (Elabd et al., 2014). However, oxytocin and vasopressin remaining stable in healthy ageing, instead suggests that these peptides do not underpin these physiological declines. It may even be that oxytocin and vasopressin are maintained in healthy ageing to help mitigate physiological losses. Vasopressin regulates extracellular fluid and blood pressure. Therefore, changes within these functions, such as fluid loss/gain, can trigger an increase or decrease in vasopressin release (Convertino, Keil, Bernauer, & Greenleaf, 1981; Morton, Connell, Hughes, Inglis, & Wallace, 1985). Thus, instead of following the typical ageing trajectory of decline, vasopressin production may remain stable in ageing in compensation for other age-related losses such as impaired renal concentrating capacity (Os et al., 1987). This fits with an allostatic account of the oxytocin system, whereby the oxytocin system is thought to be an adaptive mechanism that changes throughout the lifespan to meet the needs of the current environment (Quintana & Guastella, 2020). Notably, the current study was based on a sample of healthy older adults, and as such none of the participants had a history of heart disease, self-reported any neurological or psychological illness or were smokers. Perhaps a decline in peripheral oxytocin or vasopressin would therefore be observed in older adults with some ill health (e.g. cardiovascular diseases). Future research could investigate this possibility and identify if there is a correlation between peripheral oxytocin or vasopressin and health outcomes.

It is unclear if older adults maintaining the peripheral concentration of oxytocin and vasopressin would also protect them against psychological losses. Our finding that oxytocin remains stable in healthy ageing may initially seem contrary to the theory that the oxytocin system is a mechanism that underpins age-related psychological losses, such as diminished social processing (Ebner et al., 2013). Studies investigating the impact of intranasal oxytocin administration on behavioural outcomes provide evidence that oxytocin within the brain is

involved in social processes (Ellenbogen, 2018; MacDonald & MacDonald, 2010). Moreover, initial studies on the effects of intranasal oxytocin in healthy ageing report that intranasal oxytocin improved older adults' social processing (Campbell et al., 2014; Ebner et al., 2015; Grainger et al., 2018b), but see also (Grainger et al., 2018a). However, it is important to consider the evidence that baseline peripheral and central oxytocin concentrations are not correlated (Kagerbauer et al., 2013; Valstad et al., 2017). As such, the current finding of peripheral oxytocin remaining stable with age does not infer that central oxytocin levels also remain stable with age. Indeed, there is evidence in animal studies whereby peripheral oxytocin remains stable but central oxytocin declines with age (Martin et al., 2014; Melis et al., 1992). Furthermore, given our finding that testosterone declines with age, and the link between testosterone and loss of neural oxytocin receptors (Arsenijevic & Tribollet, 1998), it may be that it is oxytocin receptors rather than concentration levels that are compromised in ageing. Thus, the central oxytocin system may still be associated with age-related psychological losses, despite peripheral levels remaining stable. Since it is the central oxytocin system that is directly involved in social processes, and baseline central and peripheral levels are not associated, we are cautious making links between our peripheral oxytocin findings and psychological declines.

However, despite peripheral oxytocin not being directly involved in social cognitive processes, it may still serve to indirectly protect from social cognitive impairment. There is evidence that, even though the central and peripheral pathways are distinct and do not demonstrate the parallel release of oxytocin (Ludwig & Leng, 2006), they are coordinated under some circumstances, such as post intranasal oxytocin administration (Valstad et al., 2017), after inducing stress (Valstad et al., 2017), and in response to some neuroactive substances (Jurek & Neumann, 2018). Furthermore, intranasal oxytocin, which is the primary source of evidence that the central oxytocin system is involved in social cognitive deficits,

also has peripheral effects. Although there is evidence that a functionally significant amount of intranasally administered oxytocin crosses the blood-brain barrier and exert effects on the central oxytocin system (Neumann & Landgraf, 2012), much of the nasal spray is absorbed peripherally (Dal Monte, Noble, Turchi, Cummins, & Averbeck, 2014; Striepens et al., 2013). As such it is unclear if the effect intranasal oxytocin has on social processing is purely due to the oxytocin that crosses the blood-brain barrier, or is in part because of feedback from receptors within the periphery (Quintana et al., 2015a). Intranasal oxytocin may not selectively influence target behaviours, rather broader physiological effects such as the anxiolytic effect of oxytocin (MacDonald & Feifel, 2014) may drive the improved task performance (Churchland & Winkielman, 2012). Indeed, oxytocin administered intravenously, which is not considered to cross the blood-brain barrier, has been shown to improve social memory in adults with autism (Hollander et al., 2007). However, see also Quintana et al. (2016) which showed that intranasal oxytocin influenced amygdala activation in response to emotional faces but intravenous oxytocin did not. In summary, the relationship between the central and peripheral oxytocin systems remains unclear, but there is evidence that the peripheral system interacts with central functions in some way, and maintenance of the peripheral system in ageing could therefore indirectly protect against psychological decline.

The present study was the first to compare older adults' oxytocin levels to that of younger adults' while also measuring sex hormone levels. This allowed us to compare the trajectory of these sex hormones in ageing to the trajectory of oxytocin. Our finding whereby older adults had significantly less testosterone than young adults, but relatively the same amount of estradiol, fits with the pattern of decline we observed whereby peripheral oxytocin is maintained in ageing. This is because estradiol is positively linked to oxytocin concentration levels (Boos et al., 1994; Caldwell et al., 1989; Chibbar et al., 1995), and thus a

shared trajectory of oxytocin and estradiol is to be expected. In contrast, testosterone is shown to negatively impact on central oxytocin receptors (Arsenijevic & Tribollet, 1998; de Kloet et al., 1986), rather than oxytocin levels, and thus a shared trajectory of oxytocin and testosterone is not expected. However, caution is required when interpreting our sex hormone findings given that an unusual sex-effect emerged whereby males had more estradiol than females.

Our finding that there were no age differences for vasopressin conflicts with previous research that report age-effects either in favour on younger adults (Forsling et al., 1998), or in favour of older adults (Plasencia et al., 2019). Although, the culmination of the current null age-effect with these two previously reported effects produced a null-effect, here we discuss how the time of testing might contribute to these conflicting results. Firstly, it is important to note that Forsling et al. (1998) took 7 to 8 samples from participants over 24 hours. Although they found that overall older adults had less vasopressin than young adults, it was at night time that the two groups differed the most. Young adults had a significant rise in vasopressin levels at night but older adults did not. In the current study, participants were all tested during the day. A limitation of the current study is that participants were tested at different times of the day, and thus it is difficult to use the diurnal patterns reported by Forsling et al. (1998) to account for our findings. However, we note that many of our participants were tested in the afternoon, and the afternoon was when young and older adults have the most similar levels (Forsling et al., 1998), which may account for why we observed no age-differences in vasopressin levels. Conversely, Plasencia et al. (2019) tested all participants at 9am and reported that older adults had higher vasopressin levels than young adults, which is inconsistent with the diurnal patterns reported by Forsling et al. (1998). Future research could explore this by replicating the Forsling et al. (1998) study with a larger sample size.

Overall, the finding that peripheral oxytocin and vasopressin remain stable in human ageing, for both men and women, is one piece of a complex puzzle. Such evidence has implications for oxytocin and ageing research, as may suggests that this component of the oxytocin system does not underpin age-related cognitive or physical declines, or is a compensatory response to minimise age-related declines of associated functions. However, other aspects of the oxytocin system, such as central concentration levels and oxytocin receptors, may still be negatively impacted by age and underpin age-related declines. Further research investigating the human oxytocin system in ageing is required to investigate oxytocin receptor expression and central levels in the healthy ageing brain. Further research is also required that investigates the oxytocin system across various levels of cognitive or physical decline, such as dementia.

CHAPTER 8: GENERAL DISCUSSION

8.1 Chapter Guide

The general aims of this thesis were to first clarify the pattern and magnitude of older adults' difficulties recognising different facial expressions of emotions, and second to advance the current understanding of the mechanisms underlying these difficulties. This was achieved by studying the role of the neuropeptide oxytocin in this context and identifying the task conditions for which these difficulties are strongest. Through a compressive meta-analytic review of the facial emotion recognition and ageing literature (Chapter 3), this thesis first explored the moderating effects of task characteristics on older adults' facial emotion recognition performance. To provide the clearest picture of older adults' emotion recognition difficulties to date, the culminated age-effects were reported separately for each task type and emotion, which served to highlight the impact that task characteristics had on older adults' performance. Next, the oxytocin system in healthy ageing was explored, both centrally (Chapter 6) and peripherally (Chapter 7). In Chapter 6, the central oxytocin pathway was investigated as a potential biological mechanism behind older adults' facial emotion recognition difficulties through trialling the effects of intranasal oxytocin on older and young adults' facial emotion recognition accuracy. In Chapter 7, the trajectory of peripheral oxytocin (in addition to vasopressin, estradiol, and testosterone) was investigated by contrasting young and older adults' baseline oxytocin levels as measured from saliva samples.

The following general discussion provides an overview of the findings from both the meta-analytic review and the empirical studies. The contributions and theoretical implications of each finding are discussed. The strength and limitations of this research are detailed and directions for future research are outlined. Lastly, this general discussion ends with the overall conclusions of the thesis.

8.2 Summary of Main Findings

There were three main findings from the research presented in this thesis. The first finding from the meta-analysis was that older adults had difficulty recognising facial expressions of emotions on *all* emotion recognition tasks, but the strength of difficulty that they exhibited for each emotion type was moderated by key task features. The combined variance accounted for by the number of emotions included in the task, the stimulus format used, and the image set from which the stimuli were sources was significant. Furthermore, once task variation was accounted for, the most parsimonious pattern of age-related difficulty was one of impaired recognition for *all* emotions, rather than selective preservation of the recognition of disgust and positive emotions. This finding contrasts with outcomes of the most commonly cited meta-analysis on this topic (Ruffman et al., 2008).

The second finding was that the administration of oxytocin nasal spray did not modulate older adults' facial emotion recognition accuracy, and as such did not eliminate age differences in performance. Contrary to our expectations, the outcomes of this clinical trial of intranasal oxytocin do not provide evidence for the oxytocin system being a biological mechanism behind older adults' facial emotion recognition difficulties. It is worth noting, however, that the findings from this thesis are not sufficient to eliminate the oxytocin system as a biological mechanism behind these difficulties, as more direct testing would be required.

The third finding was that older adults' baseline peripheral oxytocin and vasopressin concentrations are equivalent to that of young adults' for both males and females, and as such peripheral oxytocin does not appear to decline with age. This finding provides further insights into the oxytocin system in an older adult cohort. Notably, in this cohort, vasopressin and estradiol also remained stable across the two age groups and only testosterone declined significantly with age.

The findings of this thesis contribute to the current understanding of healthy ageing in several ways. Next, these contributions and their theoretical implications are outlined and discussed.

8.3 What does this Research Contribute to the Current Understanding of Healthy Ageing?

8.3.1 The influence of task design

A key insight to have come from this thesis is the finding that task characteristics influence older adults' facial emotion recognition performance. A prominent example is that in the meta-analytic review (Chapter 3), where older adults only demonstrated equivalent or superior recognition of disgusted faces to young adults when presented with full-intensity images from the POFA image set (Ekman & Friesen, 1976). In contrast, on all other image sets and stimulus formats older adults were inferior to young adults for disgust recognition. Notably, the empirical study examining the effects of oxytocin nasal spray on older and young adults' facial emotion recognition accuracy (Chapter 6), mirrored this finding with a sample of 60 older adults demonstrating significantly inferior disgust recognition accuracy at baseline to a sample of 60 young adults on stimuli from the FACES database (Ebner et al., 2010). The findings from this thesis that older adults' disgust recognition ability depends on the image set used, is pivotal because the most commonly cited meta-analysis on this topic reported that older adults retain the ability to recognise disgust (Ruffman et al., 2008) and has been cited almost 700 times. Notably, the majority of the studies included in that meta-analysis used stimuli from the POFA (Ruffman et al., 2008), which likely accounts for this finding. Given these different image sets are not used exclusively with older adults, the potential uniqueness of the disgust stimuli from the POFA image set may also have influenced emotion recognition findings from other fields of research, which warrants investigation.

