Heart-brain interactions underlying emotion in autism spectrum conditions

James Stephen Mulcahy

A thesis submitted in partial fulfilment of the requirements of the University of Brighton for the degree of Doctor of Philosophy

Brighton and Sussex Medical School

March 2021

Abstract

Interoception refers to the afferent signalling, central processing and neural and mental representation of internal (visceral) bodily signals. Interoceptive signals are integrated by a set of cortical and sub-cortical regions, namely insula, cingulate cortices and amygdala, to regulate autonomic control and guide emotional experience. Autism Spectrum Conditions (ASCs) are a set of neurodevelopmental conditions characterised by altered sensory sensitivity and difficulties with social communication and interaction. ASC individuals may present with an altered interoceptive profile that could contribute to atypical emotional experiences in this population. Emerging work implicates interoceptive differences in the manifestation of anxiety in autistic individuals. This thesis thus aims to better quantify the interoceptive profile of autistic adults, using a combination of behavioural, physiological and neuroimaging techniques, and seeks to better understand how interoceptive signals may contribute to atypical emotional processing and, finally, aims to establish the usability of a novel interoceptive training paradigm to mitigate anxiety in this population.

In the first study, I found that increased interoceptive insight (confidence-accuracy correspondence) mitigated emotional recognition difficulties from the intonation of speech (affective prosody). This suggests that a reduction in consciously perceived interoceptive signals may contribute to atypical emotional processing and social interaction in autistic adults. In a second study, autistic adults did not differ from non-autistic adults when processing emotional (fear) faces but did show reduced activation and functional connectivity of regions involved in interoceptive and autonomic control, namely right insula cortex, during systolic cardiac signalling suggesting a dysregulated interoceptive system that may contribute to the manifestation of anxiety. In a more targeted study employing an interoceptive task during functional MRI scanning, I showed significant group

differences in functional connectivity of insula cortices across distinct dimensions of interoception (accuracy and insight), despite no group differences at the behavioural level. In the application of a novel interoceptive training paradigm, I found interoceptive training significantly increased interoceptive accuracy and functional connectivity of insular cortices, and, in a parallel investigation, interoceptive training subtly enhanced intensity ratings towards emotional faces. Finally, in the application of a novel exteroceptive training paradigm (affective prosody), I found training enhanced affective prosody recognition but did not impact interoceptive dimensions. Together, these findings elucidate the altered interoceptive profile of autistic adults and demonstrate how cardiac interoceptive signals influence emotional experience in this population. Finally, they show how interoceptive and exteroceptive training paradigms can increase emotion sensitivity in this population, which has important clinical implications for mitigating emotional and social difficulties in autistic adults.

Contents

List of Figures

List of Tables

Acronyms and definitions

ADIE	Aligning Dimensions of Interoceptive Experience
ANCOVA	Analysis of Covariance
ANOVA	Analysis Of Variance
APTP	Affective Prosody Training Protocol
ASC	Autism Spectrum Condition
AQ	Autism Quotient
BF	Bayes Factor
BOLD	Blood Oxygen Level Dependent
BPQ	Body Perception Questionnaire
CSF	Cerebral Spinal Fluid
CTU	Clinical Trials Unit
EPI	Echo-Planar Imaging
FC	Functional Connectivity
fMRI	Functional Magnetic Resonance Imaging
gPPI	Generalized PsychoPhysiological Interaction
HEP	Heartbeat Evoked Potential
HR	Heart rate

HRV	Heart Rate Variability
IAS	Interoceptive Accuracy Scale
IAQ	Interoceptive Awareness Questionnaire
IBI	Inter-Beat Interval
ITPE	Interoceptive Trait Prediction Error
MAIA	Multidimensional Assessment of Interoceptive Awareness
MCAR	Missing Completely at Random
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NTS	Nucleus of the Solitary Tract
PHQ-9	Patient Health Questionnaire
PPI	PsychoPhysiological Interactions
PTT	Pulse Transit Time
RCT	Randomised-Control Trial
ROC	Receiver Operating Characteristic
SCR	Skin Conductance Response
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences
STAI	State and Trait Anxiety Inventory
TAS-20	Toronto Alexithymia Scale
TE	Echo Time
TR	Repetition Time
VAS	Visual Analogue Scale
WM	White Matter

Acknowledgements

Firstly, I would like to thank my supervisors, Professor Sarah Garfinkel and Professor Hugo Critchley, for your invaluable advice, support and guidance throughout this journey. I would also like to thank my unofficial 'third supervisor', Doctor Lisa Quadt, for your help in making this project happen. Without all of you, none of this would have been possible and for that, I am immensely grateful.

To everyone who has worked in the PhD write-up room over the past three years, Dennis, Marta, Marisa, Diana, Sophie, and to everyone else in Trafford, thank you for making the last three years of working here enjoyable and helping me with numerous challenges.

Next, thank you to my friends and family, Mum, Dad, Rebecca, Louise, and to my partner, Alice, for your continued support over the last three years, and throughout my academic career, I am not sure any of us thought I would end up doing a PhD but here we are!

Finally, thank you to all the volunteers who took part in this research and thank you to the numerous different people, far too many to mention here, who I have met over the past three years that have contributed to making this project possible.

Author's Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed J.Mulcahy

Dated 30.09.2020

Some parts of this thesis have been published in peer-reviewed journals or are currently under preparation for publication:

- Mulcahy, J. S., Davies, M., Quadt, L., Critchley, H. D., & Garfinkel, S. N. (2019). Interoceptive awareness mitigates deficits in emotional prosody recognition in Autism. *Biological psychology*, 146, 107711.
- Mulcahy, J. S., Larsson, D. E., Garfinkel, S. N., Critchley, H. D. (2019). Heart rate variability as a biomarker in health and affective disorders: A perspective on neuroimaging studies. *Neuroimage*, 202, 116072.
- Mulcahy, J. S., Quadt, L., Silva, M., Rae, C. L., Critchley, H. D., & Garfinkel, S. N. (under review). Understanding anxiety in autistic adults: central dysregulation of interoceptive tuning to threat. *submitted to Social Cognitive and Affective Neuroscience.*

Chapter 1

Introduction

1.1 Overview

The ancient Egyptians considered the human heart to be the 'house of our mind' and it was the heart, not the brain, that they carefully preserved after death as a way to secure passage to the afterlife. We now know that what we consider 'our mind' is predominantly located in our brains, however the influence of the heart on human experience has not been forgotten. The study of how the human heart interacts with the brain to influence behaviour, emotion and cognition has in fact become increasingly prevalent in the last decade. This research has employed novel techniques, including psychophysiological, neuroimaging and computational approaches, to delineate this relationship and this 'interoceptive pathway' is now widely recognized as crucial for human experience, perhaps causally influencing affective disorders across diverse populations.

In this chapter, I address the construct of emotion and how this is causally influenced by interoceptive signaling. I delineate the construct of interoception to explain and understand how the different, often dissociable, facets of interoception differentially impact behaviour and emotion. I then frame this construct in a view to understand how these dimensions contribute to the development and maintenance of affective symptomatology, particularly anxiety. Next, I introduce Autism Spectrum Conditions (ASCs) as a population of interest, where a deeper understanding of interoceptive functioning may explain common autistic characteristics and often co-occurring affective symptomatology. Lastly, I layout the aims and hypotheses of this thesis and discuss the novel contribution of each chapter toward a better understanding of interoception and emotion in autism.

1.2 Emotion

Emotions are subjective feeling states, thought to be shaped by physiological, contextual, motivational and cognitive factors. Considerable work has sought to understand the purpose of human emotion, for example evolutionary theories propose emotions are essential for effective communication thus favouring survival. Equally, while some sought to understand the function of emotion, others, such as the somatic theorists, sought to understand brain-body experiences underpinning emotional behaviour. Such theorists place the body at the centre of emotional

experience, arguing that bodily sensations shape and inform emotional feeling states; 'We feel sorry because we cry, angry because we strike, afraid because we tremble' (p.190 James, 1884). This relationship is, however, nuanced; visceral changes (e.g. faster heart rate) can be shared among emotion constructs and some changes are too slow (e.g. parasympathetic reactivity) to adequately inform quick emotion judgements (Cannon, 1914, 1927).

A more holistic view, the somatic marker hypothesis (Damasio, 1996), argues that cognitive processes are guided by the representation of bodily responses. In this theory, emotions and bodily changes are associated with situations and outcomes; 'somatic markers'. Thus, emotions can be evoked via two pathways; the soma changes in response to stimuli which is then relayed to the brain, the 'body loop', or the body is bypassed and cognitive representations (somatic markers) of the emotions, and co-occurring bodily changes, are activated, the 'as-if body loop', allowing for a quick response to stimuli. In the brain, the medial pre-frontal cortices were proposed as the system responsible for 'somatic markers', which was evidenced in a series of studies that found patients with medial pre-frontal cortices damage showed an altered skin conductance response (SCR) during a gambling task which led to persistent choices with bad outcomes, i.e. a failure to develop and deploy appropriate integration of cognitive and bodily processes (Bechara, Tranel, Damasio, & Damasio, 1996; Damasio, Tranel, & Damasio, 1990). In this view, emotion induced activation in the brain influences physiological changes which are relayed, via interoceptive pathways, and re-expressed to inform emotional feeling states. To better understand the influence of somatic sensation on emotional experience, we must introduce and define the concept of interoception.

1.3 Interoception

In the early 1900's, Sherrington defined the 'surface field', the area of an organism exposed to the environment, which he partitioned into the 'intero-ceptive' surface; the internal surface of an organism, the 'extero-ceptive' surface; the external surface of an organism, and 'proprio-ceptors'; receptor involved in the movement of an organism (Sherrington, 1907). Thus, interoception is historically restricted to signals coming from the internal, visceral, environment. The accurate monitoring of these interoceptive signals is crucial for the maintenance of internal homeo-

stasis, allowing dynamic flexibility (i.e. allostasis) in response to an unpredictable external world. In this sense, 'interoception refers to the process by which the nervous system senses, interprets, and integrates signals originating from within the body, providing a moment-by-moment mapping of the body's internal landscape across conscious and unconscious levels' (Khalsa et al., 2018).

1.4 Interoceptive pathways

To understand how the brain integrates signals originating from the internal viscera, we must consider the interoceptive pathways involved in interoceptive signalling. Homeostatic afferent information is conveyed to the brain via two broad routes; those that carry motivational information, such as hunger, nausea and respiratory sensations, via cranial (e.g. vagus and glossopharyngeal) nerves to terminate in the nucleus of the solitary tract (NTS), and spinal visceral afferents that project into the spinothalamic tract, via spinal laminar 1, and tend to be involved in signalling tissue damage (Critchley & Harrison, 2013).

The NTS receives input from cranial nerves, notably the vagus nerve, where ascending motivational information projects to parabrachial nucleus and periaqueductal gray, which are both anatomically and functionally connected (via the thalamus) to forebrain regions, namely, the hypothalamus, insula, amygdala and mid cingulate cortex (Critchley & Harrison, 2013). In the spinal pathway, the dorsal root ganglion receives input from small diameter fibres, mainly A-delta and C-fibres, that are present in the skin, viscera, muscles and joints throughout the body (Watson, Paxinos, & Kayalioglu, 2009), which relay homeostatic information, including temperature, touch, muscle contraction and hormonal activity (Craig, 2002), to Lamina 1 neurons. Traveling via the spinothalamic tract, which has been proposed as a 'dedicated channel of viscerosensory information and related affectively-laden sensations' (Craig, 2003; Critchley & Harrison, 2013), these afferent signals project into the NTS and subsequently the ventromedial nucleus of the thalamus which extends via projection fibres to both cortical and subcortical regions, including insula cortex (Kumar, van Oort, Scheffler, Beckmann, & Grodd, 2017). Both pathways thus provide a constant mapping of internal bodily state to support autonomic function. Importantly, the representation of interoceptive state in forebrain regions allows the integration of autonomic signals with cog-

nitive processes to inform emotional and behavioural responses. One prominent example of such integration is evidenced by the baroreflex control of circulation.

1.5 The baroreflex

The arterial baroreflex is the principal mechanism for coordinated control of the heart and vasculature, allowing integration with other organs systems, and changing behavioural, emotional and cognitive demands (Smith, Thayer, Khalsa, & Lane, 2017). The baroreflex controls arterial blood pressure, modulating cardiac output and cardiovascular resistance on a beat-by-beat basis, thereby setting the baseline for organ perfusion. Patches of specialised stretch receptors (baroreceptors) within the aortic arch and carotid sinuses are innervated by afferent branches of the vagus and glossopharyngeal nerves respectively. These arterial baroreceptors discharge in response to mechanical changes in the vessel walls caused by changes in arterial blood pressure as the heart ejects blood at ventricular systole. Baroreceptor afferents synapse within the NTS, within the medulla. Here the signal is integrated with contextual descending neurochemical influences mediated, in part, by local GABAergic neurons (Dampney et al., 2005; Potts, 2006; Spyer, 1994). Through a chain of medullary nuclei, baroreceptor signalling adjusts the efferent autonomic drive to the heart and vasculature. Via the dorsal motor nucleus of the vagus, a strong baroreceptor afferent input enhances the vagal parasympathetic outflow to the primary pacemaker of the heart, the sinoatrial node, and the next heartbeat is slowed. Concurrently, via effects on descending sympathetic pathways relayed through spinal cord and paravertebral ganglia, a strong baroreceptor afferent input inhibits the sympathetic outflow to the heart and muscle vascular beds, the latter reducing blood pressure by decreased peripheral resistance and pooling of blood in the periphery. Similarly, a weak baroreceptor input signal accelerates the onset of the next heartbeat and reduces peripheral pooling. The net effect of these responses is to stabilise blood pressure, for example in response to postural changes.

The set-point and sensitivity of the baroreflex is adjusted in situations of actual or anticipated increased metabolic demand. In particular, cognitions, emotions and action policies exert dynamic top-down influence on the baroreflex, allowing for a simultaneous increase of both arterial blood pressure and heart rate, to sup-

port, for example 'fight or flight' motor behaviours. During stress, projections from dorsomedial and paraventricular nuclei of the hypothalamus and periaqueductal grey matter to the NTS are implicated (Dampney et al., 2005), whereas during physical exercise, signalling from posterior hypothalamus or muscle afferents can reset the baroreflex (Raven, Fadel, & Ogoh, 2006). Both illustrate the principle that baroreflex function (hence parasympathetic and sympathetic drive to the cardiovascular system) is tuned by descending signals from higher-order integrative systems that monitor needs of the organism and select adaptive behavioural polices (action and autonomic) (Riganello et al., 2018; Shaffer & Venner, 2013). In this sense, baroreceptor signalling operates a bi-directional relationship with the brain to control autonomic behaviour and influence, or be influenced by, emotion and behaviour.

1.6 Measuring interoception

As we have discussed, interoceptive signals can operate at unconscious levels, guiding behaviour and altering physiological state to ensure allostasis. These signals can also be accessible to consciousness. Thus, interoception spans a continuum raging from low level measurements of afferent signals expressed in brain (e.g. heartbeat evoked potentials) to higher level processes (e.g. interoceptive metacognition; see figure 1.1). By parsing and measuring different dimensions of interoception we are able to establish how distinct facets of interoception influence cognition, emotion and behaviour. In this thesis, I focus on lower level, preconscious impact of the afferent signal up to, and including, higher level metacognitive processes.

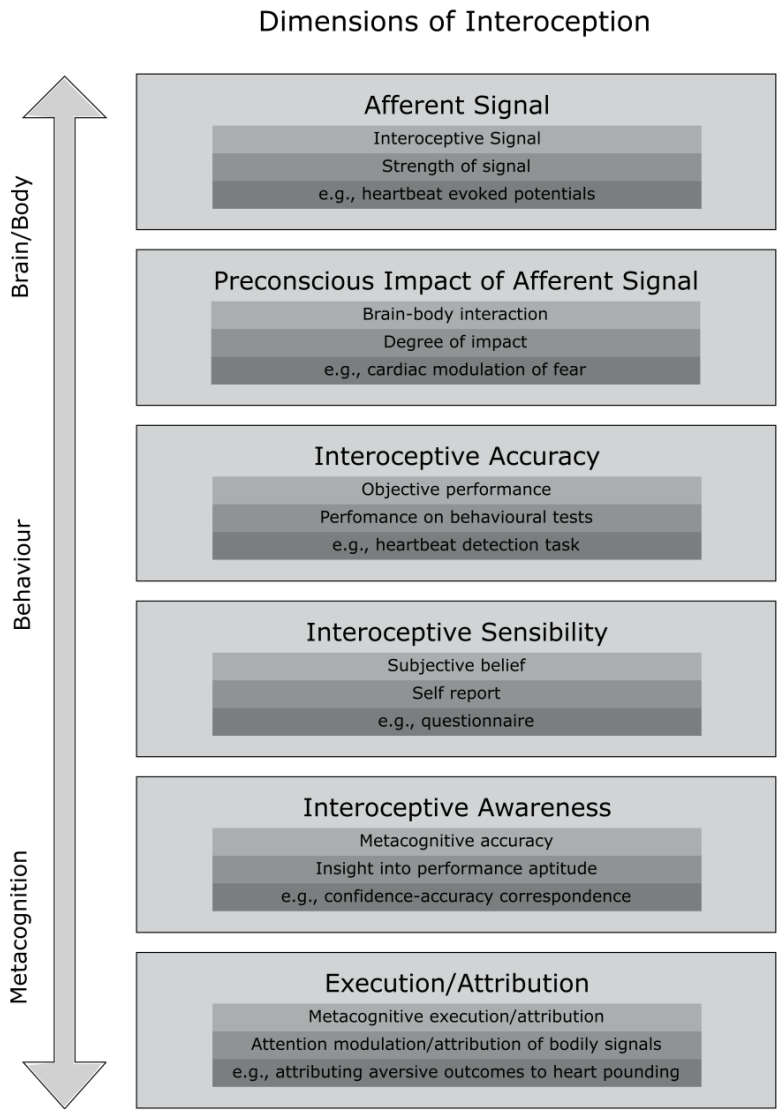


Figure 1.1. Dimensions of interoception.

Interoception spans a continuum from brain and body, to behaviour and metacognition. We can measure each facet to study their unique, or sometime co-contributing, effect on emotion and behaviour (figure taken from Quadt et al., 2018).

Preconscious impact of afferent signal: As we have discussed, interoceptive signals are continuously unconsciously monitored by the brain to maintain homeostasis, as in the case of the baroreflex, where the brain receives afferent and exerts efferent information to stabilise blood pressure. Due to the phasic nature of the heartbeat, and thus baroreflex sensitivity, we can study the top-down effect of cognitive processes, when stimuli are coupled to distinct phases of the cardiac

cycle; at systole, when baroreceptors fire, and at diastole, when baroreceptors are quiescent. Indeed, work has shown a dominant inhibitory effect during ventricular systole for pain (Edwards, Mcintyre, Carroll, Ring, & Martin, 2002; Gray, Rylander, Harrison, Wallin, & Critchley, 2009; McIntyre, Kavussanu, & Ring, 2008; Wilkinson, McIntyre, & Edwards, 2013), touch (Gahery & Vigier, 1974), startle eye blink (Schulz et al., 2009) and word processing and memory (Garfinkel et al., 2013). This relationship is however nuanced, systole can enhance familiarity during face recognition (Fiacconi, Peter, Owais, & Köhler, 2016), motor reactivity (Makowski, Sperduti, Blondé, Nicolas, & Piolino, 2020), active inhibition of motor responses (Rae et al., 2018), racial bias (Azevedo, Garfinkel, Critchley, & Tsakiris, 2017) as well as emotion specific effects, notably for fear (Garfinkel & Critchley, 2016; Garfinkel et al., 2014) and disgust (Gray et al., 2012).

Interoceptive accuracy: Interoceptive accuracy is a measurement of an individual's ability to perceive internal bodily sensations. In the case of cardiac interoception, numerous researchers have designed and implemented a range of different tasks aimed at objectively measuring cardiac interoception (Brener & Jones, 1974; Clemens & MacDonald, 1976; Epstein & Stein, 1974; Epstein, Cinciripini, McCoy, & Marshall, 1977), although two have emerged as the dominant methods; the heartbeat tracking task (Schandry, 1981), where participants are tasked with counting their own heartbeats over a time window, and the heartbeat discrimination task (Whitehead, Drescher, Heiman, & Blackwell, 1977; Katkin, Reed, & Deroo, 1983), where participants judge whether an auditory tone is synchronous or asynchronous with their own heartbeat. Both tasks have faced heavy criticism, notably the argument that using a fixed temporal window in which participants judge synchrony on the heartbeat discrimination task (250ms and 550ms after the R-wave) does not account for individual differences in heartbeat detection (Brener & Ring, 2016; Ring & Brener, 2018; Zamariola, Maurage, Luminet, & Corneille, 2018) and that the heartbeat tracking task is heavily influenced by prior knowledge of heart rate. Attempts have been made to mitigate these limitations, including adding in a time control task (Shah, Hall, Catmur, & Bird, 2016), carefully selecting task instructions (Desmedt et al., 2020; Murphy, Millgate, et al., 2018) and the implementation of a 'multi-interval task' (Brener & Ring, 2016), that presents tones at different time-intervals across the cardiac cycle to measure

the consistency of reporting to define individual optimum synchronous and asynchronous temporal windows. Nonetheless, despite criticism, there is still strong evidence that accuracy on both tasks is linked to activation in the interoceptive network, namely insula cortices, performance correlates with the heartbeat evoked potential (HEP) and accuracy scores are consistently linked to dimensions of emotional experience (for an overview, see Ainley, Tsakiris, Pollatos, Schulz, & Herbert, 2020; Corneille, Desmedt, Zamariola, Luminet, & Maurage, 2020; Zimprich, Nusser, & Pollatos, 2020).

Interoceptive sensibility: Interoceptive sensibility refers to subjective belief about sensitivity to interoceptive state, often measured via questionnaires. The two most commonly used are the Porges Body Perception Questionnaire (BPQ) (Porges, 1993) and the Multidimensional Assessment of Interoceptive Awareness (MAIA) (Mehling et al., 2012), although others have also been used, e.g. the Body Responsiveness Questionnaire (Daubenmier, 2005) and the Body Awareness Questionnaire (Shields, Mallory, & Simon, 1989). The commonly employed awareness subscale of the BPQ consists of 46 questions pertaining to bodily sensations and participants indicate their awareness of each sensation using a five point scale ranging from 'never' to 'always'. The MAIA was developed to build upon the shortcomings of previous questionnaires and aims to distinguish between 'different interoceptive attention styles that can be adaptive or maladaptive in processing interoceptive sensations to regulate emotions and behaviour' (Machorrinho, Veiga, Fernandes, Mehling, & Marmeleira, 2019). More recent scales have been developed, such as the Interoceptive Accuracy Scale (IAS) which measures self-perceived interoceptive accuracy (Murphy, Brewer, et al., 2018) or the Interoceptive Awareness Questionnaire (IAQ) (Bogaerts, Walentynowicz, Houte, Constantinou, & den Bergh, 2018), however neither are, as of yet, common place in the literature.

Interoceptive insight; (previously termed awareness; Garfinkel, Seth, Barrett, Suzuki, & Critchley, 2015): Interoceptive awareness, metacognitive interoception, henceforth referred to as interoceptive insight (Khalsa et al., 2018), refers to the level of insight individuals have into their own interoceptive performance. In this sense, interoceptive insight provides a trial-by-trial measure of how confidence predicts accuracy. Interoceptive insight on the heartbeat dis-

crimination task can be computed using type 2 receiver operating characteristic (ROC) curve analysis (Green, Swets, et al., 1966). Type 2 ROC analysis provides a non-parametric measure of metacognitive sensitivity independent of metacognitive bias, i.e. the extent to which confidence reflects accuracy, independent of the propensity to report high confidence (Sherman, Barrett, & Kanai, 2015). Interoceptive insight is dissociable from accuracy (Garfinkel et al., 2015) and, as we have found, is linked to distinct facets of emotional experience (Mulcahy, Davies, Quadt, Critchley, & Garfinkel, 2019).

1.7 Interoception and emotion

Emotions are commonly associated with feelings arising from the body, for example increased heart rate and shallow breath are both associated with the feeling of fear; as James stated, 'peripheral autonomic changes as they occur is the emotion' (James, 1884). As such, individual arousal levels can influence emotional experience (Schachter & Singer, 1962), individuals who are more accurate at judging their body states report more intense emotional experiences (Barrett, Quigley, Bliss-Moreau, & Aronson, 2004; Pollatos, Traut-Mattausch, Schroeder, & Schandry, 2007; Wiens, 2005) and increased interoceptive accuracy has also been associated with better emotional regulation (Füstös, Gramann, Herbert, & Pollatos, 2013).