A further contribution of the findings from the meta-analysis (Chapter 3), is the finding that the age-effects for video-based tasks and full-intensity photograph tasks were statistically similar in magnitude. There has been disagreement about whether static facial emotion recognition stimuli provide an accurate estimation of older adults' emotion recognition ability, or instead inflate older adults' difficulties. For example, Isaacowitz and Stanley (2011) suggest that older adults would be less impaired on tasks involving videos and/or context, which are more reflective of everyday social situations. Indeed, it has been reported that providing contextual cues does eliminate or reduce age-differences in facial emotion recognition accuracy (Richter et al., 2011; Wieck & Kunzmann, 2017). This may not mean that older adults do not have difficulty recognising emotions, rather it could be that older adults are able to rely on their crystallised intelligence and years of experience to more accurately postulate the emotional content of the expression when there is context. Due to a lack of available data, this thesis was unable to explore the impact of context on older adults' facial emotion recognition difficulties. This thesis was, however, able to examine the different age-effects produced by static versus dynamic stimuli. The meta-analytic review showed that older adults still demonstrate difficulty recognising facial expressions of emotions on videos, and the magnitude of this age-effect did not differ significantly from that of full-intensity static photographs. For videos, however, older adults demonstrated difficulty across all emotions, which was not the case for full-intensity photographs whereby older adults exhibited significantly more difficulty recognising anger, fear, and sadness, than disgust and happiness.

Different effect patterns being produced by different stimulus formats suggests that these tasks may utilise, and therefore measure, slightly different processes. This has implications not only for the field of healthy ageing, but also for all research examining facial emotion recognition regardless of the cohort being investigated. It may seem that the key

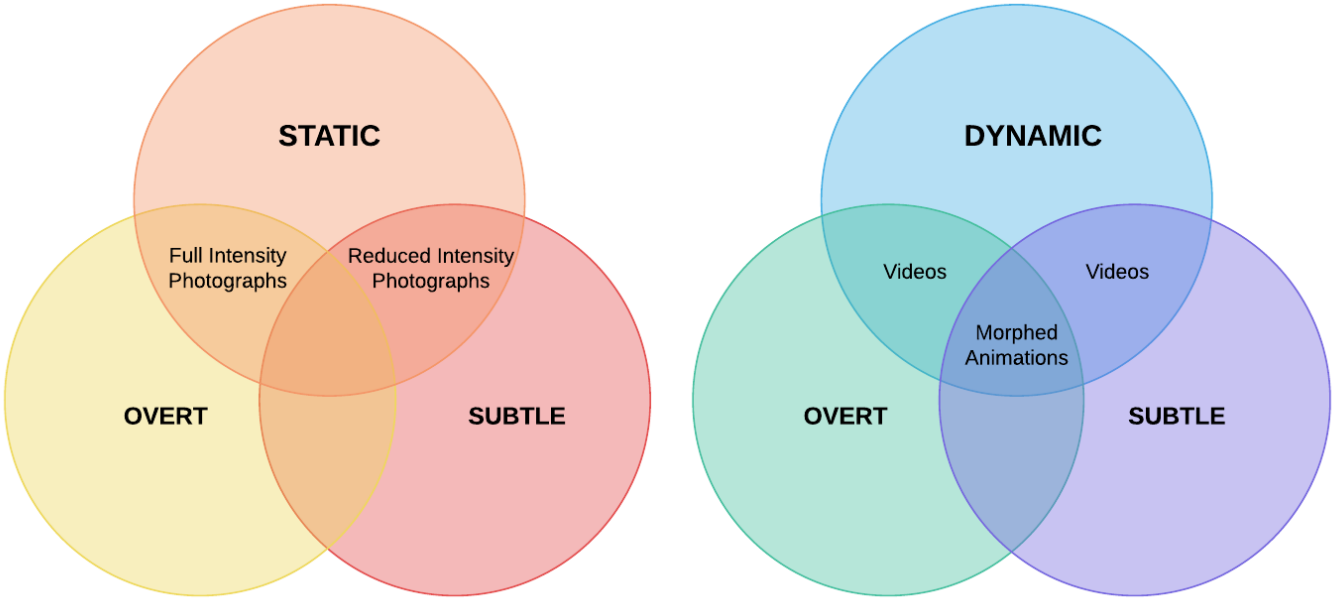
difference between videos and full-intensity photographs is that the first is dynamic whereas the latter is static. As such, speed of processing could play a role in these stimulus formats producing different patterns of effects. However, a complication in interpreting the impact of stimulus format on older adults' performance, is that videos are not only dynamic but are also likely to depict subtler expressions than full intensity photographs. Thus, there may be two factors separating different stimulus formats, whether the stimuli are static or dynamic, but also how subtle or overt the facial expressions of emotions are depicted. It is therefore unclear if older adults demonstrate different patterns of recognition difficulties across emotion for videos compared to full-intensity photographs because they are dynamic vs. static, or because they are subtle vs. overt, or an interaction between the two. Figure 8.1 schematically depicts these two factors and how each of the stimulus formats could be categorised, thereby highlighting that these stimulus formats should be carefully interpreted and one should not assume that they are measuring the same construct.

In support of the notion that different stimulus formats involve different processes, there is evidence that both static and dynamic, as well as overt and subtle, facial expressions of emotions are processed in different neural regions, at least for happiness. First, people of all ages who have damage to the orbitofrontal cortex, are impaired in recognising subtle, but not overt, facial expressions of happiness (Willis et al., 2014). Second, different patterns of neural activation are observed for recognition of static versus dynamic facial expressions, and the prefrontal cortex is one region that is activated differently for each of these stimulus formats, which is particularly evident for happiness (Kessler et al., 2011; Kilts et al., 2003). If different facial emotion recognition stimulus formats activate different neural regions, this may explain why older adults demonstrate variation in performance across the tasks, as the ageing brain does not deteriorate in a uniform manner (Lamar & Resnick, 2004; Raz et al., 2010; Raz & Rodrigue, 2006). However, it also has broader implications for research

investigating biological factors that modulate older adults' performance, as the interventions or mechanisms being tested may target specific neural regions.

Figure 8.1

Venn diagrams depicting how the different facial emotion recognition stimulus formats can be categorised in terms of static vs. dynamic and also overt vs. subtle.



Note. Videos that are naturalistic are likely to be subtle, however, if the actors were instructed to produced extreme emotions they could be overt. Morphed animations often transition from a neutral face to and overt expression. Participants are typically instructed to respond as soon as they identify the emotions and as such are recognising a subtle expression. However, if participant wait until the end of the animation to respond, they are recognising an overt expression.

Indeed, when the intranasal oxytocin effects reported in both the current thesis and prior research are considered, the stimulus format used does appear to be a potential moderator of whether or not oxytocin effects were observed. The intranasal oxytocin study reported in the current thesis (Chapter 6) tested the effects of intranasal oxytocin on young and older adults' facial emotion recognition accuracy using reduced-intensity photographs and therefore stimuli that are static and subtle. In contrast, Campbell et al. (2014) used full-

intensity photographs that were static but overt. Grainger et al. (2018b) and Horta et al. (2019) used videos and morphed animations respectively, which are both dynamic and subtle. Of these studies, only Campbell et al. (2014) reported an oxytocin effect for older adults (i.e., improved emotion recognition in older men), and they were the only study to use overt static stimuli.

Given the knowledge that oxytocin's effects are context-dependent (Shamay-Tsoory & Abu-Akel, 2016), oxytocin effects may be most sensitive to overt static stimuli (at least in the healthy older adult cohort). However, there are multiple possibilities as to why this is the case. One possibility is that the difference in oxytocin effects is due to task difficulty. This would fit with the finding that oxytocin effects for theory of mind seem to be moderated by both participant skill and item difficulty (Bartz et al., 2011). Although overt and subtle static photographs produce relatively similar age-effects across most emotions (Chapter 3), it may be that both young and older adults find subtle emotional stimuli more difficult to recognise. However, it is worth noting that older adults also find different emotions more and less difficult to recognise, yet no oxytocin effects were observed for any of the emotions (Chapter 6). Thus, it may not be task difficulty that drives the difference in oxytocin effects but an alternative difference between the stimulus formats. For example, overt and static stimuli have clear and unchanging areas of emotional information, whereas the emotional information in subtle and dynamic stimuli can be ambiguous and/or fleeting. Thus, if oxytocin increases attention to emotional cues, it may be more beneficial for stimuli where the cues are clear (i.e., overt stimuli) and remain stable (i.e., static stimuli). This hypothesis is supported by findings on other social cognitive tasks whereby intranasal oxytocin improved theory of mind performance in older adults, only when presented with minimal context and not with enriched context (Grainger et al., 2018b). Alternatively, older adults may be less motivated to engaged their limited cognitive resources in unrealistic overt and static stimuli

than stimuli that are more true to life (Phillips et al., 2013), which is in line with the selective engagement hypothesis (Hess et al., 2001). Perhaps oxytocin then increases their motivation to engage in this task type by increasing their approach urges, as per the prosocial account of the effects of oxytocin (Shamay-Tsoory & Abu-Akel, 2016). Conversely, it may be that older adults process overt static stimuli in a brain region that is more susceptible to oxytocin effects. However, more research is needed that maps how the different patterns of age-effects across the different emotions *and* stimulus formats map onto changes in the ageing brain. A final possibility is that oxytocin effects are not more sensitive to overt and static stimuli, rather it was the methodology enlisted by Campbell et al. (2014) that led to their finding of an emotion recognition oxytocin effect for healthy older men. These methodological differences are discussed in greater detail in the upcoming section '8.4 More Oxytocin-Ageing Research is Needed'.

8.3.2 The oxytocin system in healthy human ageing

There are strong theoretical reasons to expect that the oxytocin system would be negatively impacted by age. First, skills such as emotion recognition and other social cognitive processes decline with age and oxytocin has been implicated in these processes (Heinrichs et al., 2009; Lee et al., 2009). Second, peripheral oxytocin has been shown to play a role in bone density, metabolism, and muscle tissue regeneration (Colaianni et al., 2014; Elabd et al., 2014; Kiss & Mikkelsen, 2005; Quintana et al., 2019), which also decline with age. Third, there is evidence of sex hormones declining with age, and as these are positively associated with the oxytocin system (Arsenijevic & Tribollet, 1998; de Kloet et al., 1986; Gordon et al., 2017), a decline of sex hormones would be expected to reflect a decline in oxytocin. Calls have therefore been made to extend oxytocin research to ageing (Ebner et al., 2013; Huffmeijer et al., 2013), however, with so little research examining the oxytocin system in ageing humans, the trajectory of the oxytocin system in ageing has lacked

evidential support. Thus, the findings from this thesis, are some of the first insights into the impact that ageing has on the oxytocin system, both centrally and peripherally.

That this thesis found that peripheral oxytocin is maintained in healthy ageing (Chapter 7) and that intranasal oxytocin does not improve older adults' facial emotion recognition ability (Chapter 6), has meaningful implications for the current understanding of the oxytocin system in human ageing. While these results are consistent with previous findings of peripheral oxytocin being maintained in healthy ageing (Forsling et al., 1998; Plasencia et al., 2019), the results contrast with previous oxytocin-ageing research that has shown that intranasal oxytocin administration improved older adults' ability to postulate what others' are thinking (theory of mind) (Grainger et al., 2018b) and increased older males' attention to feelings (an aspect of meta-mood) (Ebner et al., 2015) and facial emotion recognition accuracy (Campbell et al., 2014). However, given the issues with intranasal oxytocin research lacking reproducibility (Leng & Ludwig, 2016), which is discussed in greater detail in the next section, these effects still need to be confirmed. Indeed, much more research is needed to confirm the trajectory of the oxytocin system in ageing, in particular direct testing of the central oxytocin system and the effects of chronic administration of oxytocin.

However, the oxytocin system maintaining function in ageing, as may be suggested by the results of the current thesis, fits with a recent allostatic theory of oxytocin (Quintana & Guastella, 2020). Quintana and Guastella (2020) propose that the oxytocin system is not only homeostatic (i.e., responds post hoc to changes within the system) but is also allostatic, in that it can predict and prepare for future events. Thus, it is plausible that the oxytocin system adapts to the foreseeably age-related decline of other biological functions, such as neurological degeneration, and because of this adaption is able to maintain functioning in an

environment of decline. This may be most relevant to the findings from this thesis where the cohort of older adults was particularly healthy and high functioning.