The shared neural architecture underlying both interoceptive and emotional processes also highlights this bi-directional relationship; the insular cortex is consistently associated with interoceptive (Craig & Craig, 2009; Critchley, Wiens, Rotshtein, Öhman, & Dolan, 2004; Kurth, Zilles, Fox, Laird, & Eickhoff, 2010; Pollatos et al., 2007; Zaki, Davis, & Ochsner, 2012) and emotional (Lamm & Singer, 2010; Lindquist, Wager, Kober, Bliss-Moreau, & Barrett, 2012; Singer, Critchley, & Preuschoff, 2009; Wicker et al., 2003) processes, and its reciprocal connections, with regions included anterior cingulate cortices and amygdala, form a social-emotional network (Adolfi et al., 2017) that show consistent activation on heartbeat detection paradigms (Canales-Johnson et al., 2015; Critchley et al., 2004; Pollatos et al., 2007). Additionally, injury to the insula cortex yields a reduced ability to recognize or name emotions (Adolphs, Tranel, & Damasio, 2003; Calder, Keane, Manes, Antoun, & Young, 2000) and results in impaired intero-

ceptive ability (Couto et al., 2015). Thus, the cortical conscious or unconscious processing of interoceptive information shapes and informs subjective emotional experience (Critchley & Garfinkel, 2017).

In this sense, interoceptive signals contribute to the development of affective symptomatology. Anxiety disorders are often accompanied by exaggerated somatic sensations such as palpitations, difficulty breathing and increased heart rate (Stern, 2014). The misinterpretation of somatic sensation is a key feature of anxiety symptomatology (Clark, 1986) and patients consistently report worrying about bodily signals (Antony et al., 1995; Yoris et al., 2015). Increased responsiveness of the autonomic nervous system may sensitize anxious individuals to bodily signals (Lyyra & Parviainen, 2018) resulting in increased attention to somatic sensations (Anderson & Hope, 2009; Gupta, 2013). Indeed, increased interoceptive accuracy has been show to relate to increased anxiety traits (Domschke, Stevens, Pfleiderer, & Gerlach, 2010; Dunn et al., 2010; Lyyra & Parviainen, 2018) and anxious individuals have been found to be more accurate at detecting their heartbeat compared to controls (Stevens et al., 2011; Van der Does, Antony, Ehlers, & Barsky, 2000). At the neural level, interoception and anxiety both share overlapping neural networks of regions implicated in the generation of autonomic states of arousal (Ottaviani et al., 2016). Indeed, aberrant activation in anxiety is reported in amygdala (Babaev, Chatain, & Krueger-Burg, 2018; Davis, 1992; Tye et al., 2011), insula (Paulus & Stein, 2006; Stein, Simmons, Feinstein, & Paulus, 2007) and cingulate cortices (Brooks & Stein, 2015; Duval, Javanbakht, & Liberzon, 2015; Paulus & Stein, 2010).

Anxiety has been associated with increased physiological arousal (Eckman & Shean, 1997) but results are not always consistent (Anderson & Hope, 2009; Hoehn-Saric, McLeod, Funderburk, & Kowalski, 2004). Some work also shows no relationship between interoceptive accuracy and anxiety (Antony et al., 1995; Barsky, Cleary, Sarnie, & Ruskin, 1994; Ehlers, Margraf, Roth, Taylor, & Birbaumer, 1988) or the inverse relationship where reduced interoceptive accuracy is related to increased anxiety (De Pascalis, Alberti, & Pandolfo, 1984). Other models, such as the 'active-inference' account of aberrant interoceptive processing, may explain these inconsistent findings. In these models, top down interoceptive predictions meet bottom up interoceptive sensation to produce a dynamic integrative map of

the internal and external environment to guide optimal functioning. When these predictions do not align 'prediction errors' are produced requiring autonomic corrective action (Khalsa et al., 2018; Paulus & Stein, 2006, 2010). In anxiety, these prediction errors are thought to be exaggerated and persistent which in turn may dysregulate the body causing an altered perception of bodily state. Such persistent errors might lead to attentional bias toward threat or increased worry and can cause the body to continue making homeostatic changes which promote anxious states. Additionally, since these errors may guide adaptive behaviour, persistent errors could result in a chain of poor choices which promotes further prediction errors further strengthening maladaptive behaviour and cognition. In a recent model, aberrant interoceptive prediction errors have been implicated in casually influencing anxiety symptomatology in autistic adults (Garfinkel, Tiley, et al., 2016). Given the high prevalence of anxiety in this population (Hollocks, Lerh, Magiati, Meiser-Stedman, & Brugha, 2019), a deeper understanding of interoceptive ability and how this relates to emotional experience in this population is warranted.

1.8 Autism spectrum conditions

Autism Spectrum Conditions (ASC) are a set of pervasive neurodevelopmental conditions characterised by social communication difficulties, the presence of fixed interests and repetitive behaviours and the presence of atypical hypo and/or hyper sensory sensitivity (American Psychiatric Association, 2013). Autism is associated with marked impairments in emotional processing including difficulty identifying emotion in self and others (Hill, Berthoz, & Frith, 2004; Hubert et al., 2007), even in the presence of bodily responses (Gu, Hof, Friston, & Fan, 2013). Autism is also associated with impaired emotion regulation (Mazefsky et al., 2013), altered emotional face recognition (Harms, Martin, & Wallace, 2010) and impaired theory of mind (Baron-Cohen, 1997). Indeed, neuroimaging research supports these alterations by demonstrating aberrant activation and altered functional connectivity in brain circuity involved in autonomic and emotional control, including the insula (Ebisch et al., 2011; Francis et al., 2019; Guo et al., 2019; Hogeveen, Krug, Elliott, & Solomon, 2018; Odriozola et al., 2016; Xu et al., 2018), anterior cingulate cortex (Agam, Joseph, Barton, & Manoach, 2010; Mundy, 2003; Simms, Kemper,

Timbie, Bauman, & Blatt, 2009; Thakkar et al., 2008), prefrontal cortex (Gilbert, Bird, Brindley, Frith, & Burgess, 2008; Gilbert, Meuwese, Towgood, Frith, & Burgess, 2009; Shalom, 2009) and amygdala (Ibrahim et al., 2019; Lassalle et al., 2017; Leung, Pang, Anagnostou, & Taylor, 2018; Tam et al., 2017; Top Jr et al., 2016).

The current literature examining interoception in autism is in its infancy however a trend of altered interoception in this population has started to emerge, though it should be noted that such differences are not always observed (Nicholson et al., 2018; Mash, Schauder, Cochran, Park, & Cascio, 2017; Schauder, Mash, Bryant, & Cascio, 2015; Shah et al., 2016). Research has reported reduced interoceptive accuracy in autistic children (Palser, Fotopoulou, Pellicano, & Kilner, 2018) and adults (Garfinkel, Tiley, et al., 2016), and increased interoceptive sensibility compared to controls (Garfinkel, Tiley, et al., 2016; Mul, Stagg, Herbelin, & Aspell, 2018). Subjectively, evidence suggests autistic participants report a hyper sensitivity to external stimuli yet a blunted, hypo sensitivity to internal sensations (Elwin, Ek, Schröder, & Kjellin, 2012; Fiene & Brownlow, 2015), although it should be noted that it is possible autistic individuals experience both a hyper and hypo sensitivity to external stimuli, both across and within sensory modalities (e.g. Robertson & Simmons, 2015). Conversely, some argue that interoceptive impairments are not a core feature of autism (Nicholson et al., 2018) and interoceptive impairments actually underlie the manifestation of alexithymia (Bird et al., 2010; Cook, Brewer, Shah, & Bird, 2013; Shah et al., 2016), defined as difficulty identifying and describing one's own emotional feelings (Apfel & Sifneos, 1979), which is highly prevalent in autism (Hill et al., 2004). Thus far, relatively little work has investigated the neural underpinnings of interoceptive ability however one study (Failla et al., 2020) found no objective accuracy differences between autistic and control participants and no group differences in insula activation. They did, however, find that insular response interacted with group to predict autistic symptoms which, speculatively, could represent intact interoceptive accuracy yet aberrant interoceptive processing in a different dimension, i.e. sensibility or insight.

The high concordance between interoception and emotion, and the high prevalence of anxiety in autistic individuals, is demonstrated by work that has re-

lated impaired interoception to the manifestation of anxiety in autistic individuals (Garfinkel, Tiley, et al., 2016; Palser et al., 2018). Here, the 'interoceptive trait prediction error' (ITPE), defined as the difference between subjective sensibility (as measured by the Body Perception Questionnaire (Porges, 1993)) and objective accuracy (i.e. performance on a heartbeat detection task), which is distinct from interoceptive insight, which is a metacognitive measure of trial-by-trial confidence-accuracy correspondence, predicted anxiety. Notably, the ITPE scores can be considered a trait measure, i.e. enduring over time, whilst interoceptive insight can be considered more of a state measure, i.e. providing insight at that moment in time, on a trial-by-trial basis (Koreki et al., 2020). Placed in a predictive-coding framework, this postulates that autism, and subsequent co-morbid anxiety, may be associated with a failure to incorporate ascending interoceptive error signals with descending predictions that inform subjective sensibility. It thus follows that a training paradigm aimed at aligning these dimensional signals may reduce subjective and autonomic states of anxiety.

1.9 Aims and hypotheses of this thesis

The overall aim of this thesis is to develop a deeper understanding of emotion processing in autism and determine how this may relate to distinct facets of interoception, from a behavioural and neuroimaging perspective.

In chapter 2, I sought to investigate the relationship between interoception and affective prosody recognition in autistic adults. I hypothesised that autistic, relative to non-autistic, participants would show reduced performance on a test of prosodic emotional discrimination and that this deficit would correspond to a reduction in both interoceptive accuracy and metacognitive interoceptive insight.

In chapter 3, my aim was to extend our understanding of how unconscious interoceptive signals influence emotion perception, particularly towards fear, in autism from a behavioural and neuroimaging perspective. I hypothesised that all participants (autistic and non-autistic) would show a relative enhancement of fear processing when stimuli were presented at cardiac systole, an effect that would be exaggerated in individuals scoring high in trait anxiety. At the neural level, autistic participants would present with atypical functional reactivity and connectivity of regions that integrate interoceptive signals with emotional processes.

In chapter 4, I sought to further our understanding of interoceptive ability, and the accompanying neural activation and functional connectivity, in autistic adults, relative to non-autistic controls. I hypothesised that autistic adults would show reduced interoceptive accuracy on heartbeat tracking and discrimination tasks and that this would correspond with altered activation and reactivity in interoceptive neural regions, namely insula cortices. In a two-part study, I also employed a novel interoceptive training paradigm to investigate neural and behavioural changes in interoceptive ability as a result of targeted interoceptive training. I hypothesised that interoceptive accuracy and insight would significantly increase following training and the activation and functional connectivity of insula cortices would also increase following training. I also hypothesised that this increase would correspond to a reduction in subjective and autonomic levels of anxiety.

In chapter 5, my aim was to assess the impact of interoceptive training on emotional processing. I hypothesised that fear processing at cardiac systole would be enhanced following interoceptive training. Additionally, the relationship between systolic enhancement of fear and anxiety would be reduced as a result of interoceptive training induced reduction in anxiety levels. In brain, I predicted that, following training, we would observe increased activation and functional connectivity of amygdala and insula when viewing fearful stimuli and increased functional connectivity of right insula during systole.

In chapter 6, I sought to investigate the impact of a novel affective prosody recognition training paradigm on subsequent emotional prosody recognition and how this would relate to interoceptive insight. I hypothesised that we would observe a significant increase in affective prosody recognition following training and this improvement will correspond with improvement in subjective states of anxiety, depression and with a reduction in self-reported alexithymia traits.