Conversely, it may be that the oxytocin system is able to maintain oxytocin secretion, but is impaired in other ways in older adults. For example, while concentration levels may remain stable, if oxytocin receptors were to diminish, this would result in decreased functioning of the system. An age-related loss of oxytocin receptors, despite concentration levels remaining stable with age, would fit with the ageing trajectories that we observed for testosterone and estradiol. One of the reasons that oxytocin has been expected to decline with ageing is the evidence that sex hormones decline with age, and the positive association between sex hormones and the oxytocin system (Arsenijevic & Tribollet, 1998; de Kloet et al., 1986; Gordon et al., 2017). However, it is estradiol, more so than testosterone that is linked with oxytocin production. The findings from this thesis suggest that of the sex hormones only testosterone declines significantly with age, whereas estradiol remains stable. Note, however, that this finding is inconsistent with observed declines in females' estrogen levels post-menopause (Eskes & Haanen, 2007; Horstman, Dillon, Urban, & Sheffield-Moore, 2012). Nevertheless, estradiol remaining stable in our cohort is therefore consistent with oxytocin concentration levels remaining stable. In contrast, age-related decline of testosterone levels is linked to loss of central oxytocin receptors in aged rats (Arsenijevic & Tribollet, 1998), rather than oxytocin production (Goudsmit et al., 1988). Since the older adults' in our study had significantly lower testosterone levels than the young adults, it could be that intranasal oxytocin did not improve the older adults' emotion recognition accuracy they lacked central oxytocin receptors and not oxytocin concentration levels

As highlighted in the introductory chapters of this thesis (Chapter 4), it is important to note the limitation of this thesis, and oxytocin research more generally, of not being able to directly test the central oxytocin system. Although this thesis reports on older adults'

peripheral oxytocin concentration levels, this research could not examine central oxytocin concentration levels, and as such central oxytocin levels may still decline with age despite intranasal oxytocin not modulating older adults' facial emotion recognition accuracy. Although the findings of this thesis do not implicate the oxytocin system in older adults' emotion recognition difficulties, the findings also do not eliminate the oxytocin system as a potential mechanism behind older adults' facial emotion recognition difficulties. Especially since there is evidence that intranasal oxytocin does influence older adults' neural activation whilst completing a facial emotion recognition task using magnetic resonance imaging (MRI; Horta et al., 2019), and also modulates other social cognitive behaviours, for example, see Ebner et al. (2015). Thus future research should continue to interrogate oxytocin as a possible mechanism behind older adults' emotion recognition difficulties, especially in studies testing the effects of chronic oxytocin administration on emotion recognition behaviour and related brain functions.

8.4 More Oxytocin-Ageing Research is Needed

The null effects reported in this thesis in testing the effects of intranasal oxytocin on older adults' facial emotion recognition accuracy, add to the growing body of evidence that oxytocin effects lack reproducibility. Despite Campbell et al. (2014) reporting a clear oxytocin effect for older men, the current well-designed (repeated measures) and highly powered study was unable to reproduce this finding. In the initial years of oxytocin nasal spray research, the effects produced by intranasal oxytocin were very promising and intranasal oxytocin was being put forward as a possible intervention for people with social cognitive difficulties, such as those with autism. However, more recently, the findings from studies examining the effects of intranasal oxytocin on social cognitive performance, have been mixed, with many studies reporting null effects (Leppanen et al., 2017). A recent study by Tabak et al. (2019), for example, reported null effects (evidenced by Bayesian statistics)

for social processes such as empathetic concern, social working memory, deception detection, trustworthiness perception, and expressed empathy in healthy adults. Results such as these have led to intranasal oxytocin effects being critiqued as lacking reproducibility and being underpowered (Leng & Ludwig, 2016; Walum et al., 2016).

There are two main accounts for the inconsistent intranasal oxytocin findings across the literature. One perspective is that the initial effects were exaggerated and used statistical techniques that increased the magnitude and significance of the reported effect (Leng & Ludwig, 2016). From this perspective, it is argued that the oxytocin field of research is tainted by publication bias (Jurek & Neumann, 2018). That is, multiple studies have gone unpublished due to null effects, and the published effects have been biased towards analysing the data in a way that produced statistically significant, and thus publishable, results. From this perspective, the null oxytocin effects may represent the truer finding (Walum et al., 2016). However, Quintana (2018) rebutted this argument by using equivalence testing to show that a quarter of both published and unpublished null effects did not represent the oxytocin and placebo groups being equal. Rather the data lacked power and was not sensitive to oxytocin effects. Leppanen et al. (2017) also report in a meta-analysis of emotion recognition in healthy and clinical populations that the majority of the 33 studies included in the review lacked statistical power due to small sample sizes. Their power analysis indicated that studies need to have 64 participants per group for between-group designs and 34 participants for within-group designs (Leppanen et al., 2017). However, the study presented in the current thesis was well powered, with a total of 120 participants in a within-group design, yet still produced null effects. Therefore, the oxytocin null-effects reported in this thesis would be considered further evidence that intranasal oxytocin does not produce effects as strong or as significant as previously believed. Furthermore, with the exception of disgust, the Bayesian analysis revealed that there was moderate evidence in favour of a null-oxytocin

effect for older adults across all emotions. This finding therefore suggests that the null oxytocin effects were not due to Type II error.

The alternative perspective is that intranasal oxytocin does have effects on social cognition, but that these effects are context-dependent and are therefore only present for certain groups under certain conditions. From this perspective, the null oxytocin effects reported in this thesis do not indicate that intranasal oxytocin does not affect older adults' emotion recognition. Rather, that intranasal oxytocin did not affect this cohort of older adults on this specific task. For example, a recent study reported that adults with a history of childhood trauma demonstrated improved emotion recognition from intranasal oxytocin, but those without a trauma history did not (Schwaiger et al., 2019). As such Quintana and Woolley (2016) highlight that oxytocin research needs to go beyond simply reporting whether or not oxytocin had an effect and instead explore for whom, how, and where oxytocin has its effects. In the current thesis, the factors of age, sex, personality (BFI personality domains), trait anxiety, and autistic traits were explored. However, only age and sex appeared to have any impact on oxytocin effects, and this was minimal (i.e., a very small oxytocin effect emerged for young females' sadness recognition), perhaps relating to the potentially healthy and high functioning nature of the cohort studied in this thesis.

The null oxytocin effects presented in this thesis could also be explained by intranasal oxytocin modulating social processes indirectly via the periphery. In a report by Leng and Ludwig (2016), they critique the viability of oxytocin nasal spray as a social cognitive intervention by highlighting the lack of knowledge about how intranasal oxytocin exerts effects on social processes. Social processing is a cognitive skill and as such is a central function. Yet, as detailed by Leng and Ludwig (2016), the majority of intranasally administered oxytocin enters the periphery, with only small amounts crossing the blood-brain barrier to the central system. Leng and Ludwig (2016) evidence the known effects of

intranasal oxytocin on the periphery by citing its historic use in childbirth. In current practice, intranasal oxytocin is used to increase lactation. In a rebuttal paper, Quintana and Woolley (2016) agreed that there is a current lack of understanding in terms of how intranasal oxytocin affects social processes. However, they add that the evidence remains that it does affect social cognitive processes, which are a function of the central system (Quintana & Woolley, 2016), especially since studies using functional MRI in other populations (e.g., anxiety disorders and neurodegenerative disease) report significant changes in brain function during social cognitive processes following intranasal oxytocin (Labuschagne et al., 2010; Labuschagne et al., 2018). Thus, it may be that the small, but functionally significant, amount of intranasal oxytocin that does cross the blood-brain barrier (Landgraf & Neumann, 2004; Neumann & Landgraf, 2012) has a central effect. However, it may also be that the large amounts of intranasal oxytocin that circulate the periphery (Jurek & Neumann, 2018) have indirect effects on central processes.

Although central and peripheral oxytocin secretion are unique processes (Ludwig & Leng, 2006), it is unclear if the effect intranasal oxytocin has on social processing is purely due to the oxytocin that crosses the blood-brain barrier, or is in part because of feedback from receptors within the periphery (Quintana et al., 2015a). Intranasal oxytocin may not selectively influence target behaviours. Rather, broader physiological effects, such as the anxiolytic effect of oxytocin (MacDonald & Feifel, 2014), may drive the improved task performance (Churchland & Winkielman, 2012). Indeed, oxytocin administered intravenously, which is not considered to cross the blood-brain barrier, has been shown to improve social memory in adults with autism (Hollander et al., 2007). However, see also Quintana et al. (2016) reporting that intranasal oxytocin influenced amygdala activation in response to emotional faces but intravenous oxytocin did not. If the effects of intranasal oxytocin are primarily due to indirect peripheral effects, this may account for why the older

adults in the study presented in the current thesis did not benefit from the intranasal oxytocin, as they maintained peripheral oxytocin concentration levels.

At this stage, intranasal oxytocin lacks evidence as a viable intervention for older adults' social cognitive difficulties. However, intranasal oxytocin, and the study of the oxytocin system more generally, is still an emerging field. It appears that intranasal oxytocin effects are context and person dependent (Bartz et al., 2011). Thus, future research examining the effects of intranasal oxytocin need to not only report if an effect was present, and report null findings, but also examine moderating variables. It is therefore important that future research use sample sizes with enough power (Leppanen et al., 2017) to examine group differences. Ideally, a more direct (and isolated) way of testing the central system would be preferable. Although MRI provides indirect evidence of oxytocin's central effects, research is advancing with developments of tracers used in PET to trace oxytocin's behaviour in the brain (Smith et al., 2012; Smith et al., 2013). There is also a need for chronic studies that administer multiple doses of oxytocin over a period of time, which is discussed further in the 'Limitations' section.

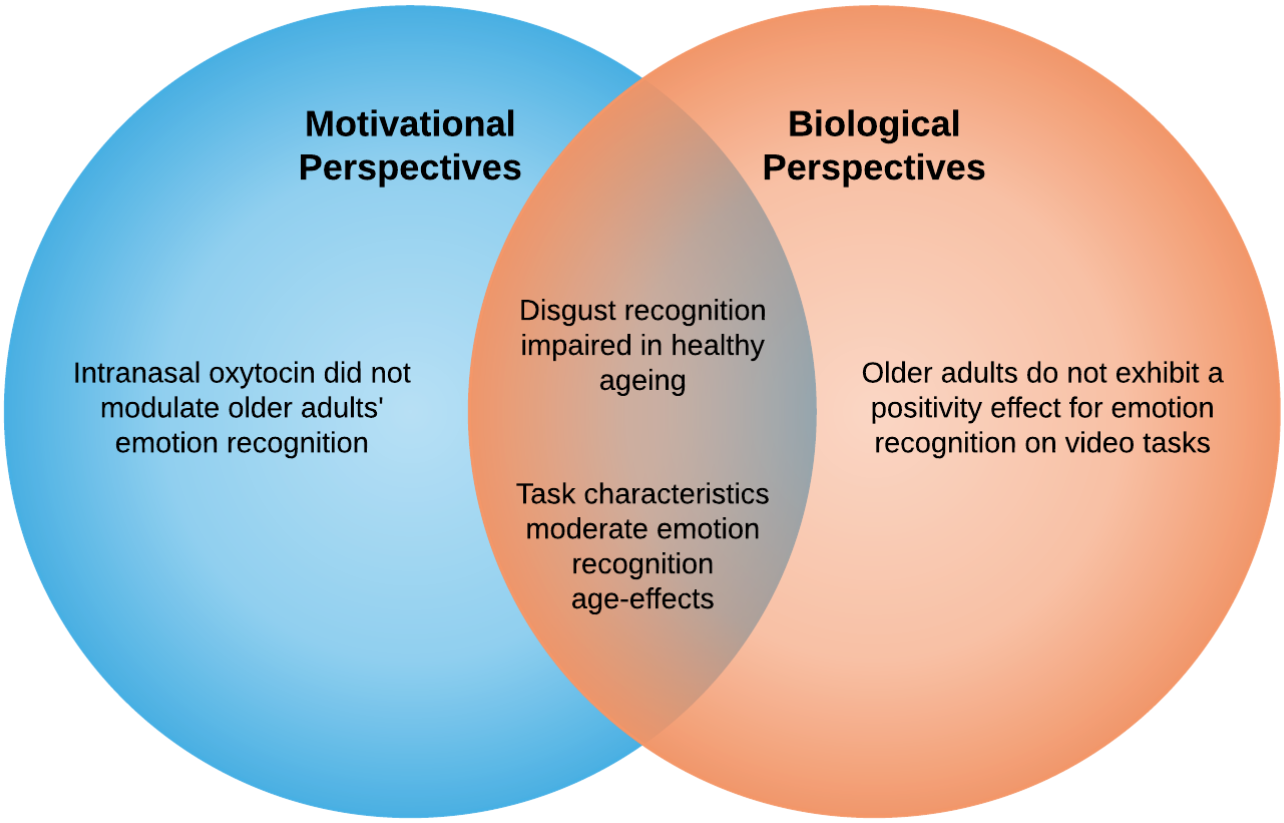
8.5 Theoretical Implications

This section will firstly discuss the theoretical implications relating to the outcomes from the meta-analysis (Chapter 3) and then a discussion of the theoretical implication of the oxytocin findings (Chapters 6 and 7). Overall, this thesis provides evidence for and against both motivational and biological models of facial emotion recognition and ageing; see Figure 8.2. Firstly, age-related difficulty recognising disgust (see meta-analysis; Chapter 3), rather than disgust recognition being retained in ageing (Ruffman et al., 2008), is a better fit for both motivation and biological perspectives. Although, it is unclear which unique feature of the POFA stimuli provides older adults with an advantage that is not present for alternative facial emotion recognition stimuli, from both motivational and biologicals perspectives,

retention of disgust recognition in healthy ageing has been difficult to account for. Motivational theory is used to account for the positivity effect (Reed & Carstensen, 2012), a phenomenon whereby older adults demonstrate a preference for and greater accuracy recognising positive stimuli versus negative stimuli (Reed et al., 2014). It is thought that older adults' motivation to improve their current experience and reduce the load on their limited cognitive resources are reasons why the positivity effect occurs, with positive stimuli typically being easier to recognise and more pleasant to attend to. However, the retention of disgust recognition in healthy ageing (Ruffman et al., 2008) never fit well with the positivity effect, as disgust is typically a negative stimulus in the populations studied. From the biological perspective, age-related retention of disgust recognition was argued to be due to the selective preservation of the basal ganglia and insula (Calder et al., 2003; Williams et al., 2006). However, with growing evidence that these brain regions are also susceptible to age-related degeneration (Persson et al., 2014; Raz et al., 2010), this argument is somewhat tenuous. Thus, the finding from this thesis of there being an age-related difficulty recognising disgust better fits with the current theoretical understanding of facial emotion recognition decline in healthy ageing.