1.10 Methodological considerations

There are a few noteworthy considerations in the methodology employed throughout this thesis. Firstly, all autistic participants referenced throughout this thesis were recruited as part of a larger cohort for the 'Aligning Dimensions of Interoceptive Experience' (ADIE) study. The sample size for this study was calculated

based on experimental data (Garfinkel, Tiley, et al., 2016). This data showed that the mean trait anxiety levels, as measured on the State and Trait Anxiety Inventory (STAI; Spielberger, 2010), in individuals with Autism was 52.65 (sd = 12.03). The primary outcome measure of the study was the concordance between levels of trait anxiety and the interoceptive trait prediction error, with the hypothesis that decreasing the interoceptive trait prediction error will reduce anxiety levels, as measured on the STAI (Spielberger, 2010). A clinical meaningful difference would be 7.65 points following treatment (Critchley et al., 2004). Thus, with a threshold of significance set at 5% for a two-sided test, power set at 90% and a 1:1 allocation ratio, a sample size of 53 participants is needed per arm (total study size, N = 100) to detect a difference in means of 7.65 between the treatment group and the control group (s.d. = 12.03) based on a t-test. Recruitment was increased to 120 to allow for 10% drop out. The sample size (n=40) within the neuroimaging study was informed by budget constraints and previous neuroimaging work to permit across group regressions analyses of individual differences in baseline measures and treatment response expressed as change in task-related activation differences in amygdala and insula during emotional processing in autism (Critchley et al., 2000; Garfinkel, Tiley, et al., 2016; Garfinkel et al., 2014; Makovac et al., 2016). The subset of participants who were scanned pre and post interoceptive training, in chapter 4, were not sufficiently powered to observe the primary outcome measure (i.e. the drop in anxiety in the main sample). Therefore, the autistic sample analysed in chapters 3, 4, 5 and 6 was based on the final available data collected whilst the sample analysed in chapter 2 was based on the data collected up until that point (i.e. analysis was conducted mid-way through the trial). The control group recruited in chapter 2 were recruited as part of a separate pilot study and thus the sample size of this control group was limited. The control group, as seen in chapter 3 and 4, were selected to match the autistic scanning group (n=40 in each group, matched on age, gender and education) and thus the sample size is adequate, as we have already mentioned, for between group regression analyses. Throughout all data collection there was some drop out/excluded participants in both groups and the drop in statistical power as a result is acknowledged.

Next, as autistic participants were recruited as part of a clinical randomised-

control trial (RCT), allocation to study group (interoceptive training or prosodic training, as seen in chapters 4 and 6 respectively) was randomised. Participants were allocated to either the treatment arm or control arm using a 1:1 ration and permuted block randomisation by the Brighton and Sussex Clinical Trials Unit (CTU). After allocation, the researcher liaised with the CTU regarding participant group allocation. Unfortunately, double-blinding was not possible as both the experimenter and researcher were aware of which group was expected to deliver therapeutic benefit (with reference to the primary outcome measure). However, in line with the pre-defined protocol, the experimenter who collected data from the follow-up session, i.e. the session in which the primary outcome measures (anxiety and interoceptive ability post interoceptive training) were measured, was blind to the participants group allocation.

Regarding statistical analyses employed throughout this thesis, my approach for all statistical analyses was to ensure that I selected the most robust test to identify significant effects whilst mitigating type 1 and type 2 errors. Details of statistical methods employed can be seen in the relevant methods section of each data chapter. For all behavioural analyses, I used 'Statistical Package for the Social Sciences' (SPSS) whilst all neuroimaging data were analysed using Statistical Parametric Mapping version 12 (SPM-12), a MATLAB toolbox built on consensus approaches to optimal neuroimaging analyses grounded upon general linear models.

One final noteworthy consideration is that some chapters of this thesis contain missing data (reported in the relevant methods section of each chapter). I thus computed Little's Missing Completely at Random (MCAR) test to test that data was missing completely at random and thus not related to any of the dependent variables. In all cases, Little's MCR test was not-significant ($p > 0.05$) meaning I failed to reject the null hypothesis and thus conclude that the data was missing completely at random. As such, I opted to perform complete case analysis (Jakobsen, Gluud, Wetterslev, & Winkel, 2017) by selectively removing participants with missing values from the analysis, with the assumption that the observed data will not be biased (Sterne et al., 2009), and thus should represent the overall sample, however I do acknowledge the reduction in statistical power as a consequence of the reduced sample size.

Chapter 2

Interoceptive awareness mitigates deficits in emotional prosody recognition in autism

2.1 Abstract

The sensing of internal bodily signals, a process known as interoception, contributes to subjective emotional feeling states that can guide empathic understanding of the emotions of others. Individuals with Autism Spectrum Conditions (ASC) typically show an attenuated intuitive capacity to recognise and interpret other peoples' emotional signals. Here we test directly if differences in interoceptive processing relate to the ability to perceive emotional signals from the intonation of speech (affective prosody) in ASC adults. We employed a novel prosody paradigm to compare emotional prosody recognition in ASC individuals and a group of neurotypical controls. Then, in a larger group of ASC individuals, we tested how recognition of affective prosody related to objective, subjective and metacognitive (awareness) psychological dimensions of interoception. ASC individuals showed reduced recognition of affective prosody compared to controls. Deficits in performance on the prosody task were mitigated by greater interoceptive awareness, so that ASC individuals were better able to judge the prosodic emotion if they had better insight into their own interoceptive abilities. This data links the ability to access interoceptive representations consciously to the recognition of emotional expression in others, suggesting a crossmodal target for interventions to enhance interpersonal skills.

2.2 Introduction

Emotions fall into categories that are broadly differentiable by their affective and motivational flavour and by their individual behavioural response repertoires. These are underpinned by patterned changes in both central neural responses and peripheral bodily physiology (Kreibig, 2010; Tracy & Randles, 2011). Affective and physiological representations undergo higher contextual and retrospective appraisal, from which the specific emotional experience is ultimately constructed (Barrett, 2017; Seth, Suzuki, & Critchley, 2012). Importantly, it has been argued that the sensing of changes in bodily physiology shape and inform subjective emotional feeling states (Lange, James, & Dunlap, 1967).

Interoception encompasses the afferent signalling, central processing, neural and mental representation of internal (visceral) bodily signals (Critchley & Garfinkel, 2017). Interoception can be partitioned according to channel (e.g. humoral or neural; spinothalamic /vagal) and organ (e.g. cardiac, vascular, gastrointestinal). Moreover, at the psychological level, interoception can be parsed into dissociable objective, subjective and metacognitive dimensions (Garfinkel et al., 2015). Objective measures of 'interoceptive accuracy' can be derived from performance on behavioural tests of interoception (e.g. tests of heartbeat perception). Subjective interoception, 'interoceptive sensibility', reflects self-reported measures of interoceptive experience, which can be quantified using questionnaires. Metacognitive interoception, 'interoceptive awareness', refers to the level of insight of individuals into their own interoceptive performance. This can be computed from the correspondence between objective and subjective interoceptive measures (e.g. trial-by-trial judgments of task performance accuracy and confidence). Across normative populations, these dimensions are dissociable (Garfinkel et al., 2015). Relationships are reported between heightened interoceptive accuracy and the intensity of subjective emotional experiences (Pollatos et al., 2007; Wiens, Mezzacappa, & Katkin, 2000). Moreover, the mismatch between subjective / objective and the related metacognitive aspects of interoception are implicated clinically in the genesis of psychological symptomatology (Garfinkel, Tiley, et al., 2016; Yoris et al., 2015). More broadly, the established relationships between interoceptive processing and emotional experience (e.g. Barrett et al., 2004; Craig, 2003; Seth, 2013) support the notion that human emotions encompass feeling states that draw

upon interoceptive abilities.

Autism Spectrum Conditions (ASC) are a set of pervasive neurodevelopmental syndromes characterised by social and emotional impairments, restrictive, repetitive behaviours, atypical sensory sensitivity and communication difficulties. Particular impairments are described in identifying emotions in self and others (Hill et al., 2004; Hubert et al., 2007). Within the ASC population, explicit deficits in empathy can occur in the presence of empathic bodily responses (Gu et al., 2015), suggesting that ASC individuals have difficulty integrating their intact (or even heightened) physiological responses to emotional cues into overt emotional judgements and subjective empathy. At the neural level, circuits involving the 'viscerosensory' insular cortex support the representation of autonomic and visceral information (Critchley et al., 2004; Harrison, Gray, Gianaros, & Critchley, 2010) and, through the anterior insula, conscious access to interoceptive signals and their integration with sensory representations in other modalities. By extension, the insular cortex is considered a critical neural substrate for emotional awareness (Craig & Craig, 2009; Critchley et al., 2004; Harrison et al., 2010; Pollatos, Kirsch, & Schandry, 2005; Singer et al., 2009; Terasawa, Shibata, Moriguchi, & Umeda, 2013). Insula reactivity is reported to be atypical in ASC individuals when engaged in processing emotional and motivational information, including; the appraisal of social rewards (Leung et al., 2018); active inhibition of responses to affective stimuli (Duerden et al., 2013); interpretation of bodily expressions (Hadjikhani et al., 2009), and; evaluation of incongruent emotional information (Watanabe et al., 2012). ASC individuals also show alterations in the intrinsic functional connectivity between insular regions and other brain centres involved in emotion and sensory processing (Anteraper et al., 2019; Cheng et al., 2017; Xu et al., 2018). Together, these findings are consistent with the hypothesis that deficits in emotional processing in ASCs may arise, in part, through neurobiological differences in substrates for interoceptive representation, integration, and appraisal.

ASC individuals are reportedly impaired at translating salient interoceptive signals into higher order brain representations (Fiene & Brownlow, 2015; Uddin, 2015). Sensory differences associated with ASC extend to a reported hyposensitivity to interoceptive cues, impairing accurate detection of internal bodily sen-

sations (Elwin et al., 2012). ASC individuals also manifest atypical temporal binding of information across sensory modalities: there is an expansion of audio-visual, visual-tactile and cardio-visual temporal binding windows, referring to the temporal window over which participant's judge two events as occurring in synchrony (Noel, Lytle, Cascio, & Wallace, 2018). This observation is relevant to the interpretation of the heartbeat discrimination task commonly used to quantify interoceptive accuracy from synchrony judgements between heartbeat and external stimuli (Brener & Kluvitse, 1988; Whitehead et al., 1977). The wider temporal binding window of ASC individuals suggests a core difference in higher-order cross-modal sensory integration. Putatively, this difference may specifically compromise emotional flexibility, in part through the sluggish central integration of interoceptive signals with prior affective representations and/or new exteroceptive information. In ASCs, objective interoceptive accuracy can be impaired in both adults (Garfinkel, Tiley, et al., 2016; Mul et al., 2018) and children (Palser et al., 2018). However, deficits in heartbeat detection accuracy are not always observed (Nicholson et al., 2018; Schauder et al., 2015). Variability in interoceptive accuracy reported across studies of ASC may be driven by variation in symptom profiles, e.g. the extent of anxiety or, notably, the presence or absence of alexithymia (Shah et al., 2016).

Simulation of neural and bodily states may underpin and facilitate the recognition of (and empathy for) emotional states of other individuals (Gallese & Goldman, 1998; Jackson, Meltzoff, & Decety, 2005; Lee, Dolan, & Critchley, 2008; Singer et al., 2009). There is evidence within the visual domain for interoceptive facilitation of emotional judgements, e.g. from facial expressions (Garfinkel et al., 2014; Gray et al., 2012). However, in the auditory domain, the relationship between interoception and the discrimination of emotional intonation of speech (affective prosody) is underexplored. Affective prosody refers to the use of non-linguistic features of speech, for example varied pitch and volume, to convey emotional information in support of adaptive interpersonal communication and social exchange (Hubbard, Faso, Assmann, & Sasson, 2017; Shriberg et al., 2001). Affective prosody is distinct from pragmatic prosody, defined as the accenting of words or syllables to convey meaning, and syntactic prosody, which refers to the use of boundary markers or pauses or the segmentation of utterances (Peppé,

Cleland, Gibbon, O'Hare, & Castilla, 2011).