Figure 8.2

Venn diagram depicting the key findings from this thesis and the theoretical perspective(s) that they support.



Note. Each finding has been placed under the model for which it is the best fit. For example, the finding that intranasal oxytocin did not modulate older adults' emotion recognition does not support the biological perspective and therefore could be considered to support motivational perspectives.

In terms of the moderating effect of task design, it is unclear if the findings of this thesis provide support for or against motivational and biological accounts. This is because the different task characteristics do not clearly map onto each model as discussed in Chapter 3. For example, videos being more realistic of everyday interactions than static photographs may mean older adults' are more motivated to engage in video-based tasks. However, videos place greater demands on the speed of processing than static photographs, which would instead suggest that older adults are less motivated to engage with videos as older adults are

also motivated to conserve cognitive resources. Thus, it is unclear how the finding that older adults demonstrate more consistent difficulties across emotions on videos compared to static photographs, links to motivational models, as it is unclear if videos are overall more or less motivating for older adults than static photographs. However, the finding that for videos, older adults also demonstrate difficulty recognising happiness, is difficult to account for from motivational perspectives because it does not fit with a positivity effect. Even though older adults are thought to be motivated to conserve cognitive resources, and as such may not allocate resources to demanding video-based tasks, they are still thought to allocated their limited cognitive resources to increase positive experiences, such as attending to a video of a happy person. Many of the null age-effects for happiness recognition reported across the literature, including the findings from the intranasal oxytocin study presented in this thesis, were hampered by ceiling effects. Thus, it appears that on more challenging tasks, such as video-based tasks, where ceiling effects do not hamper performance, that older adults do not demonstrate a positivity effect.

In relation to the oxytocin findings from this thesis, intranasal oxytocin not impacting older adults' facial emotion recognition performance seems to go against a biological theory. While previous research has suggested an oxytocin effect is present for older males' emotion recognition (Campbell et al., 2014), the study presented in the current thesis had substantially more power, to the extent that each emotion could be tested separately, and used repeated-measures design, which ensures groups are matched for ability. There are differing accounts for the effects of intranasal oxytocin; that it is a prosocial effect (oxytocin facilitates affiliation), that it is an anxiolytic effect (oxytocin reduces stress, which in turn increase prosocial behaviour), or that it is a social salience effect (oxytocin modulates the perception of social salience, which in turn increase prosocial behaviours towards in-group members, but potentially increases anti-social behaviours towards out-group members) (Shamay-Tsoory &

Abu-Akel, 2016). Given this thesis did not observe an oxytocin effect on facial emotion recognition in healthy ageing, it does not provide support for any of these accounts. However, it may be that the lack of effect can be better understood from one account more than the others. A null-oxytocin effect is difficult to account for from the prosocial account, as facial emotion recognition would be expected to increase for all participants if their prosocial behaviours were increased. From the anxiolytic account, a null-oxytocin effect for older adults could be accounted for by the older adults in our sample having significantly lower trait anxious (as per the STAI) than the young adults. However, it is worth noting that the results indicated that trait anxiety levels were not a significant moderator of oxytocin effects for any of the emotions. From the social salience account, one could argue that oxytocin may have increased attention and accuracy for recognising emotion of same-age posers, but decrease it for different-age posers. Thus, a null oxytocin effect could be accounted for by stimuli in this thesis comprising 50% older adult posers and 50% young adult posers.

While the findings of this thesis do not implicate the oxytocin system in older adults' emotion recognition difficulties, the findings also do not entirely eliminate the oxytocin system as a potential mechanism behind older adults' facial emotion recognition difficulties. More direct testing of the central oxytocin system would be required to confirm this, including examining the effects of chronic administration of oxytocin, before firmer conclusions can be drawn. Alternatively, it may be that the oxytocin system is not a mechanism behind older adults' facial emotion recognition difficulties, but neurological degeneration or even interactions among neurotransmitter and neuropeptides systems are presenting a better biological mechanism behind the declines in emotion recognition in older adults.

8.6 Strengths and Limitations of the Research in this Thesis

Concerning the strengths of this thesis, all of the research presented in this thesis was well powered, comprehensive, and used current leading-edge statistics. The meta-analytic review included 81 studies, and the empirical study tested 120 participants in a within-group design, which allowed for explorations of moderating factors; task characteristics in the meta-analytic review, and participant characteristics in the empirical study. Moreover, the meta-analytic review utilised multi-level meta-analysis modelling and the empirical study incorporated Bayesian statistics, both of which are current analyses that are considered by some to superseded older methods. There has been a recent shift away from sub-group comparisons within meta-analyses to multi-level modelling methods. Sub-group comparisons are restricted to two groups (e.g., videos and full-intensity photographs), whereas multi-level modelling is a complex statistical procedure that allows for comparison of more than two groups within the same analysis (e.g., videos, full-intensity photographs, reduced-intensity photographs, and morphed animations) due to being able to control for levels of dependence (Van den Noortgate, Lopez-Lopez, Marin-Martinez, & Sanchez-Meca, 2013). Secondly, significance testing using *p*-values can be used to accept an alternative hypothesis (i.e., that the observed effect is true and unlikely due to Type I error), but cannot be used to accept null hypotheses (i.e., that the null-effect is true and unlikely due to Type II error) (Dienes, 2016). Bayesian statistics are becoming a common way to test the strength of evidence within the data in support of *either* the null hypothesis or alternative hypothesis (Stern, 2016). Thus, the use of Bayesian ANOVA in the current thesis to test the level of evidence for null age/sex/oxytocin effects indicated whether the null-effects reported were more likely to be Type-II error or a true absence of an effect.

To achieve the level of detail demonstrated in this thesis, the focus was restricted to facial emotion recognition. Yet, facial emotion recognition is a component of emotion

recognition, and emotion recognition is only one social cognitive process. It is therefore unclear if the outcome of this thesis can be generalised to social cognition in ageing more broadly, or are unique to facial emotion recognition. Indeed, emotion recognition has been categorised as a low-level perceptual process, and as such may lack inference to social cognitive processes such as theory of mind, which involve higher-level integration of social information (Mitchell & Phillips, 2015). Thus, future research could further investigate the modulating effects, and moderating factors, of intranasal oxytocin on older adults' performance for other social cognitive skills given evidence of this in young adults; for example, oxytocin improves social memory (Guastella et al., 2008; Rimmele et al., 2009), theory of mind (Domes et al., 2007; Sun et al., 2020), social understanding (Kéri & Benedek, 2009), and increases in-group trust (Van IJzendoorn & Bakermans-Kranenburg, 2012) in young adults.

A further point regards the comprehensiveness of this thesis is the examination of both central and peripheral components of the oxytocin system in ageing. While it is the central system that is speculated to be implicated in older adults' facial emotion recognition difficulties, examining one part of a complex system can be limiting. Given the lack of knowledge about the oxytocin system as a whole in healthy ageing (Ebner et al., 2013; Huffmeijer et al., 2013), and the possibility that intranasal effects on central processes are an indirect peripheral effect (Churchland & Winkielman, 2012), it was important to also examine peripheral oxytocin in our older adult cohort. Notably, there is a trend for researchers to take this exploration further and draw links between peripheral oxytocin concentration levels and cognitive abilities (Hayashi et al., 2019; Mutu Pek et al., 2019; Plasencia et al., 2019; Strauss et al., 2015; Unti et al., 2018). However, we were cautious to make such connections between peripheral levels and emotion recognition, due to the growing evidence that peripheral oxytocin concentration levels do not infer central oxytocin

concentration, with central and peripheral oxytocin secretion being two distinct processes that are not coordinated (Kagerbauer et al., 2013; Lefevre et al., 2017; Ludwig & Leng, 2006).

There are some broader limitations of this thesis worth noting. This thesis examined the effects of a single dose of intranasal oxytocin, yet there is evidence that social behavioural effects can emerge for chronic oxytocin administration that are not present when only a single dose is given (Teng et al., 2013). It may be that a single dose is enough to observe differences in older adults' brain activation when labelling facial expressions of emotion (Horta et al., 2019), but that multiple doses are required to observe behavioural change. To truly investigate intranasal oxytocin as a means of changing psychopathology, future oxytocin and ageing research needs to investigate the effects of multi-dose intranasal oxytocin. Research investigating chronic oxytocin administration as a treatment for social cognitive impairments has been conducted in several clinical groups (i.e., autism, schizophrenia, obsessive-compulsive disorder, and one study on dementia) (Horta, Kaylor, Feifel, & Ebner, 2020). Within these clinical group studies, the effects of chronic oxytocin administration on social cognitive skills have been mixed. For ASD, chronic oxytocin administration has been shown to improve theory of mind (Anagnostou et al., 2012), social communication (Tachibana et al., 2013), and increase social interactions/social reciprocity (Munesue et al., 2016; Watanabe et al., 2015). However, other studies have reported no effects (e.g., Dadds et al., 2014; Guastella et al., 2015a). For schizophrenia, some studies report that chronic oxytocin administration with adjunct therapy showed a reduction in positive and/or negative symptomology (Feifel et al., 2010; Gibson et al., 2014; Jarskog et al., 2017; Ota, Yoshida, Nakata, Yada, & Kunugi, 2018), but other studies report no significant reduction of symptoms (Bradley & Woolley, 2017; Buchanan et al., 2017; Dagani et al., 2016). More research is clearly still needed that clarifies the most effective course of chronic oxytocin administration, which may differ across clinical groups and/or individuals (Horta et al., 2020). Yet to date there has been no

research on the effects of chronic oxytocin administration on healthy older adults' social cognition, so this first step is required.

An additional limitation is that there are emerging potential moderators of oxytocin that were not included in this thesis. For example, there is evidence to show that individual variability, such as childhood adversity and socio-economic status, moderate intranasal oxytocin effects on emotion recognition theory of mind respectively (Schwaiger et al., 2019; Sun et al., 2020). Despite this current thesis considering some moderating variables (i.e., age, sex, trait anxiety, autistic traits, and personality traits) assessments of childhood adversity and socio-economic status were not included. Thus, there are grounds for these assessments to be included in future intranasal oxytocin research as potential moderators.

Moreover, there is evidence that oxytocin effects are moderated by participant skill and item difficulty (Bartz et al., 2011; Domes et al., 2007; Guastella et al., 2010), yet item difficulty was unable to be explored systematically in this thesis. Throughout this thesis, the impact of task difficulty has been discussed in relation to the design of the facial emotion recognition task (e.g., the stimulus format used), performance across emotions (e.g., sadness being the most difficult emotion for participants to recognise), and the skill level of the groups (e.g., younger adults demonstrating superior performance to older adults). However, we were unable to analyse and separate task items based on difficulty, as per Domes et al. (2007). This is because we used a repeated measure design, and as such used two slightly different variations of the task to reduce practice effects. Thus, participants did not view the exact same items in the oxytocin condition as they did the placebo session. Note, that counterbalancing ensured that any marginal difference in difficulty between the two versions was accounted for. While the decision to use repeated measures design prevented the analysis of individual task items, it did provide sufficient power for each emotion to be analysed separately. Analysing each emotion separately was considered to be important given that

much of the difference in item difficulty occurs across emotion types (i.e., some emotions are more difficult to recognise than others) than within emotion types.

A final limitation of this thesis is that the population being investigated was *healthy* older adults. Moreover, the sample of older adults included in this thesis was highly educated and high functioning. Thus, the results cannot be inferred to older adults with any level of cognitive impairment. Due to screen measures that we employed, the sample also had no history of heart disease, neurological or psychological illness, and were not smokers. Perhaps a decline in peripheral oxytocin or vasopressin would, therefore, be observed in older adults with some ill health (e.g. cardiovascular diseases). Future research could include older adults with a broader spectrum of cognitive function and health, to identify if oxytocin levels and/or effects are more or less present for older adults with mild cognitive impairments or poorer health outcomes.