ASC individuals can manifest marked deficits in the production and recognition of affective prosody. This is consistent with other emotional processing deficits commonly associated with ASCs (Hadjikhani et al., 2009; Hill et al., 2004). Possible basic mechanisms that have been proposed to underlie these deficits include altered perceptual processing (Adolphs, Sears, & Piven, 2001; Williams, Goldstein, & Minshew, 2006), impaired multimodal sensory integration (Lerner, McPartland, & Morris, 2013), impaired integration of perceptual information and social contextual information (Mottron, Dawson, Soulieres, Hubert, & Burack, 2006), dysfunctional mirror neuron system (Dapretto et al., 2006), atypical gaze and attention toward facially expressed emotions (Black et al., 2017), and impaired theory of mind (Baron-Cohen, 1997). Aberrant interoception may also provide a plausible account extending evidence for impaired sensory integration in ASC to the interoceptive (rather than exteroceptive) domain. Individuals with ASC may be impaired in sensing and integrating the affective information contained within their own bodily responses when inferring the emotions of others. The recognition of emotional prosody may thus rely on such interoceptive reference.

Affective prosodic information is important to smooth social interaction (Wang & Tsao, 2015). For many individuals with ASC, prosodic impairment may exacerbate awkward social communication. However, difficulties in processing affective prosody vary across ASC individuals. Correspondingly, some studies report marked impairments (Golan, Baron-Cohen, & Hill, 2006; Lindner & Rosén, 2006; Peppé et al., 2011; Rosenblau, Kliemann, Dziobek, & Heekeren, 2017), while others fail to show significant differences between ASC individuals and controls (Brennand, Schepman, & Rodway, 2011; Grossman, Bemis, Skwerer, & Tager-Flusberg, 2010; Le Sourn-Bissaoui, Aguert, Girard, Chevreuil, & Laval, 2013). Male-female differences may contribute to some of this variability; observed gender specific dissociation (e.g. Rosenblau et al., 2017; Schneider et al., 2013), is not always replicated (e.g. Hubbard et al., 2017; McLennan, Lord, & Schopler, 1993; Rivet & Matson, 2011). Other factors that may further account for this inconsistency include small group size, methodological differences, wide variance in performance and study-particular features of research participants. We also hypothesize that individual differences in interoception may be an im-

portant contributing factor, wherein deficits in interpreting affective prosody may be amplified when coupled to aberrant interoceptive processing.

Here, based on the notion that the sensing and representation of interoceptive bodily signals underpins emotional feeling states, and hence the capacity to understand emotional information in self and others, we investigated the relationship between affective prosody recognition and interoceptive abilities in ASC individuals. We hypothesized that ASC adults, relative to neurotypical controls, would show reduced performance on a test of prosodic emotional discrimination. Moreover, within a larger group of ASC adults, we hypothesized that reduced prosodic accuracy would correspond with reductions in both interoceptive accuracy and metacognitive interoceptive awareness.

2.3 Methodology

2.3.1 Participants

Seventy Four participants with a confirmed ASC diagnosis (38 male, 36 female; mean age 36.7; range 18–64 yrs) and 20 neurotypical controls (9 male, 11 female, mean age 34; range 22–51 yrs) took part in the study. 20 participants from the ASC group (mean age=34.95, range 20–57 yrs) were age and sex matched to controls, with equal numbers of males and females in each group, to allow for a direct comparison between groups. All ASC participants were fluent English speakers, 6 were left handed and the remaining 68 were right handed. None of the ASC participants had a history of past head injury or organic brain disorders, cognitive impairment or a learning disability (general mental impairment); none had asthma/respiratory illnesses, epilepsy or evidence of psychotic experiences. Ten ASC participants reported that they had completed GCSE's or similar, 16 A level or similar, 13 attended university or business college but did not receive a degree, 23 had received an undergraduate degree and 12 had received a postgraduate degree.

Control participants were recruited from the University of Sussex and members of the local community. ASC participants were recruited from the Sussex Partnership Neurobehavioral Clinic as well as through advertisements placed on social media and via leaflets and posters. All participants provided written in-

formed consent with all procedures approved by the local ethics committee at the University of Sussex, School of Psychology, and the NHS Research Ethics Committee.

2.3.2 Prosody paradigm

The affective prosody protocol was designed using Paradigm Experiments software (2016). All emotions were taken from the EU Emotion stimulus set (O'Reilly et al., 2012) which comprises 507 audio files and 166 photographs depicting 21 different emotions. The stimulus set features a diverse balance of adults and children of different genders and various races. All photographs and audio files have been validated in three languages to confirm they represent their assigned emotional labels (O'Reilly et al., 2016). Emotions included feature the six basic emotions; happy, sad, disgusted, surprised, angry, afraid (Ekman, 1992). These were presented in two levels of intensity - regular and mild. In addition, thirteen complex emotions were also included; bored, kind, jealous, unfriendly, hurt, disappointed, interested, joking, ashamed, proud, excited, frustrated and worried. The audio clips were content neutral to ensure that emotion may only be detected through prosodic cues. Any audio clips deemed to include semantic content were removed and omitted from the study.

Three different trial types were utilised; matching voices to faces (face-only), matching voices to emotion descriptors (text-only) and matching voices to faces and emotion descriptors combined (face with text) (Figure 2.1). Each domain was further divided into positive and negative valence. In total 114 trials were completed (38 face-only, 38, text-only and 38 face with text). Each of the 19 verbally expressed emotions were presented twice for each domain but remained novel. The presentations were randomised and no trials were repeated. Out of 114 trials, 72 were of a negative valence (24 out of each trial type).

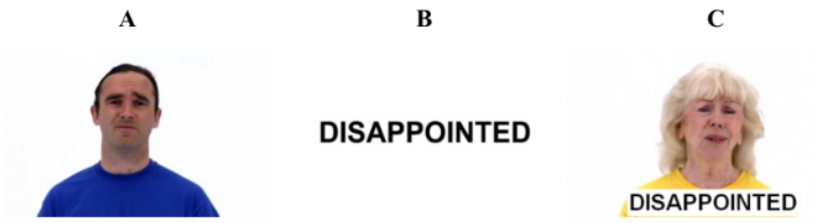


Figure 2.1. Prosody paradigm: stimuli examples.

Example of stimuli displayed during prosody paradigm for face-only (A), text-only (B) and face with text (C) trials. Each trial displayed one stimulus type with four different emotion choices.

Participants were first instructed to put on over-the-ear headphones and were presented with on screen instructions explaining that they would hear audio clips of different phrases and that they should "focus on the tone of voice as much as possible". After each audio clip, they were presented with different emotion options in the form of facial expressions (figure 2.2A, face only condition), words (figure 2.2B, text only condition) or faces with words (figure 2.2C, face/text combined condition). Their task was to decide which of the emotions best matched the tone of voice in the clip that they had just heard. Once it was clear that participants fully understood the task, they then progressed to the main experiment. This comprised 114 trials, where the voice was played while the four different emotion options were presented simultaneously on the screen (figure2.2). These represented the correct emotion plus 3 distractors; two emotions of same valence and one of an opposing valence, randomly selected from the remaining stimuli. Depending on trial type, these were either in the form of face only, text only or face/text combined, all four options remained on screen until the user responded. The dependant variable was response accuracy, measured as the correct selection of the matching emotion.

Figure 2.2. Prosody paradigm: example trials.

Example trial displayed during the prosody paradigm. Each trial was either a face only (A), text only (B) or face with text (C) trial.

2.3.3 Interoceptive accuracy

Two measures were used to determine objective behavioural interoceptive accuracy in the ASC group: the heartbeat-tracking task (Schandry, 1981) and the heartbeat discrimination task (Katkin et al., 1983; Whitehead et al., 1977), which showed no correlation in the current sample ($r = 0.193$, $p = 0.102$). Participants' heartbeat was measured at rest using a medical-grade pulse oximeter (Nonin4600 pulse oximeter, Nonin Medical Inc. Plymouth MN USA) fitted with soft finger cuff (not tension / spring-loaded).

Participants first completed the heartbeat-tracking task, and were required to concentrate on their heartbeat and without physically checking, silently count how many heartbeats they felt in their body from the time they heard "start" to when they heard "stop". Six durations, presented in a random order, of 25, 30, 35, 40, 45 and 50s were used. After each trial, participants completed a visual analogue scale (VAS), with a scale of 0–10, to signal confidence of their decision.

Previous research has demonstrated a positive relationship between heartbeat-tracking performance accuracy with IQ (Mash et al., 2017; Murphy, Millgate, et al., 2018). Although years of education and educational attainment provide a pragmatic measure for general intelligence, only a subset of our participants had formal IQ measures (N=39). We therefore did not enter performance on the heartbeat-tracking task into further analyses. Consequently, the present study focused on results obtained from the heartbeat discrimination test.

The heartbeat discrimination task involved the presentation of a periodic external stimulus and participants were tasked with identifying whether the tones were presented synchronous or asynchronous with their own heartbeat. Participants were presented with 10 auditory tones, 20 times to form 20 trials. Tones were presented at 440Hz with a 100ms duration. In the heartbeat discrimination task, tones were triggered at the rising edge of the pulse pressure wave, representing mid ventricular systole, on synchronous trials. On the delayed trials, tones were triggered 300ms after the rise of the pulse pressure wave, representing early diastole. Adjusting for an average pulse transit time of 250ms, these tone timings corresponded to 250ms or 550ms after the ECG R-wave, putatively the time of peak perceptual differentiation. At the end of each trial, participants reported whether the tone was synchronous or asynchronous with their heartbeats, and then provided a confidence rating using the VAS scale. The auditory tones were always presented at the participant's own heart rate, hence the participant was unable to use the tempo of tones or knowledge about their own heart rate to inform their response (Garfinkel et al., 2015).

2.3.4 Interoceptive sensibility

All participants in the ASC group completed the awareness section of the Porges Body Perception Questionnaire (BPQ) (Porges, 1993). The scale comprises of 45 questions pertaining to bodily sensations and participants indicate their awareness of each sensation using a five-point scale ranging from 'never' to 'always'. ASC participants also completed the Multidimensional Assessment of Interoceptive Awareness (MAIA) (Mehling et al., 2012). Confidence judgments were also taken after each trial in both the heartbeat tracking and heartbeat discrimination tasks to determine confidence in task performance accuracy.

2.3.5 Interoceptive awareness

Interoceptive awareness, also termed interoceptive insight (Khalsa et al., 2018) and interoceptive metacognition (Garfinkel, Manassei, et al., 2016) is a metacognitive measure derived from confidence-accuracy correspondence (Garfinkel et al., 2015). For the discrimination task, interoceptive awareness was quantified using receiver operating characteristics (ROC) curve analysis (Green et al., 1966) for confidence-accuracy correspondence on a trial-by-trial basis. ROC analysis indexes the strength of correspondence between confidence (measured by VAS) and a binary state variable, i.e. correct or incorrect asynchrony judgements during heartbeat discrimination. Confidence judgements were divided by hit rate, the proportion of correct trials on which confidence was high (y-axis), and the false alarm rate (x-axis), the proportion of incorrect trials on which confidence was high. The area under the curve then gives a measure of the extent to which confidence reflects accuracy, independent of the participant's propensity to report high confidence (Garfinkel et al., 2015), with higher scores indicating better interoceptive metacognition.

2.3.6 Questionnaires

In addition to completing the awareness section of the BPQ, and the MAIA, participants in the ASC group also completed the Autism Quotient (AQ) (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001), the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Patient Health Questionnaire (PHQ-9) (Kroenke, Spitzer, & Williams, 2001) and the Toronto Alexithymia Scale (TAS-20) (Bagby, Parker, & Taylor, 1994). Participants in the control group completed the AQ and the STAI. For each questionnaire, no sub-scales were used and thus the total scores were computed and used in the analysis.

2.4 Data analysis

Group differences in age, anxiety and AQ scores were determined using independent sample t-tests. Between-group differences in performance of the prosody task were assessed using a 2×3 analysis of variance (ANOVA) with group as the between-subjects factor (ASC, control) and trial type as the within-subject factor

(face, face with text, text). We also tested for effects of emotional valence and emotional complexity by conducting 2 mixed $2 \times 2 \times 3$ ANOVAs with group as the between-subjects factor (ASC, control) and trial type (face, face with text, text) and emotion (positive vs negative / basic vs complex) as within-subject factors. State and trait anxiety were subsequently entered as separate covariates to check that group differences could not be ascribed to individual differences in anxiety symptomatology.

The relationship between interoception and prosody was investigated in the larger ASC sample (N=74) by separately entering the three dimensions of interoception, accuracy, sensibility (BPQ and MAIA separately) and awareness, as covariates into a one-way analysis of covariance (ANCOVA), with trial type as the within-subject factor. We also examined the effect of emotional valence and emotional complexity by conducting 2, 2×3 ANCOVAs (with emotion – positive vs negative / complex vs basic, and trial type as within-subject factors) and subsequently entering the three dimensions of interoception as covariates. Significant effects pertaining to interoceptive awareness and emotional prosody were followed up with correlational analyses to explore the effects of sex. The significant differential relationship between interoceptive awareness and prosody accuracy in males versus females was ascertained by computing a Fisher's r to z transformation so z scores could be compared and analysed for statistical significance (Lenhard & Lenhard, 2014). Notably, the interoception analyses included only the autistic participants as the non-autistic control participants were recruited as part of a separate study where interoception was not measured.

Within-group individual differences in prosody performance were examined and AQ scores, TAS-20 scores, trait anxiety and depression scores were added individually to each ANCOVA to understand the relative contribution of ASC, alexithymia and affective symptomatology to prosodic accuracy. Heart rate was controlled for in all ASC analyses not involving the control group by entering mean BPM as a covariate (3 participants had missing BPM data so were not included in these analyses). Significant interactions were further explored using paired sample t-tests and bivariate Pearson's correlations.

To better understand the contribution of interoception to affective prosody recognition, and to demonstrate the relative contribution of each variable while

controlling for the influence of the other factors, a multiple regression analysis was performed. Heartbeat discrimination accuracy and awareness (metacognitive confidence-accuracy correspondence) scores, mean BPM, average confidence ratings, AQ scores, STAI (trait), TAS-20 scores, age, sex and the interaction between sex and interoceptive awareness were entered as predictor variables. All p values in the results section are uncorrected.

2.5 Results

2.5.1 Demographic data

Twenty participants from the ASC group were age ($t(38)=0.244$, $p= 0.808$) and sex matched to neurotypical controls. ASC participants had significantly higher state (mean 45.85; SD 9.6; $t(37) = 2.843$, $p= 0.007$) and trait (mean 57.1; SD 8.3; $t(37) = 5.080$, $p< 0.001$) anxiety scores compared to controls (mean 36.26; SD 11.42; mean 41.9; SD 10.33 for state and trait respectively). As expected, AQ scores were significantly higher in the ASC group (mean 35.05; SD 6.2) compared to controls (mean 14.65; SD 5.7; $t(38) = 10.825$, $p<0.001$).

2.5.2 Prosody accuracy in ASC vs controls

Participants in the ASC group were significantly impaired in affective prosody recognition relative to control participants across all trial types, as signified by a main effect of group ($F(1, 38)=5.283$, $p= 0.027$). Within-subject effects revealed a main effect of trial type ($F(2, 76) = 21.464$, $p<0.001$) although no interaction effect between trial type and group was observed ($F(2, 76) = 0.097$, $p= 0.784$). Thus all participants, irrespective of whether they had an ASC diagnosis, were significantly poorer at matching emotional prosody for face alone stimuli relative to both face with text ($t(39) = 6.009$, $p<0.001$) and text alone ($t(39) = 4.762$, $p<0.001$) (figure 2.3). The main effect of group was maintained when both trait and state anxiety were separately entered as a covariate ($F(1, 36)=7.101$, $p= 0.011$ and $F(1, 36) = 5.394$, $p = 0.026$, respectively), indicating that the reduction in prosody performance in the ASC group was not driven by elevated anxiety levels. Anxiety also did not exert any influence over prosody accuracy, as signified by a non-significant main effect of state ($F(1, 36) = 0.086$, $p = 0.771$) and trait ($F(1,$

36) = 1.435, p = 0.239) anxiety.

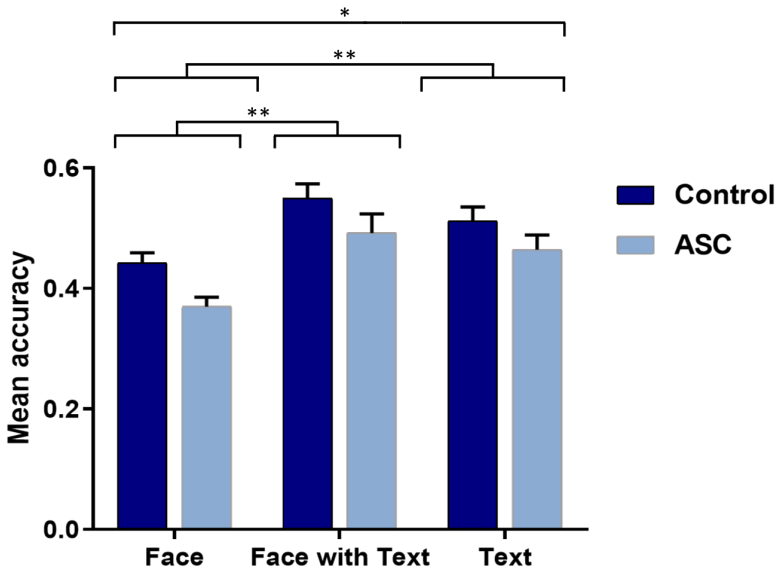


Figure 2.3. Mean prosodic accuracy scores in the ASC and control groups across each trial type; face, face with text, and text.

A main effect of group signified that the ASC group was impaired for all types of stimuli and the main effect of trial type revealed all participants performed worse on face vs text trials and face vs face with text trials. Bars represent standard deviation. * Significant at the 0.05 level, ** significant at the 0.01 level.

There was no main effect of emotional valence (F(1,38)=0.102, p= 0.751), but emotional valence significantly interacted with trial type (F(2, 76) = 3.738, p= 0.028). Here, negative emotions were recognized significantly better than positive emotions for text trials (t(39) = 2.35, p=0.024), while no negative emotion advantage was conferred to either face trials (t(39) = 0.97, p=0.34) or face with text trials (t(39) = 0.80, p=0.43). A significant main effect of emotion complexity was identified (F(1, 38) = 26.139, p<0.001) but no interaction effect was observed between emotion and group (F(1, 38) = 0.615, p = 0.438). Thus, regardless of an ASC diagnosis, all participants were significantly poorer at identifying complex emotions compared to basic emotions (t(39) = 5.138, p<0.001). Emotional complexity also interacted with trial type (F(2, 76) = 17.670, p<0.001) indicating all participants were worse at identifying complex emotions on face trials (t(39) = 6.461, p < 0.001) and on text trials (t(39) = 4.360, p<0.001) but not on face with

text trials (t(39) = 0.944, p = 0.351).

2.5.3 Interoception in ASC:relationship with prosody

Accuracy: We observed no main effect of interoceptive accuracy (F(1, 68)=2.129, p=0.149) suggesting interoceptive accuracy did not reliably influence the accuracy with which ASC individuals judged affective prosody. No significant interactions were identified between emotional valence and interoceptive accuracy (F(1. 68)=1.138, p=0.290), interoceptive accuracy and trial type (F(2, 136)=0.663, p=0.517) or emotional complexity and interoceptive accuracy (F(1, 68)=3.432, p=0.068).

Sensibility: Interoceptive sensibility scores, from both the BPQ and the MAIA, revealed no main effect of the BPQ (F(1, 59)=0.568, p=0.568) or the MAIA total score (F(1, 54)=2.123, p=0.151) on prosody accuracy. No significant interactions were identified between emotional valence, emotional complexity, trial type and interoceptive sensibility. There was no main effect of average confidence and all interactions also did not meet threshold significance.

Awareness: Interoceptive awareness scores revealed a main effect of metacognitive interoceptive awareness on the discrimination task (F(1,68)=4.077, p=0.047) suggesting prosodic accuracy varied as a function of interoceptive awareness. This relationship between overall prosody accuracy and interoceptive awareness was significant in the overall sample (r=0.238, p=0.047) (figure 2.4A) and in males (r=0.384, p= 0.021) (figure 2.4B), but not females (r = 0.144, p= 0.422) (figure 2.4C). The correlations in males and females differed significantly (p= 0.023). Interoceptive awareness did not significantly interact with emotional valence (F(1, 68)=0.450, p=0.505), emotional complexity (F(1, 68)=0.046, p=0.831) or trial type (F(2, 136)=0.618, p=0.540).

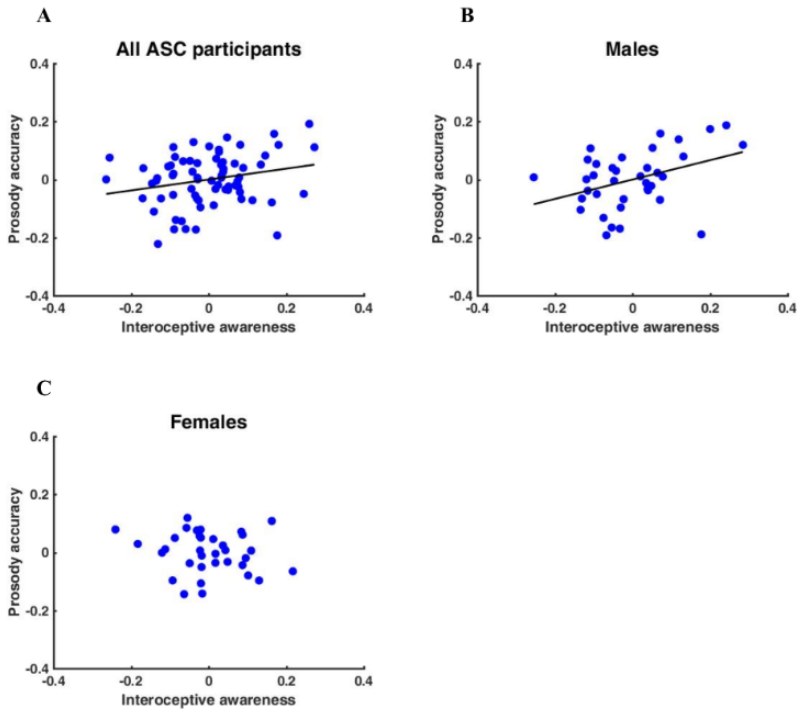


Figure 2.4. Correlations between prosody accuracy and interoceptive awareness.

Relationship between overall prosody accuracy and interoceptive awareness (r=0.238, p=0.047) (A). This was driven by a significant relationship between prosody accuracy and interoceptive awareness in (B) males (r=0.384, p=0.021), a relationship not seen in (C) females (r = 0.144, p=0.422). The correlations in males and females differed significantly (p=0.023).

2.5.4 Emotional prosody deficits in ASC: related factors

We investigated the relationship between prosody performance and individual differences between ASC individuals. In the extended sample of ASC participants (N=74), performance did not differ across emotion categories, as reflected by a non-significant effect of emotional valence ($F(1, 69)=0.123$, $p=0.727$), and a non-significant effect of basic vs complex emotions ($F(1, 69)=1.823$, $p=0.181$). Accuracy scores significantly differed across trial types ($F(2, 69)=4.072$, $p=0.019$) indicating ASC participants were significantly worse at identifying prosodic emotion on face vs text ($t(73) = 8.380$, $p<0.001$), face vs face with text ($t(73) = 8.541$, $p<0.001$) but not face with text vs text ($t(73)=1.939$, $p=0.056$) trials. There was no interaction effect between emotional valence and trial type ($F(2,$

138)=0.809, p=0.447), nor between emotional complexity and trial type (F(2, 138)=0.346, p=0.708), suggesting that neither positive vs negative nor basic vs complex emotions provided a consistent recognition advantage across trial types.

AQ: Analysis of AQ scores revealed no significant effect of AQ on prosody accuracy (F(1, 66)=1.640, p=0.205) suggesting that prosodic accuracy did not differ as a function of autism severity (as reflected by AQ scores). There were also no interactions between AQ and emotional valence (F(1, 66)=0.001, p=0.979), emotional complexity (F(1, 66)=2.586, p=0.113), or trial type (F(2, 132)=0.595, p=0.553).

Alexithymia (TAS-20): No main effect of alexithymia was observed (F(1, 67)=3.735, p=0.058). No significant interactions were found between alexithymia and trial type (F(2, 134)=0.895, p=0.411), emotional valence (F(1, 67)=3.203, p=0.078) or emotional complexity (F(1, 67)=1.186, p=0.280).