8.7 Conclusions

In conclusion, this thesis confirms that older adults have difficulty recognising facial expressions of emotion. Moreover, this thesis provides evidence that these difficulties are more universal across emotion types than previously believed. In particular, this thesis implicates disgust recognition, and possibly even happiness recognition, in ageing deficits. The clinical trial presented in this thesis indicates that a single dose of intranasal oxytocin (24 IU) does not modulate older adults' emotion recognition accuracy. This null effect emerged despite the study being well powered to detect drug effects, and remained present even after investigating potential moderating characteristics; sex, personality traits, trait anxiety, and autistic traits. However, given the evidence that oxytocin effects are context-dependent, it may be that there are moderators that were not explored in the current thesis that reveal for which participants, and under what conditions, oxytocin effects emerge for facial emotion recognition. This avenue of inquiry is essential given the other key finding from this thesis,

that task characteristics moderate older adults' facial emotion recognition performance. It may, therefore, be that intranasal oxytocin only modulates older adults' facial emotion recognition accuracy on certain facial emotion recognition tasks, due to either the level of difficulty of the task or the neural regions activated by the task design. Oxytocin and ageing research is still an emerging field. While this thesis does not provide conclusive evidence for or against oxytocin playing a role in older adults' emotion recognition deficits, it makes important contributions to the growing foundation of knowledge, which will serve to inform future research.

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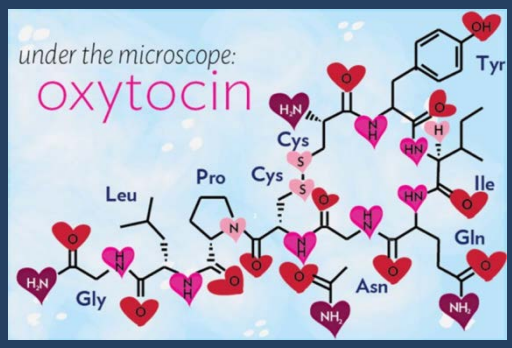
Appendices

Appendix A – Study Material

Appendix A – 1. Advertising flyer for young adults

Can you be part of our research study?

The Cognition & Emotion Research Centre at Australian Catholic University (ACU) is looking for looking for HEALTHY YOUNG ADULTS to be part of their research.



www.facebook.com/OXYSTUDY/

How long will it take?
2 sessions, each about 2½ - 3 hours

Location: ACU campus in Fitzroy

You can participate if you:
- Are aged 18-30 years
- Have no psychiatric/ neurological illness
- English first language

What will I have to do?
- Answer questionnaires
- Complete computer activities
- Provide saliva and mouth cell samples
- Administer a nasal spray containing **oxytocin** or placebo

What is oxytocin?
- A natural occurring hormone in the body
- Known to play a role in social behaviours
- Also called the 'love hormone' that promotes bonding

Participation is voluntary and you can withdraw at any time.

We provide a small financial reimbursement for your time and travel.

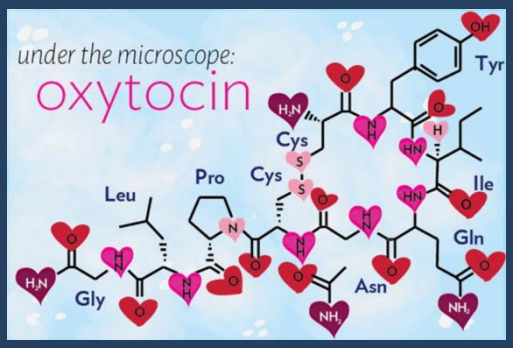
This research has been granted ethics approval HREC No.2 015-181H

Contact:
grace.hayes3@myacu.edu.au
T: (03) 9230 8131

Appendix A – 2. Advertising flyer for older adults

Can you be part of our research study?

The Cognition & Emotion Research Centre at Australian Catholic University (ACU) is looking for healthy older adults to be part of their research.



www.facebook.com/OXYSTUDY/

How long will it take?
2 sessions, each about 2½ - 3 hours

Location: ACU campus in Fitzroy

You can participate if you:
- Are aged 65-90 years
- Have no psychiatric/ neurological illness
- English first language

What will I have to do?
- Answer questionnaires
- Complete computer activities
- Provide saliva and mouth cell samples
- Administer a nasal spray containing **oxytocin** or placebo

What is oxytocin?
- A natural occurring hormone in the body
- Known to play a role in social behaviours
- Also called the 'love hormone' that promotes bonding

Participation is voluntary and you can withdraw at any time.

We provide a small financial reimbursement for your time and travel.

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Contact:
grace.hayes3@myacu.edu.au
T: (03) 9230 8131

Appendix A – 3. Expression of interest form

PROJECT TITLE: The effects of intranasal oxytocin on social cognitive performance in ageing
LOCATION: 115 Victoria Parade, Fitzroy VIC 3065
PHONE: 03 9230 8131 or 0490 061 534 **EMAIL:** grace.hayes3@myacu.edu.au

Investigators:	**Research Officers:**	**Student Investigators:**
Professor Peter Rendell	Ms Natalie De Bono	Ms Grace Hayes
Dr Izelle Labuschagne		Ms Vanessa Curmi
Dr Skye McLennan		
Professor Gill Terrett		

INVITATION TO PARTICIPATE

Dear Potential Participant,

You are invited to take part in an exciting research project being carried out by Professor Peter Rendell's research centre in the School of Psychology, Australian Catholic University (ACU).

The research project aims to assess whether various social and cognitive skills known to change as people get older may be improved by administration of a natural occurring hormone called oxytocin. Recent evidence suggests that oxytocin is particularly important for everyday social and emotional behaviours including our ability to recognise emotions and read the intentions of others, as well as stress and anxiety. This research will provide answers to questions such as whether hormonal levels change as people get older, and whether this change may relate to changes in social and emotional behaviours.

If you are aged **60-85 years** or **18-35 years**, we invite you to take part in this exciting research project.

The study involves the use of a nasal spray that contains one dose of oxytocin or one dose of saline solution (salt solution).

Testing will include:

- Collection of a sample of your saliva
- One dose of a nasal spray of either oxytocin or a saline solution (salt solution)
- Filling out questionnaires where you will have to look at photos and videos of people and guess how those people might be feeling

Yours Sincerely,

Professor Peter Rendell
Principal Supervisor

Expression of interest to participate

I ..... am interested in hearing more about the research projects being conducted by Cognition and Emotion Research Centre, and I agree to be contacted by telephone for this purpose. I understand that I don't have to decide whether to take part until I have been provided with more detailed information.

Signature: .....

Date of birth: ..... Phone: .....

Professor Peter Rendell
Tel: 03 9953 3126 **Fax:** 03 9953 3205
Email: peter.rendell@acu.edu.au **Web:** www.acu.edu.au

Australian Catholic University Limited, ABN 15 050 192 660
Melbourne Campus,115 Victoria Parade Fitzroy Vic 3065.

Locked Bag 4115 Fitzroy MCD VIC 3065 Australia
CRICOS registered provider: 00004G, 00112C, 00873F,088

Appendix A – 4. Phone screening script

Participants – Initial Phone Call

STUDY MOBILE NUMBER: 0490061534

1. Greeting and Introductions
   a. Collect Participant name_____
2. How did you hear about the study?
3. Discuss study

What is the research study about? We want to know if people's social and cognitive abilities change as they age, and if these abilities can be improved by administration of a natural occurring hormone called oxytocin. *Oxytocin* is a hormone that is produced in the brain and is best known for its role in pregnancy, i.e., commonly administered to pregnant women to induce labour. Our research is not interested in the pregnancy effects of oxytocin, but instead in the role that oxytocin plays in our everyday social and emotional behaviours. Recent evidence has suggested that oxytocin is particularly important for everyday social and emotional behaviours including our abilities to recognise emotions and read the intentions of others, as well as stress and anxiety. This research has not been conducted with an Australian sample before and will provide answers to questions such as whether hormonal levels change as people get older, and whether this change may relate to changes in social and emotional behaviours.

Who are the researchers conducting the study? My fellow researchers are Master of Psychology students at Australian Catholic University, supervised by experienced academic research staff. Would you like to know more about any of the researchers involved in this study? IF YES: This project is being supervised by Professor Peter Rendell, Dr Izelle Labuschagne, and Professor Gill Terrett, researchers and lecturers in the School of Psychology at Australian Catholic University (ACU). Researchers have conducted multiple trials in the fields of oxytocin (Dr. Izelle Labuschagne) and in general ageing (Prof Peter Rendell). Mrs Grace Hayes is student conducting the project under the supervision of these supervisors for her PhD. Various other student researchers have and will continue to help conduct the project as part of their Master of Psychology (Clinical) degree at ACU also under the supervision of Prof Peter Rendell and Dr Izelle Labuschagn..

Confidentiality: All personal information gathered during this research project will remain confidential and secure. All participants will be given a code number and names will not be retained with the data. Individual participants will not be able to be identified in any report of the study, as only aggregate data will be reported.

4. Determine Age

Young Participant (18-30 years old)	Older Participant (65-90)

5. Exclusion Criteria (Ask these 3 at a time, to protect confidentiality):

Neurological illness	
Psychiatric illness	
Substance abuse	
Antipsychotic or antidepressant medication	
Currently taking hormone supplements	
Heart Disease or problematic cardiac history	
Current smoker	
Pregnant	
Current cold or flu symptoms	
English is not first language	
** Older adults – Fail TICS	

→ *If they meet exclusion criteria --- "Unfortunately the requirements of taking part in this study are extremely strict. It seems that you do not quite meet the requirements that we are looking for at this time. However I really appreciate you calling me today. If you would like I can take your details and put you on a list that allows you to stay updated with the latest ACU studies that are recruiting participants?

6. Thanks for answering those questions for me. The final thing to determine eligibility for this study, is to complete a short questionnaire that examines cognitive skills. This is an 11 question questionnaire that I can do with you now, do you consent to taking part in this final eligibility questionnaire?

Yes → administer TICS	No → 1) reassure that their answers will remain completely confidential and reassure that it will only take 4-5 minutes. 2) Ask if there is a more convenient time to do the questionnaire

7. You are eligible for the study and we would appreciate having you on board for this study. But before I ask for your contact details, I will like to tell about what participating in this study will involve:
   - You will be asked to participate in 2 sessions that will go for approximately 3 hours each
   - The sessions will be held at ACU, and be about 4 weeks apart.
   - You will be reimbursed for your time (with a $60 Coles-Myer gift card)

8. PROCEDURE: First, the researcher will collect a sample of your saliva into small storage tubes via a straw. After this, we will collect buccal cells (or mouth cells) sample using a cotton swab on the inside of your cheek. Both the saliva and buccal cell samples will be used to examine the concentration levels of hormones such as oxytocin.

   Following this, you will be given one dose of a nasal spray of either oxytocin or a placebo (i.e., saline solution) and you will be asked to wait 40 minutes for it to take effect. You can bring a book to read or something to do in this time. After 40 minutes you will be asked to complete several tasks where you will have to look at photos and videos of people and guess how those people might be feeling. These tasks are designed to measure aspects of social cognitive function. Finally, you will be asked to complete a questionnaire related to mood and general quality of life.

   During the test sessions, you will be encouraged to take breaks between tasks as needed. Water will be provided, feel free to bring some sugar free biscuits or snack.

   You will also be asked to take home series of questionnaires that ask you questions about your life and social interactions.

9. Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences.

10. Some questions will be personal and about your medical history. Everything you report to us will be kept entirely confidential in accordance with research guidelines. However if at any time you feel uncomfortable with answering questions, you can just let the researcher know.

11. Do you have any questions about any of the information I have told you today? YES/NO

12. If all of the information we have spoken about today sounds okay and if you have no more questions, at this point I would like to formally as you if you would like to participate in this research study examining the effects of oxytocin on ageing? YES/NO

13. Great! That is very exciting to hear. Now if it's okay with you, I would like to send out an initial package for the study that includes information about the study, and some questionnaires that we would like you to fill out and bring to our first session. I just need to get some contact details of you.

Phone Number
Mobile:_____
Home:_____

Email Address:_____

Residential Address:_____

Is your residential address the same as your postal address: YES / NO

Postal Address:_____

First session booked on:
DATE_____
TIME_____
LOCATION_____

1. Please remember to bring your glasses or contact lenses if you require them.
2. Additionally, in order for us to get a good sample of your saliva, we ask if participants do not eat a major meal within 60 minutes of attending the session.
3. Please also limit you caffeine intake on the day of the session to 1 cup of tea or coffee and not in the 3 hours preceding the session
4. Please call us if you have a cold on the day of testing, we may have to reschedule as cold symptoms (such as a blocked nose) can interfere with the administration of the nasal spray.

Looking forward to meeting with you.

Admin duties
1. Assign participant a participant number 2. Add booking to room booking Calendar 3. Let Jennie know that a new booking has been made so that she can order oxytocin 4. Write participant on oxytocin counterbalancing list 5. Send participant an email with data and time of their session, location of session (include map of campus), information letter and the 4 points above (i.e., not to eat beforehand)

Appendix A – 5. Participant information letter

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Cognition & Emotion Research Centre

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: The Effects of Oxytocin on Ageing
PRINCIPAL INVESTIGATOR: Prof Peter Rendell
CO-INVESTIGATORS: Dr Izelle Labuschagne, Dr Skye McLennan, Associate Prof. Gill Terrett
EXTERNAL INVESTIGATOR: Professor Markus Heinrichs, University of Freiburg, Germany
STUDENT RESEARCHER: Ms Casandra Murphy; Ms Rebecca Gilchrist; Ms Grace Hayes; Ms Nicole Kearns; Ms Vanessa Curmi
STUDENT'S DEGREE: Masters of Clinical/Educational and Developmental Psychology
ACU HREC REGISTER NUMBER: HREC 2015-181H

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project aims to assess whether various social and cognitive skills known to change as people get older may be improved by administration of a natural occurring hormone called oxytocin.

Oxytocin is a hormone that is produced in the brain and best known for its role in pregnancy, i.e., commonly administered to pregnant women to induce labour. Our research is not interested in the pregnancy effects of oxytocin, but instead in the role that oxytocin plays in our everyday social and emotional behaviours. Recent evidence has suggested that oxytocin is particularly important for everyday social and emotional behaviours including our abilities to recognise emotions and read the intentions of others, as well as stress and anxiety.

The research will include healthy young (aged 18-30) and older participants (aged 65-90).

This research is novel and will provide answers to questions such as whether hormonal levels change as people get older, and whether this change may relate to changes in social and emotional behaviours.

Who is undertaking the project?
This project is being supervised by **Professor Peter Rendell**, **Dr Izelle Labuschagne**, and **Dr Skye McLennan**, researchers and lecturers in the Cognition and Emotion Research Centre in the School of Psychology at Australian Catholic University (ACU). Researchers have conducted multiple trials in the fields of oxytocin (Dr Labuschagne) and in general ageing (Prof Rendell and Dr McLennan). Four student researchers, **Ms Cassandra Murphy, Ms Grace Hayes, Ms Nicole Kearns** and **Ms Rebecca Gilchrist**, will be conducting the project. These students are carrying out this research as part of their Master of Psychology (Clinical) degree at ACU under the supervision of Prof Rendell, Dr Labuschagne, and Dr McLennan.

What will I be asked to do?
Initially, you will be asked some questions over the phone to ensure that you are eligible to participate in the study. This will involve some questions about your medical history. If you are eligible, you will be asked to participate in two sessions (each session will take approximately 3 hours) which will be held at mutually convenient times and locations, approximately 4 weeks apart. Additionally, researchers will ask for your consent in order to send out an information pack that includes information about the study, and several questionnaires for the study. You will be asked to complete this questionnaire pack and bring along to your first session.

During the first session, the student researcher will record your answers on paper and some answers will be audio recorded for scoring purposes. Then the researcher will collect a sample of your saliva into small storage tubes via a straw. We will then collect buccal cells (or mouth cells). Buccal cells will require using a

AUSTRALIAN CATHOLIC UNIVERSITY

Cognition & Emotion Research Centre

cotton swab to collect the cells on the inside of a person's cheek. Saliva and buccal cell samples will be used to examine the concentration hormones such as oxytocin.

Following this, you will be given one dose of a nasal spray of either oxytocin or a placebo (i.e., saline solution) and you will be asked to wait 40 minutes for it to take effect. After 40 minutes you will be asked to complete several tasks where you will have to look at photos and videos of people and guess how those people might be feeling. These tasks are designed to measure aspects of social cognitive function. Finally, you will be asked to complete questionnaires related to mood and general quality of life.

During the test sessions, you will be encouraged to take breaks between tasks as needed. Refreshments will be provided. If you have a partner who is willing to participate in the study, the researchers can organise a mutually convenient time for them to take part in the same tasks.

Are there any risks associated with participating in this project?
During the two sessions, you will be asked to complete several questionnaires and tasks that will measure your social cognitive function. These tests are relatively easy, will take no longer than one hour to complete, and will pose no risk.

We anticipate that the collection of saliva samples will produce minimal risk. You will collect the saliva yourself and the researcher will guide you through the process. This will be done using small tubes and the 'passive drool method', after which you will place the tubes containing saliva in a bag and hand this to the researcher.

The administration of oxytocin and placebo nasal sprays also poses minimal risk. The placebo spray consists of a saline solution. The oxytocin spray consists of a concentration of synthetic oxytocin diluted in a saline solution. We and other researchers have conducted several studies with intranasal oxytocin over the past years in human studies, and have found it to be safe and well tolerated. To date, hundreds of studies have been published that use intranasal oxytocin and no adverse effects have been reported. We are also administering a very small amount of oxytocin that is significantly less than what is commonly administered to pregnant women during labour.

In the unlikely event that you become unwell during a session, the research staff will assist you in obtaining appropriate medical treatment. If you feel unwell after participating in the study and the symptoms you are experiencing are uncommon or of particular concern to you, then please seek medical assistance. You may take this information sheet with you so that you can inform the doctor of procedures that you were involved in. ACU will not be responsible for medical costs.

How much time will the project take?
The research will involve two individual testing sessions lasting approximately 3 hours.

What are the benefits of the research project?
Participants will be financially reimbursed for participating in this research at a rate of $10 per hour (in the form of a $60 Coles-Myer gift card). There are no immediate benefits to participants. However, the study will provide the researchers with a better understanding of age-related social and emotional changes and will assist researchers in exploring the role of oxytocin in these changes.

Can I withdraw from the study?
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. If you are an ACU student, withdrawal from this study will in no way affect your ACU studies. On withdrawal, if participants would also like data to be removed, it will be removed.

Will anyone else know the results of the project?

All participants will be given a code and names will not be retained with the data. The student researchers will be reporting the findings in a thesis and we plan to also report the findings at a conference and/or in a scientific journal. It is emphasized that individual participants will not be able to be identified in any report of the study, as only aggregate data will be reported.

Will I be able to find out the results of the project?

Findings of the study will be made available to participants upon request.

Who do I contact if I have questions about the project?

Any questions regarding this project can be directed to the Principal Investigator: **Professor Peter Rendell** in the School of Psychology, St. Patrick's Campus (Australian Catholic University, Level 5, The Daniel Mannix Building, Young Street, Fitzroy 3065, phone 03 9953 3126).

What if I have a complaint or any concerns?

The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (review number 2014 145V). If you have any complaints or concerns about the conduct of the project, you may contact the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
North Sydney Campus
PO Box 968
NORTH SYDNEY, NSW 2059
Ph.: 02 9739 2519
Fax: 02 9739 2870
Email: res.ethics@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

Your support for the research project will be most appreciated.

Yours sincerely,

Rebecca Gilchrist Student Research	Professor Peter Rendell Principal Investigator	Dr Skye McLennan Co-investigator	Grace Hayes Student Researcher
Cassandra Murphy Student Researcher	Dr Izelle Labuschagne Co-investigator	Professor Markus Heinrichs External Investigator	Nicole Kearns Student Researcher
Vanessa Curmi Student Researcher	Associate Prof. Gill Terrett Co-investigator		

Appendix A – 6. Consent forms

ACU
AUSTRALIAN CATHOLIC UNIVERSITY

Cognition & Emotion Research Centre

CONSENT FORM
Copy for Participant to Keep

TITLE OF PROJECT: The Effects of Oxytocin on Ageing

PRINCIPAL INVESTIGATOR: Professor Peter Rendell

CO-INVESTIGATORS: Dr Izelle Labuschagne, Dr Skye McLennan, Associate Prof. Gill Terrett

STUDENT RESEARCHER: Ms Rebecca Gilchrist, Ms Cassandra Murphy, Ms Grace Hayes, Ms Nicole Kearns, Ms Vanessa Curmi

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in the activities as outlined in the information letter that involve completing two sessions, each session running approximately for two hours. The sessions will comprise of administering one of two nasal sprays, and completing several short questionnaires and computerised tasks on background information, mood and social cognitive function. I understand that some of the tasks that involve reading or saying aloud lists of words will be audio-taped as a backup for scoring. Additionally, the study involves providing saliva and buccal (mouth) samples for use in analysing physiological levels of relevant hormones such as oxytocin. I understand that I can withdraw my consent at any time (without adverse consequences). I agree that the research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT.....

SIGNATURE:..... DATE.....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):.....

DATE:.....

(and, if applicable)

SIGNATURE OF STUDENT RESEARCHER: ..... DATE:.....

Please tick this box if you would be interested in hearing about future research projects conducted by the Cognition and Emotion Research Centre at ACU ☐

Please tick this box if you are interested in hearing about the outcomes of the current study ☐

If you have ticked any of the boxes above, please provide your contact details below:

Address .....

Email .....

PH ..... DOB:.....

CONSENT FORM
Copy for Researcher

TITLE OF PROJECT: The Effects of Oxytocin on Ageing

PRINCIPAL INVESTIGATOR: Professor Peter Rendell

CO-INVESTIGATORS: Dr Izelle Labuschagne, Dr Skye McLennan, Associate Prof. Gill Terrett

STUDENT RESEARCHER: Ms Rebecca Gilchrist, Ms Cassandra Murphy, Ms Grace Hayes, Ms Nicole Kearns, Ms Vanessa Curmi

I ..... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in the activities as outlined in the information letter that involve completing two sessions, each session running approximately for two hours. The sessions will comprise of administering one of two nasal sprays, and completing several short questionnaires and computerised tasks on background information, mood and social cognitive function. I understand that some of the tasks that involve reading or saying aloud lists of words will be audio-taped as a backup for scoring. Additionally, the study involves providing saliva and buccal (mouth) samples for use in analysing physiological levels of relevant hormones such as oxytocin. I understand that I can withdraw my consent at any time (without adverse consequences). I agree that the research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT.....

SIGNATURE:..... DATE.....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):.....

DATE:.....

(and, if applicable)

SIGNATURE OF STUDENT RESEARCHER: ..... DATE:.....

Please tick this box if you would be interested in hearing about future research projects conducted by the Cognition and Emotion Research Centre at ACU ☐

Please tick this box if you are interested in hearing about the outcomes of the current study ☐

If you have ticked any of the boxes above, please provide your contact details below:

Address .....

Email .....

PH ..... DOB:.....

Appendix A – 7. Protocol instructions

1

Session A only: Mouth wash

"In about 10 minutes we will be doing the saliva collection, so to make sure you don't have any food in your mouth from breakfast or snacks I am going to give you some water. I want you to take a big sip, but don't swallow! I want you to swish it around your mouth as you would with mouth wash or maybe if you are at the dentist. I am going to tell you when you can swallow the water. Okay?"

Session A only: NART

Put folder in front of participant

Start voice recording

The following task is a word reading task. I just want you to know that I will be recording your responses. This is just so I can review your response with another researcher. On each page in this folder there is a word, I want you to read each word for me. I must warn you that there are many words that you probably won't recognise; in fact most people, including myself, don't know them, so just have guess at these, O.K. Go ahead.

STAI

Page with questions 1-20

"This next task is a simple mood scale. Here are a number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to her right of the statement to indicate how you <u>feel right now, that is, at this moment.</u> There is no right and wrong answers. Do not spend much time on any one statement but give the answer which seems to describe your present feelings best."

Session A only: Saliva sample collection

a) Give participant plastic tube and collection aid. Let participant open the collection aid and put the aid into the tube (with smooth part of the aid to the top)

"Please open the bag. Inside is a strawlike object. Please put it on top of the little container with the smooth part at the top."

b) Instruct participants to allow saliva to pool in their mouth. To help them do this, tell the participant to imagine slowly chewing their favourite food

2

"Now we are going to collect 1 ml of your saliva. First you need to let some saliva pool in your mouth. It might help to imagine you are eating your favourite food!"

c) Once they feel the saliva collecting under their tongue, instruct the participant to tilt their head forward and collect the saliva in the vial (rather than following the natural instinct of swallowing)

"Once you feel the saliva collection under your tongue, instead of swallowing it I want you to collect the saliva in the vial. Just let it drip in through the straw." [Can make a comment about it being a bit weird if they look uncomfortable] – "do NOT blow into the straw".

d) Instruct the participants to repeat the procedure until they reach the 1 ml mark on the tube.

e) Participant to remove the aid and replace the cap on the tube.

"When done, please remove the straw and put it on the second tube and put the lid back on the first tube. Now repeat the process with the second tube until you reach the 1ml line"

f) Repeat the process with the second tube until 1 ml of saliva is in the tube.

g) Ask the participant to put the cap on their tube/s and put the tubes in the ziplock bag, squeezing the air out.