Affective symptoms (PHQ-9 and STAI_T): No main effect of depression (F(1, 52)=2.977, p=0.090) or anxiety (F(1, 63)=2.141, p=0.148) was found. No significant interactions were found between depression and emotional valence (F(1, 52)=2.057, p=0.157), emotional complexity (F(1, 52)=0.007, p=0.932) or trial type (F(2, 104)=2.540, p=0.084). There were also no significant interactions between anxiety and emotional valence (F(1, 63)=2.150, p=0.081), emotional complexity (F(1, 63)=3.177, p=0.079) or trial type (F(2, 126)=0.804, p=0.450). See also table 2.1 below for a correlation matrix demonstrating the relationship between interoception and affective symptomatology.

	Heartbeat discrimination	Awareness	Mean confidence	AQ	Trait anxiety	TAS total	BPQ (awareness section)	Mean BPM
Heartbeat discrimination	1							
Awareness	0.182	1						
	0.120							
Mean confidence	0.375**	0.158	1					
	0.001	0.178						
AQ	0.088	0.192	−0.153	1				
	0.462	0.107	0.201					
Trait anxiety	0.094	0.086	−0.061	0.100	1			
	0.441	0.482	0.618	0.413				
TAS total	0.150	0.120	−0.092 0.441	0.375**	0.140	1		
	0.206	0.311		0.001	0.251			
BPQ (awareness section)	0.216	0.003	−0.122	0.095	0.346**	−0.063	1	
	0.085	0.982	0.332	0.453	0.006	0.617		
Mean BPM	−0.122	0.204	−0.296*	−0.090	−0.078	0.097	−0.053	1
	0.311	0.088	0.012	0.461	0.532	0.425	0.683	

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Table 2.1. Interoception and affective symptomatology correlation matrix.

Correlation matrix to demonstrate the relationships between the three psychological dimensions of interoception during heartbeat discrimination and their relationship with affective symptomatology. The first number denotes the r value, the second number denotes the p value.

2.5.5 Regression analysis

The regression model was not significant for prosodic accuracy ($F(10,65)=1.870$, $p=0.070$, $R^2=0.254$). However, the contribution of metacognitive interoceptive awareness was the only predictor variable to prevail as significant for the heartbeat discrimination model, $p = 0.044$, providing evidence of its contribution to affective prosody recognition. A summary of the predictor variables can been seen in table 2.2 below.

Prosody accuracy					
	B	*SE B*	*β*	*t*	*p*
AQ	−0.001	0.002	−0.115	−0.821	0.415
Trait anxiety	0.001	0.001	0.144	1.031	0.307
Alexithymia	−0.002	0.001	−0.252	−1.922	0.060
Interoceptive accuracy	0.027	0.081	0.046	0.333	0.740
Confidence	−0.002	0.005	−0.058	−0.406	0.686
Interoceptive awareness	**0.680**	**0.329**	**0.832**	**2.065**	**0.044***
Mean BPM	−0.001	0.001	−0.104	−0.664	0.510
Age	−0.001	0.001	−0.158	−1.048	0.299
Sex	0.187	0.120	1.061	1.568	0.123
Sex * Interoceptive awareness	−0.307	0.208	−1.175	−1.481	0.144

* Significant at the 0.05 level.

Table 2.2. Prosody accuracy regression table.

Regression table to demonstrate the relative contribution of each predictor variable to individual differences in prosody accuracy.

2.6 Discussion

Recognition of emotion from the intonation of speech (affective prosody) was significantly impaired in ASC participants, compared to neurotypical controls, as demonstrated by reduced performance accuracy on a novel prosody paradigm. In a larger ASC sample, prosody performance was linked to the degree of metacognitive interoceptive awareness during the heartbeat discrimination task. Thus, those individuals with better interoceptive insight (on this task) had enhanced prosody recognition. Importantly, our results emerged in the domain of interoceptive insight and thus significant results cannot be ascribed to heightened rhythm processing, i.e. better able to detect heartbeat rhythm and spoken prosody rhythm, which may confound findings had they emerged in the domain of interoceptive accuracy. This relationship between affective prosody and interoceptive awareness provides a fresh perspective into brain-body interactions in ASC individuals, where the capacity for conscious insight into one's perception of interoceptive signals appears to facilitate the recognition of emotional prosody. It is however worth noting that uncorrected p values make our results preliminary.

Influential 'peripheral' theories of emotion relate the sensing of internal physiological states of bodily arousal to the emotional experience (Damasio, 1996; Lange

et al., 1967). Successful cardiac interoception is moreover an important factor in the perception, regulation and expression of emotional information (Critchley & Garfinkel, 2017; Garfinkel et al., 2014). Even low-level afferent signals concerning cardiac arousal (arterial baroreceptor firing with each individual heartbeat) influence the detection and experience of emotional facial expression (Garfinkel et al., 2014). However, the results of the current study did not find a simple and reliable relationship between prosody and objective measures of interoceptive accuracy. In fact, our findings highlight an effect of a higher-level representation of interoceptive state: metacognitive interoceptive awareness.

Metacognitive interoceptive awareness is, unlike interoceptive performance accuracy, an expression of higher-order conscious access to interoceptive signals (Garfinkel et al., 2015). The current findings suggest that understanding emotional information, in the form of emotional prosody, is functionally dependent upon understanding and interpreting one's own physiological state rather than being accurately (but potentially pre-consciously) guided by the physical sensation of interoceptive signals. Notably, other types of emotion processing (e.g. intensity ratings) are directly associated with interoceptive accuracy (Wiens et al., 2000), yet emotional prosody recognition and inference is arguably more complex, incorporating discrete and interacting processing channels, including pitch, volume and duration, which draw upon distinct neural networks (Buchanan et al., 2000). Affective prosody recognition thus aligns with an interoceptive dimension that is more connected to higher-order conscious access of interoceptive information. Our findings within this autistic sample emphasize the need to quantify interoceptive insight to derive mechanistic insight into the processing of socially relevant emotional information conveyed through speech, which appears to recruit higher level, metacognitive processes.

Interestingly, our results provide evidence to show that the association between interoceptive awareness and prosodic accuracy is most strongly driven by the male participants in our sample. Male/female differences have been a particular topic of investigation in studies of ASC, driven by influential theoretical considerations (Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003; Baron-Cohen, 2009). Sex differences in brain structure may be attenuated in ASC (e.g. Beacher et al., 2012), yet sex differences in brain function, beha-

viour and symptomatology are recognised (e.g. Lai et al., 2011; Rivet & Matson, 2011); for example, females show relative preservation in their perception and understanding of emotional information both behaviourally (McGillivray & Evert, 2018) and at the neural level (Schneider et al., 2013; Schulte-Rüther, Markowitsch, Shah, Fink, & Piefke, 2008). Indeed, even in healthy populations, females report greater attention to bodily sensations yet actually perform worse than males on the heartbeat-counting task (Grabauskaitė, Baranauskas, & Griškova-Bulanova, 2017). It should be noted, however, that the effects of sex were not significant in the main regression analysis linking effective prosody and interoceptive awareness, presumably due to shared variance with other factors. Thus, our results provide tentative evidence that males may require greater conscious awareness of their internal bodily sensations in order to comprehend affective prosody.

To date, research on the psychology of interoception has focused either on subjective reports (indexed by questionnaires) or on more objective behavioural measures, e.g. performance accuracy during the heartbeat detection task. Historically, the term awareness was used to refer to both subjective and objective measures of interoceptive sensitivity. However, drawing on advances in the cognitive psychology of consciousness awareness, recent terminology equates awareness to metacognition. Correspondingly, there is a paucity of research referring to metacognitive aspects of interoception (e.g. Canales-Johnson et al., 2015; Ewing et al., 2017; Khalsa et al., 2008), and its relative contribution to emotional processing is not fully explored. The mechanisms required to appraise one's own internal bodily sensations may be fundamental to the understanding of emotional information in self and others (Singer et al., 2009). This builds upon previous work that highlights the role of more automatic measures, such as physiological resonance and contagion (Cooper et al., 2014; Harrison, Wilson, & Critchley, 2007; Konvalinka et al., 2011). As the state of others can be mirrored in the observer, interoceptive insight into one's own bodily signals can also shape understanding of the state of others. Correspondingly, people with alexithymia (an inability to perceive and describe one's own emotions), are also impaired in the perception and recognition of emotional expressions (Lane et al., 1996; Parker, Taylor, & Bagby, 1993; Prkachin, Casey, & Prkachin, 2009). Thus, the capacity to understand one's own emotions facilitates the accurate perception of emotion

in others. Neuroimaging findings also indicate a sharing of neural architecture during both personal experience of emotion and judging the emotions of others. In particular, the insula, a key structure involved in interoception and emotional processing, shows increased activation both when observing another person's disgust and when experiencing disgust directly (Wicker et al., 2003). Engagement of insular cortex is characteristic of social emotional processing (Lamm & Singer, 2010), particularly empathy (Jackson et al., 2005; Singer et al., 2009).

Alexithymia is extremely common in ASC, but it is not (when subjectively rated) an obligatory, defining attribute of this diagnosis (Bird et al., 2010; Cook et al., 2013; Shah et al., 2016). Since alexithymia is characterised by an inability to identify and describe emotions, the relative contribution of alexithymia to prosodic impairment was also investigated in this study. While we observed a correlation between AQ scores and TAS-20 scores confirming a relationship between ASC and alexithymia (Shah et al., 2016), we saw no reliable relationship between reported levels of alexithymia and affective prosody recognition. Thus, impaired prosodic accuracy in ASC individuals appears to be driven by interoceptive metacognition, and not alexithymia. This represents a potential avenue for intervention, and future work may usefully explore whether individual differences in metacognitive interoceptive awareness predicts sensitivity to emotional prosody in neurotypical populations or if this association is more specific to ASC.

Our finding of impaired emotional prosody recognition adds to literature concerning affective prosody deficits in ASC individuals. We observed a more pronounced impairment on trials that also required face processing. This is perhaps unsurprising, consistent with previously-described difficulties in face processing in ASCs (Dalton et al., 2005; Lynn et al., 2018; Rigby, Stoesz, & Jakobson, 2018). In fact, all participants, irrespective of ASC status, showed a reduced performance on 'face-only' trials, relative to trials with accompanying text that specified the possible emotion.

Previous work has not always demonstrated clear deficits in processing affective prosody in ASC individuals compared to neurotypical controls (Brennand et al., 2011; Golan et al., 2006; Grossman et al., 2010; Le Sourn-Bissaoui et al., 2013; Peppé et al., 2011; Rosenblau et al., 2017), but discrepancies may reflect the varied methodologies employed. Some studies only employed stimuli convey-

ing 'basic' emotions (Globerson, Amir, Kishon-Rabin, & Golan, 2015; Grossman et al., 2010), which are arguably easier to detect (Brennand et al., 2011; Smith, Montagne, Perrett, Gill, & Gallagher, 2010). Other studies vary in the type of stimuli used to assess prosody (Chevallier, Noveck, Happé, & Wilson, 2011; Grossman et al., 2010; Kujala, Lepistö, Nieminen-von Wendt, Näätänen, & Näätänen, 2005; Peppé, McCann, Gibbon, O'Hare, & Rutherford, 2007; Peppé et al., 2007). and some studies have used stimuli containing semantic information thus giving emotional information that is non-dependant on prosodic cues (see Wang & Tsao, 2015). The current study accounted for these methodological discrepancies by using semantically-neutral prosodic cues, by combining a range of complex and basic emotions (e.g. Golan et al., 2006) and by employing three different trial types; face only, face with text and text only trials. Our stringent methodology may therefore encourage the use of more robust paradigms to assess the processing of affective prosody.

Notably, we quantified interoceptive dimensions using two different tasks that access both shared and distinct mechanisms (Schulz, 2016), although we focused our examination on only the heartbeat discrimination task. Strong correlations in performance accuracy between these heartbeat-tracking and discrimination tasks are not always observed especially within small samples (Ring & Brener, 2018). The heartbeat tracking task is arguably influenced by prior knowledge about heart rate (Ring, Brener, Knapp, & Mailloux, 2015) and the heartbeat discrimination task requires the integration of interoceptive and exteroceptive information (Garfinkel, Tiley, et al., 2016). Recognition of affective prosody may itself be an internal-external integration task, particularly if internal bodily changes elicited by external affective prosody guide correct comprehension and appraisal processes. Indeed, our results suggest a relationship between interoception and prosody, as measured by the cross-modal discrimination task, manifesting in the metacognitive domain only, thus indicative of a higher-level processing deficit.