"Now remove the straw and put it in this bin. Put the lid back on the second tube and place both tubes in this bag [hold open ziplock bag for them to place tube in]. You can use a tissue to clean up any saliva on your hands."

Session A only: Buccal cell collection

a) Remove cap from swab just before using, take care not to touch the swab with your fingers

b) Put soft end of swab in one side of participant's mouth and rub up and down 20 times against the inside of the cheek (A), then rub in the grooves (sulcus) next to the top and bottom gums on the same side for 10 s (B).

IF THERE IS BLOOD ON THE SWAB DISCARD IT IMMEDIATELY

3

c) Invert [hang from clip?] the swab and let is air dry for 15-20 minutes [or be VERY careful not to touch the side of the tube when putting the swab in]

d) Use the second swab for the other side, collecting a sample in the same way.

e) Invert [hang from clip?] the second swab and let is air dry for 15-20 minutes [or be VERY careful not to touch the side of the tube when putting the swab in]

f) Remove gloves and wipe hands with cleanser

OT ADMINISTRATION

Instructions for nasal spray administration:

Ask participant if they have used nasal sprays before?

Ask participant whether they feel like they need to blow their nose.

- Participants are allowed to blow their nose only before 1st puff; should avoid this for the following 20 min – they are only allowed to dab off leaking fluid or to suck it back in.

Let the participants practice the handling of the spray by letting the participant prime the spray bottle – this can be up to 1 full pre-puff in the corner of the room to ensure normal distribution of each bottle before use.

It takes a little coordination between administration and breathing.

- The order is always "**breathe normally- insert sprayhead - exhale - puff - inhale nasally."**

- To keep the bottle during puffing in an upright position. Sitting with head back is allowed. Holding the nose down is allowed.

Administration is always **"left nostril - 45 sec break - right nostril – 45 sec break – etc. until 6 puffs in total is completed"**

Tick the boxes on the left as you go to ensure you do 6 puffs!

"Please get the cap back on, and put the it in the bin. You can use hand disinfectant and tissues to remove any of the solution of your hands."

4

Hopkins Verbal Learning Test (HVLT)

Follow instructions on testing materials

Symbol Digit Modalities

Look at the boxes at the top of the page. Each box in the upper row has a symbol in it, and each box below it has a number.

Now look at the next line of boxes *[point to the first line of boxes without numbers].*

Notice that the boxes on the top have symbols, but the boxes beneath are empty. You are to fill in each empty box with the number that goes with each symbol, according to the way they are paired at the top of the page.

For example, if you look at the first symbol, [point to the first symbol in the row beneath the key], **and then look up at the key, you see that this symbol is paired with the number "1"** *[show the pairing]***. So, you would write a "1" in this box** [write a "1" in the first box]. **This next symbol** [point to the next symbol] **is paired with "5", so you would put a "5" in this box** [write "5" in the second box*].* **Now, what number goes in this box** [point to third box]***?***

Subject should say "two." If not, correct the subject and explain the error.

When the subject appears to comprehend the task, say:

Good. Now, for practice, fill in the boxes up to this double line, and then stop.

Correct immediately any errors made during the practice period, explaining the subject's error. Repeat the instructions and review the correct coding of the practice boxes as necessary until the subject understands the task.

Continue with the test by saying,

When I say "Go," write in the numbers just like you have been doing as fast as you can until I say "Stop." Work as quickly as you can, moving from one line to the next, without skipping any boxes. If you make a mistake, cross it out and write the correct answer below. Remember to work as quickly as you can. Ready? Go!

Start timing. Do not allow the subject to skip any boxes. Do not intervene if the subject records a number incorrectly.

At the end of 90 seconds, say, ***"STOP!"***. Be sure that the subject does not continue working after the time limit is reached.

5

Move chair to sit near participant

Trustworthiness and Approachability

"In this task you will be shown a number of people. For each person, you will be asked to judge how trustworthy the person is.

Imagine that you are on a crowded street while on holiday. You have been taking photographs of a famous monument. Someone comes up to you and offers to take a photograph of you in front of the monument with your camera."

- next screen:

"Use the scale to indicate for each person the extent to which you agree with the statement: I would trust this person.

You can click on any part of the scale. Please attempt to use the full range of the scale across the task when making responses.

Try to respond as fast and accurately as you can.

Press the space bar when you are ready to begin

- **Let me know when the task has finished and new instructions come up on the screen."**

FEEST

"In this task, you will be shown a series of faces. For each face, you are asked to categorise the facial expression.

To response, please click on the most appropriate label from the six shown below. These will be displayed underneath each face throughout the task.

Please respond as fast and accurately as you can.

Press the spacebar to begin.

- **Let me know when the task has finished and new instructions come up on the screen."**

6

REMET

"In this ask you will be shown various pairs of eyes accompanied by four words. For each set of eyes, select the word that best describes what the person in the picture is thinking or feeling. You may feel that more than one word is appropriate, but please choose just one word, the word which you consider to be most suitable.

Before making your choice, make sure that you have read all four words.

You should try to respond as quickly and accurately as possible.

When you are ready to complete the practise trial, please press the space bar"

Benton Facial Recognition Test

Display stimuli booklet on table

Questions 1-6

You see this person? Show me where the person is on this picture (pointing to the multiple choice display below)

Check the correct responses on the record form and record errors by circling the appropriate numbers on the right side of the record form.

Questions 7-22

You see this person? The person is shown three times on this picture (pointing to the multiple choice display below). Show me where she is. Find three pictures of her.

Record in same manner as above. If participant appears at a loss to find a picture say " **just choose the most likely choice**"

Hopkins Verbal Learning Test (HVLT) -delayed

Follow instructions on testing materials

WMS Faces 1

display stimuli manual

7

Follow instruction on administration manual, on reverse side to faces presented

Hayling Sentence Completion Task

Read out instructions as written on the form.

If subject does not fully understand, repeat the instructions again.

Stop your stop watch as soon as the participant has said the word, if the participant gives 2 responses, record the time for the first.

The Awareness of social Inference test (TASSIT)

*DVD should be ready

You will be shown some short scenes on the screen. Each one lasts for 16-60 seconds. Please watch each scene carefully. After viewing the scene you will be asked 4 short questions.

Social Inference response card should be presented to the participant

Point to Question A – **The first question will focus on what you think one of the people is *doing* to the other person. That is what they are trying to make a person do, think or feel.**

Point to Question B – **The second question will ask you what you think one person is *trying* to say to the other person. That is, what the message is that they are trying to get across. Note that this may be different to the actual words they are using. For example, a person may say "its hot in there" to mean " you should open the window".**

Point to Question C – **The third question will ask you what you think one of the people *is thinking*. That is what is their underlying belief, which may be different from what they are saying.**

Point to Question D – **The fourth question will ask you what you think one of the people *is feeling*. That is, what is the emotion that they are feeling, or how do they feel towards the other person.**

Each time you need to say Yes, No or Don't Know. If you really can't decide whether the answer is Yes or No, use the Don't Know response, but try your hardest to choose either Yes or No.

Do you have any questions?

Remove response card

Start DVD, pause after each item and ask examinee the 4 questions.

8

Trail Making Test

Give participant Part A

On this sheet as you can see there are some numbers in small circles. The numbers go from 1 through to 8. I want you to start here at number 1 (point to) and join up the numbers in their correct order until you get to number 8. Work as fast as you can without lifting the pencil from the paper. Ok? Go ahead.

If the subject makes an error, point this out immediately and require them to correct the error.

Turn page over

I want you to do much the same thing for the next task except that there are 25 numbers to connect instead of 8 numbers. Remember to work as fast as you can, get them in the correct order and don't lift your pencil from the paper. Ok? Go ahead.

record time

Give participant Part B

This part is a little more difficult than the last part. On this sheet there are letters in the circles as well as numbers. The numbers go from 1 through to 4 and the letters go from A to D. I want you to connect them by swapping between the numbers and the letters. I want you to start here at 1 (point) **the first number, and connect it to A, the first letter, then go to 2, the second number, and then to B, the second letter, and so on. Do you get the idea?**

Remember to keep swapping between letters and numbers and connect all the circle as fast as you can without lift in the pencil from the paper. OK? Go Ahead.

Point out incorrect connections and ensure that the subject correct their performance.

Turn page over

I want you to do much the same thing again except that you will have 13 numbers to connect to letters instead of 4 and the letters will go from A to L instead of A to D. I'm going to time you again so work as fast as you can without lifting the pencil from the paper. Ok? Go ahead.

If the subject makes an error, stop them, point out the error and request that they make the correct response. Note the number of errors, and note if the subject failed to complete the task.

Record time

9

WMS Faces 2

*display stimuli manual – FACES II)

Follow instruction on administration manual.

Empathic Concern Subscale of the interpersonal Reactivity Inventory

provide participant with questionnaire and pencil

For each item, indicate how well it describes you with an X in the box below the letter. Read each item carefully before responding. Answer as honestly as you can. Thank you.

STAI

Page with questions 1-20

"This next task is a simple mood scale. Here are a number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to her right of the statement to indicate how you feel right now, that is, at this moment. There is no right and wrong answers. Do not spend much time on any one statement but give the answer which seems to describe your present feelings best."

Additional Questions

- Ask participants the two additional questions on running sheet checklist

Appendix A – 9. Protocol running sheet checklist

Short checklist Date:

1) Preparation	2) Materials	3) Setup
☐ Participant call & TICs ☐ Two sessions booked ☐ Rooms booked ☐ Assign ID number ☐ Fill details in questionnaire pack ☐ Update participant excel sheet	☐ Participant folder and testing bag ☐ Reimbursement (cash or gift card) ☐ Receipt of reimbursement ☐ Consent form, information letter, questionnaires ☐ Nasal spray out of the fridge (room temperature) ☐ Eski with water & frozen icepack ☐ Extra bag as bin ☐ Hand sanitiser ☐ Tissues **Session A only:** ☐ Sample collection pack (Ziplock bags, Gloves, Labels, Buccal collection: 2 x sealed swab in tube, Saliva collection: 2 x 2ml sealed cryovials (tubes), sealed saliva collection aid (straw))	☐ Set up laptop ☐ Label testing forms ☐ Tissues, hand sanitiser placed on table within reach of participant ☐ Cup with water **Session A only:** ☐ Label questionnaire package pages ☐ Consent form and information sheet placed ☐ Label saliva, buccal swab tubes (ID, time, date) ☐ Label ziplock bag

4) Arrival	5) **Session A only:** sample collection
Session A only: ☐ Consent: Privacy of information, Right to withdraw, Overview of tasks **Session A only:** ☐ Mouth wash: Provide water ☐ start timer or check time ☐ **Session A only**: Administer NART ☐ Administer STAI	<u>Saliva sample collection (8-9 minutes after) mouth wash:</u> ☐ Put gloves on and check tube ID ☐ Explain method to participant – see instructions ☐ Get participant to place tube in ziplock bag ☐ Place on ice IMMEDIATELY <u>Buccal cell collection:</u> ☐ With gloves on check tube ID ☐ Explain method to participant – see instructions ☐ Put soft end of swab in one side of participants mouth and rub up and down 20 times against the inside of the cheek, then rub in the groves (sulcus next to the top and bottom of the gums for 10 seconds. ☐ If there is blood discard immediately ☐ Use the second swab for the other side of the mouth, collecting the same way. ☐ Place swab on window to dry

6) Nasal spray administration:
☐ Get stop watch ready, Administer spray – record time and tick boxes:

	Start time	End time
Nasal spray:	_____	_____

Tick boxes as completed: L ☐ ☐ ☐ R ☐ ☐ ☐

*****Start the stopwatch after the last puff to keep the time until the Cognitive battery, wait 40 minutes *****

7) Break	☐ Hand out questionnaires and explain ☐ **Session B only**: Received questionnaire package and check for missing items ☐ Let participant have a toilet break ☐ Provide participant with something to read ☐ Provide low sugar biscuits and cup of water	**Session A only**: buccal swaps ☐ Place swab into container ☐ Place on ice **Session A only:** take samples to freezer if possible

8) Testing battery part 1

Start 40 mins since nasal spray end time. Record the start/end times **in minutes** for each task.

☐ Hopkins Verbal Learning Test (HVLT)

	Start time	End time
[Task 1]: e.g. 40 mins	_____	_____

Time for second part (+20-25min):

☐ Symbol Digit Modalities Test

	Start time	End time
[Task 2]:	_____	_____

☐ Trustworthiness and Approachability task

	Start time	End time
[Task 3]:	_____	_____

☐ FEEST

	Start time	End time
[Task 4]:	_____	_____

☐ Reading the Mind in the Eyes Test

	Start time	End time
[Task 5]:	_____	_____

Check if you need to do Hopkins delayed recall

☐ Benton Facial Recognition Test

	Start time	End time
[Task 6]:	_____	_____

☐ Hopkins

(CHECK if 20 mins passed since part 1)

	Start time	End time
[Task 1]:	_____	_____

9) Testing battery part 2

------OFFER PARTICIPANT A BREAK----------

☐ Faces subtest of the WMS III stage 1

	Start time	End time
[Task 7]:	_____	_____

Time for second part (+25-30min):

☐ Hayling Sentence Completion Task

	Start time	End time
[Task 8]:	_____	_____

☐ The Awareness of Social Inference Test

	Start time	End time
[Task 9]:	_____	_____

Check if you need to do WMS delayed recall

☐ Trail Making Test (TMT)

	Start time	End time
[Task 10]:	_____	_____

RESPONSE TIME PART A _____
RESPONSE TIME PART B _____

☐ Empathic Concern Subscale of the Interpersonal Reactivity Inventory

	Start time	End time
[Task 11]:	_____	_____

☐ Faces subtest of the WMS III

(check that 25-30 mins have passed since part 1)

	Start time	End time
[Task 12]:	_____	_____

10) After testing battery	11) Storage of data and samples
☐ Administer STAI **Session B only:** ☐ Provide reimbursement ☐ Ensure participant signs stipend sheet ☐ **Session A only**: confirm date for second session Tests to score: ☐ HVLT & HVLT delayed recall –score Nr. correct ☐ Symbol Digit Modalities Test - score Nr. correct ☐ Benton Facial Recognition Test –two scores ☐ Faces subtest of the WMS –add up - out of 48 ☐ Hayling Sentence Completion –score and scale ☐ TASIT –score per scene –out of 4 ☐ TMT –enter completion times into checklist	☐ Data transfer: NART, SUPERLAB ☐ Take any finished testing papers to Natalie ☐ Take the participant consent forms and the TICs to Skye ☐ Take the signed reimbursement forms to Jennie ☐ Dispose of Nasal Spray ☐ **Session A only**: As soon as possible, transport samples to freezer located in Exercise Sciences lab, level 1 of Danniel Mannix Building, ACU.
Are you taking any hormone based medication (this includes contraceptive pill/device) Y/N: Which type?_____	
Which session do you think was active?:_____ Handedness: L/R	

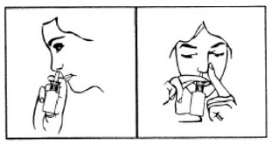
Appendix A – 10. Instructions for saliva sampling and storage as well as ordering and administering nasal sprays

All saliva samples will be collected before other oral samples (e.g. buccal cells).

Saliva Samples	We will collect a minimum of 1ml and a maximum of 2ml of saliva from each participant using the Passive drool method.
Equipment	• Labels (with participant ID, DOB, date and time of collection) • 2 x 2ml cryovials (tubes) • 2 x saliva collection aid (straw) • Esky containing wet ice (ice bath) • Cup of water • Disposable gloves • Alcohol based hand cleanser • Box tissues
Preparation	• participants will be advised to: 1. Not to eat a major meal within 60 minutes of sample collection 2. Avoid dairy products for 20 minutes before sample collection 3. Avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may compromise the assay by lowering saliva pH and increasing bacterial growth • Label saliva tubes with participant ID, DOB, date and time of collection **Wait a full ten minutes after the mouth has been washed with water before collecting the saliva sample.** *"Now make sure you don't have any food in your mouth from breakfast or snacks I am going to give you some water. I want you to take a big sip, but don't swallow! I want you to swish it around your mouth as you would with mouth wash or maybe if you are at the dentist. I am going to count to ten, and once I get to ten you can swallow the water. Okay?"* *Count to 10 – "Okay now swallow the water. If there is any water left in the cup can you finish it off?"* *"Now we are going to wait ten minutes and then we'll do the saliva collection".*
Collection	• Where possible, collect sample whilst the participant is seated • Give participant plastic tube and collection aid. Let participant open the collection aid and put the aid into the tube (with smooth part of the aid to the top) • Instruct participants to allow saliva to pool in their mouth. To help them do this, tell the participant to imagine slowly chewing their favourite food • Once they feel the saliva collecting under their tongue, instruct the participant to tilt their head forward and collect the saliva in the vial (rather than following the natural instinct of swallowing) • Instruct the participants to repeat the procedure until they reach the 1 ml mark on the tube. • Participant to remove the aid and replace the cap on the tube. • Repeat the process with the second tube until 1 ml of saliva is in the tube. • Ask the participant to put the cap on their tube/s and put the tubes in the ziplock bag, squeezing the air out.
Storage	**Short Term:** • Immediately store samples in the esky with th**e wet** ic**e until storage.** **Long term:** • All samples should be maintained at 4°C for no longer than necessary (ideally transferred within 2 hours for freezing at or below -20°C)

Buccal Cells Swab	
Equipment	• Two swabs per participant • Disposable gloves • Alcohol based hand cleanser • Box tissues • Esky containing wet ice (ice bath)
Preparation	• Label swabs (with participant ID, DOB, date and time of collection) • Wipe hands with cleanser and don disposable gloves • Remove cap from swab just before using, take care not to touch the swab with your fingers
Collection	• Put soft end of swab in one side of participant's mouth and rub up and down **20 times** against the inside of the cheek, then rub in the grooves (sulcus) next to the top and bottom gums on the same side for **10s.** • If there is blood on the swab discard it immediately. • Invert the swab and let is air dry for **15-20 minutes** • Immediately after collection swirl buccal swab into labelled and place on wet ice. • Use the second swab **for the other side**, collecting a sample in the same way. Replace both swabs in their original containers.
Storage	**Short term:** • Immediately store samples in the esky with th**e wet** ic**e until storage.** **Long Term:** • All tubes containing buccal cells are stored at 2-8oC for 24-72 hours prior to storage in the original packaging at –20°C for up to 6 months or -80°C for 12 months.

Oxytocin Nasal Spray	**Dartnells Pharmacy** 376 Canterbury Road Surrey Hills VIC 3127 Tel: 03 9888 5899 Fax: 03 9888 6911

Equipment	• Nasal spray (if blind, see randomisation schedule) • Stop watch • Alcohol based hand cleanser • Box tissues
Preparation	• Ask participant if they have used nasal sprays before? • Ask participant whether they feel like they need to blow their nose. • Participants are allowed to blow their nose only before the 1st puff; should avoid this for the following 20 min – they are only allowed to dab off leaking fluid with a tissue but should not blow their nose! • Let the participants practice the handling of the spray. This is done by asking the participant to remove the small cap, and by letting them **prime** the spray bottle – this can be up to 3 pre-puffs until a full spray (this ensures normal distribution of each bottle before use; be careful not to spray too many times!).
Administration	• The order is always: **insert sprayhead → exhale → puff & inhale nasally** (almost simultaneously) Keep the bottle during puffing in an **upright position** (not at 90°!). Participants can close the other nostril if want to.  • After the sprays (or each spray), participants can sit with their heads back if they feel the need to do so to avoid any leakage. • Administration is always: **left nostril – 45 s break – right nostril – 45 s break – etc. until 6 puffs in total** • It is important that you **tick the boxes** (see below) as you go to ensure you do 6 puffs! **Immediately start the stopwatch after the last puff to keep the time until the experiment**
Storage of Nasal Bottles	

Ordering Samples:

Please email details to:
Main pharmacist: **Les Bassin**
Email: info@dartnellspharmacy.com.au
Use the specific ordering form for your study.
Use the short study name in the email subject, e.g., **OXT-Age-Behav study**

Appendix B – Ethics Approval and Clinical Trial Registration

Appendix B – 1. Original ethics approval

From: Kylie Pashley on behalf of Res Ethics
To: Peter Rendell; Skye McLennan; Izelle Labuschagne
Cc: Res Ethics
Subject: 2015-185H Ethics application approved!
Date: Tuesday, 6 October 2015 3:21:14 PM

Dear Applicant,

Principal Investigator: Prof Peter Rendell, Dr Skye McLennan, Dr Izelle Labuschagne
Student Researcher: Cassandra Nicole Murphy, Rebecca Ellen Margaret Gilchrist (non-HDR students)
Ethics Register Number: 2015-185H
Project Title: Oxytocin's Role in Social Cognitive Changes in Ageing
Risk Level: Low Risk
Date Approved: 06/10/2015
Ethics Clearance End Date: 31/03/2017

This email is to advise that your application has been reviewed by the Australian Catholic University's Human Research Ethics Committee and confirmed as meeting the requirements of the National Statement on Ethical Conduct in Human Research.

The data collection of your project has received ethical clearance but the decision and authority to commence may be dependent on factors beyond the remit of the ethics review process and approval is subject to ratification at the next available Committee meeting. The Chief Investigator is responsible for ensuring that outstanding permission letters are obtained, interview/survey questions, if relevant, and a copy forwarded to ACU HREC before any data collection can occur. Failure to provide outstanding documents to the ACU HREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research. Further, this approval is only valid as long as approved procedures are followed.

If your project is a Clinical Trial, you are required to register it in a publicly accessible trials registry prior to enrolment of the first participant (e.g. Australian New Zealand Clinical Trials Registry http://www.anzctr.org.au/) as a condition of ethics approval.

If you require a formal approval certificate, please respond via reply email and one will be issued.

Researchers who fail to submit a progress report may have their ethical clearance revoked and/or the ethical clearances of other projects suspended. When your project has been completed a progress/final report form must be submitted. The information researchers provide on the security of records, compliance with approval consent procedures and documentation and responses to special conditions is reported to the NHMRC on an annual basis. In accordance with NHMRC the ACU HREC may undertake annual audits of any projects considered to be of more than low risk.

It is the Principal Investigators / Supervisors responsibility to ensure that:
1. All serious and unexpected adverse events should be reported to the HREC with 72 hours.
2. Any changes to the protocol must be reviewed by the HREC by submitting a Modification/Change to Protocol Form prior to the research commencing or continuing. http://research.acu.edu.au/researcher-support/integrity-and-ethics/
3. Progress reports are to be submitted on an annual basis. http://research.acu.edu.au/researcher-support/integrity-and-ethics/
4. All research participants are to be provided with a Participant Information Letter and consent form, unless otherwise agreed by the Committee.
5. Protocols can be extended for a maximum of five (5) years after which a new application must be submitted. (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

Researchers must immediately report to HREC any matter that might affect the ethical acceptability of the protocol eg: changes to protocols or unforeseen circumstances or adverse effects on participants.

Please do not hesitate to contact the office if you have any queries.

Kind regards,

Kylie Pashley
on behalf of ACU HREC Chair, Dr Nadia Crittenden

Ethics Officer | Research Services
Office of the Deputy Vice Chancellor (Research)
Australian Catholic University

THIS IS AN AUTOMATICALLY GENERATED RESEARCHMASTER EMAIL

Appendix B – 2. ANZCTR registration

From: info@actr.org.au
To: Izelle Labuschagne
Subject: Your ACTRN (registration number): ACTRN12618001343291
Date: Thursday, 9 August 2018 11:47:54 AM
Attachments: ATT00001.png

Dear Izelle Labuschagne,

Re: Oxytocin's role in social cognitive changes in ageing

Thank you for submitting the above trial for inclusion in the Australian New Zealand Clinical Trials Registry (ANZCTR).

Your trial has now been successfully registered and allocated the ACTRN: ACTRN12618001343291

Web address of your trial: http://www.ANZCTR.org.au/ACTRN12618001343291.aspx
Date submitted: 30/06/2018 10:13:46 PM
Date registered: 9/08/2018 11:47:37 AM
Registered by: Izelle Labuschagne
Principal Investigator: Izelle Labuschagne

****Please note that as your trial was registered after the first participant was enrolled, it does not fulfil the criteria for prospective registration and will therefore be marked as being Retrospectively Registered on our website.****

If you have already obtained Ethics approval for your trial, please send a copy of at least one Ethics Committee approval letter to info@actr.org.au or by fax to (+61 2) 9565 1863, attention to ANZCTR.

Note that updates should be made to the registration record as soon as any trial information changes or new information becomes available. Updates can be made at any time and the quality and accuracy of the information provided is the responsibility of the trial's primary sponsor or their representative (the registrant). For instructions on how to update please see http://www.anzctr.org.au/Support/HowToUpdate.aspx.

Please also note that the original data lodged at the time of trial registration and the tracked history of any changes made as updates will remain publicly available on the ANZCTR website.

The ANZCTR is recognised as an ICMJE acceptable registry (http://www.icmje.org/faq.pdf) and a Primary Registry in the WHO registry network (http://www.who.int/ictrp/network/primary/en/index.html).

If you have any enquiries please send a message to info@actr.org.au or telephone +61 2 9562 5333.

Kind regards,
ANZCTR Staff
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