The observed relationship between prosody and interoceptive awareness highlights the value in investigating interoceptive contributions to adaptive emotional behaviours and clinical symptomatology. Given the impaired recognition of emotional prosody that we observed in ASC individuals, and the role that interoceptive awareness plays in this impairment, targeted interventions aimed at im-

proving interoceptive awareness may be useful to improve emotional processing in this group who are at higher risk of anxiety and mood disorders. Support for this notion lies in the memory domain, wherein better memory performance is associated with a more accurate judgement of one's own performance, a relationship not observable for the interoception tasks (Meessen et al., 2016). One proposed reason of this difference is the availably of feedback: information about the accuracy of memory performance is common in everyday situations, yet feedback about interoceptive performance is not. Therefore, provision of performance feedback during interoceptive tasks, could be used to train ASC individuals to increase interoceptive awareness, and by association to improve emotional prosody recognition. Moreover, individuals who possess good metacognition may be more able to allocate attentional resources to functional domains, e.g. interoception, on which they perform poorly (Schooler et al., 2011). There may thus be synergistic benefits in improving interoceptive metacognition.

There are limitations to the current study that should be addressed in future work. Firstly, the heartbeat discrimination task served as the primary outcome interoceptive measure used. For this task, studies vary in the number of index trials, although it has been claimed that 40–60 trials are needed to ensure robust reliability on this measure of interoceptive performance accuracy (Kleckner, Wormwood, Simmons, Barrett, & Quigley, 2015). Moreover, ROC fit is also enhanced with more trials, and thus this may have also impacted our calculations of interoceptive awareness. Since the task employed here consisted of only 20 trials, this can be considered a limitation. Additionally, due to the design of the prosody paradigm we were unable to examine the effect of interoception on discrete basic emotions, since each basic emotion was only presented 6 times; we were thus underpowered to test this relationship. The absence of a significant relationship between prosodic accuracy and AQ suggests that the prosodic deficits may not be driven by core ASC symptomatology, but instead they may represent a specific feature coupled to aberrant interoceptive processing. However, interoceptive dimensions were not measured within the neurotypical control group. We therefore cannot conclude whether or not the relationship between interoceptive awareness and prosody is specific to autism, nor whether this coupling reflects a core relationship that can be extrapolated to other individuals. Future research should

investigate the relationship between prosody and interoception in normative populations to see if the manifestation of prosodic deficits are also driven by reduced interoceptive awareness. Further studies are also needed to test if the interoceptive metacognitive skill required to recognise affective prosody is modality-specific, i.e. does it solely rely on interoceptive awareness, or does the metacognition of knowing when you understand another person's emotions also affect accuracy in labelling emotional cues from speech. Ultimately, a more comprehensive understanding of metacognitive interoceptive awareness is needed to better understand its contribution to emotion and of its presentation in clinical disorders.

The results of the current study provide a novel contribution to understanding affective prosody deficits in ASC individuals, relating low-level processing of social/emotional cues to higher-level appraisal of one's own ability to process physiological changes in one's body. The relationship between interoception and emotions remains pertinent: improved detailed knowledge of their association will enhance insight into the mechanisms underlying core ASC symptomatology and enable targeted strategies to mitigate psychological distress within this population.

Chapter 3

Understanding anxiety in autistic adults: Central dysregulation of interoceptive tuning to threat

3.1 Abstract

Anxiety is a prominent symptom of autism, amplifying social and emotional difficulties. Interoceptive signaling of physiological arousal (e.g. stronger, faster heartbeats) can enhance anxious feelings. We tested whether the neural and functional integrity of this interoceptive mechanism is different in autistic adults. Participants with and without a diagnosis of autism underwent functional neuroimaging (fMRI), while they rated images of fearful and neutral faces presented at distinct phases of the heart cycle (ventricular systole vs diastole). Across all participants, systole attenuated the perceived intensity of neutral, but not fear, stimuli. Moreover, with increasing individual differences in anxiety level, systole selectively enhanced the intensity of fear stimuli. In autistic, relative to non-autistic, participants, insula and cingulate neural reactivity was blunted, and right insula functional connectivity was significantly weaker during cardiac systole. These findings suggest that a shared interoceptive mechanism, tuning responses to threat, is dysregulated in autistic adults, increasing vulnerability to anxiety.

3.2 Introduction

Interoceptive (visceral afferent) signals from the heart can inform subjective emotional experience (Lange et al., 1967) through both conscious and unconscious representations (Füstös et al., 2013; Garfinkel et al., 2014; Gray et al., 2012; Wiens, 2005). For example, afferent signals concerning cardiovascular arousal can selectively tune and amplify the processing of threat, relative to other salient or neutral stimuli (Garfinkel et al., 2014). Individual differences in aspects of cardiac interoceptive processing increase vulnerability to anxiety(Dunn et al., 2010; Garfinkel, Tiley, et al., 2016) and aberrant interoception is observed in autism, a neurodevelopmental condition characterised by altered emotional processing (Gu et al., 2013; Mulcahy, Davies, et al., 2019), including increased vulnerability to anxiety (Garfinkel, Tiley, et al., 2016).

Afferent interoceptive (viscerosensory) representation and efferent autonomic (visceromotor) control of internal bodily state are supported at multiple levels of the neuroaxis. This includes forebrain regions, e.g. insula, amygdala, anterior cingulate cortices (Craig, 2002; Critchley & Harrison, 2013; Critchley, 2005; Medford & Critchley, 2010), through which cognitive and emotional processes can exert top-down influences on more proximate (brainstem) cardiovascular control centres, modulating heart rate and blood pressure (Dampney et al., 2005; Mulcahy, Larsson, Garfinkel, & Critchley, 2019). The brain-to-body axis of cardiovascular control is informed by a body-to-brain afferent signalling (Critchley & Harrison, 2013; Critchley, 2005; Sherrington, 1952) for which arterial baroreceptors are critical to physiological regulation of the cardiovascular system (e.g. baroreflex control of blood pressure). These same baroreceptor signals impact emotional, cognitive and perceptual processes (Azevedo et al., 2017; Craig, 2002; Garfinkel et al., 2014; Gray et al., 2012, 2009; Makovac et al., 2015; Kunzendorf et al., 2019; Ohl, Wohltat, Kliegl, Pollatos, & Engbert, 2016). Arterial baroreceptors discharge as blood is ejected into the aorta (and carotids) at ventricular systole. This neural discharge informs the brainstem of the strength and timing of each heartbeat, and thus the instantaneous state of cardiovascular arousal. The phasic properties of baroreceptor firing within the cardiac cycle can be exploited to examine how interoceptive information concerning physiological arousal impacts cognitive and perceptual processes (Critchley & Garfinkel, 2017): responses

to stimuli presented at ventricular systole, when baroreceptors discharge, can be contrasted with stimuli presented at diastole, between heartbeats, when baroreceptors are quiescent (Azevedo et al., 2017; Edwards et al., 2002; Garfinkel et al., 2014; Gray et al., 2012, 2009).

Most experimental work using such cardiac timing paradigms report inhibition of sensory processing during ventricular systole, e.g. for pain (Edwards et al., 2002; Gray et al., 2009; McIntyre et al., 2008), touch (Gahery & Vigier, 1974) and startle eye blink (Schulz et al., 2009) responses are attenuated. This inhibitory effect of systole also extends to word processing and memory (Garfinkel et al., 2013) and to active sampling in visual search, where vision is dampened during cardiac systole (Galvez-Pol, McConnell, & Kilner, 2020). However, this relationship is nuanced: systole can enhance familiarity during face recognition (Fiacconi et al., 2016), motor reactivity (Makowski et al., 2020) and active inhibition of motor responses (Rae et al., 2018). There are also emotion-specific effects. Notably at systole, fear cues are detected more easily and will evoke greater ratings and stronger reactions (Garfinkel & Critchley, 2016; Garfinkel et al., 2014). These effects of cardiac arousal signals on threat extend to racial biases in perceptual judgement (Azevedo et al., 2017). Therapies for anxiety and phobia can potentially harness this systolic enhancement of fear and threat processing (Watson et al., 2019).

Exaggerated amygdala responses are commonly reported in anxiety disorders (Adhikari et al., 2015; Garfinkel et al., 2014; Tye et al., 2011), and can also be observed in autism spectrum conditions (Critchley et al., 2000; Herrington et al., 2017). The prevalence of anxiety is considerably higher in autistic individuals (Hollocks et al., 2019), which may relate to such aberrant amygdala reactivity. Differences in how interoceptive information is processed may also contribute to emotional differences and increased anxiety in autistic people (Critchley & Garfinkel, 2017). Interoception spans a continuum from low-level neural signalling of, and perceptual sensitivity to, internal bodily signals (which may occur without conscious awareness), to higher-level subjective representations of sensitivity to interoceptive signals and metacognitive insight into one's interoceptive ability (i.e. with conscious awareness) (Quadt et al., 2018). In autistic individuals, differences in cardiac interoception are reported at multiple levels. These include deficits in objective measures of interoceptive perceptual accuracy (Palser et al.,

2018), altered subjective sensitivity to interoceptive signals (Elwin et al., 2012; Fiene & Brownlow, 2015; Garfinkel, Tiley, et al., 2016), over-allocation of attentional resources to internal bodily state (Schauder et al., 2015) and, relatedly, weak central interoceptive coherence (Hatfield, Brown, Giummarra, & Lenggenhager, 2019; Quattrocki & Friston, 2014). However, there is much individual variability and not all studies demonstrate group level interoceptive differences in autism (Nicholson et al., 2018; Schauder et al., 2015). Interoceptive differences may emerge only as a function of alexithymia (Bird et al., 2010; Shah et al., 2016), defined as a difficulty in identifying and describing one's own emotional feelings (Apfel & Sifneos, 1979). Relatedly, increased interoceptive sensibility and alexithymia, both of which are highly prevalent in autism (Hill et al., 2004; Hollocks et al., 2019), may heighten risk for clinically significant anxiety (Palser et al., 2018).

At the neural level, functional brain imaging studies report differences in the activation or connectivity of 'interoceptive' insula cortex in autism (Ebisch et al., 2011; Odriozola et al., 2016; Silani et al., 2008). Even when group differences are not observed, insular reactivity when performing an interoceptive task can predict differences in social functioning among autistic people (Failla et al., 2020). Moreover, better interoceptive awareness is linked to better recognition of emotional prosody (Mulcahy, Davies, et al., 2019), while mismatch between objective and subjective measures of interoceptive sensitivity predicts a greater severity of trait anxiety in autistic adults (Garfinkel, Tiley, et al., 2016).

Based on this accumulating evidence linking interoception to emotional and social functioning, we tested the functional and neural integrity of cardiac afferent influences on the processing of anxiety-relevant emotional information in autistic adults. With stimuli presentation contingent upon different phases of the cardiac cycle, we used neuroimaging to quantify neural responses to the processing of fear vs neutral faces in autistic adults and neurotypical controls (Garfinkel et al., 2014). We measured how systole vs diastole affected ratings and neural responses to the stimuli, testing for effects of diagnosis and affective symptoms, including anxiety and depression. We hypothesised that, across participants, the processing of fear stimuli relative to neutral stimuli would be enhanced at systole (relative to diastole) and that this effect would be proportionate to anxiety level (Garfinkel et al., 2014). Further, at the neural level, the relative enhancement of fear pro-

cessing at systole would correlate with greater amygdala and insula activation, and again this effect would be exaggerated in highly anxious individuals (Garfinkel et al., 2014). Lastly, we predicted that autistic individuals, compared to neurotypical controls, would show exaggeration of this interoceptive mechanism linked to differences in functional reactivity and connectivity of regions that integrate interoceptive signals with emotional processes, including the insula, amygdala and anterior cingulate cortex (Critchley, Melmed, Featherstone, Mathias, & Dolan, 2002; Garfinkel et al., 2014; Gray et al., 2009).

3.3 Methodology

3.3.1 Participants

Initially, 40 participants with a confirmed ASC diagnosis and 40 non-autistic controls were recruited for this study. However, due to scanning abnormalities (2 incidental findings, 3 excessive movement, 3 signal loss, 2 incomplete datasets), 10 participants (5 autistic, 5 neurotypical) were excluded from the study, resulting in a final sample of 35 autistic (18 male, 17 female as assigned at birth; mean age 32.40yrs, range 18-64yrs) and 35 non-autistic neurotypical participants (18 male, 17 female as assigned at birth; mean age 30.37yrs, range 18-63yrs). Remaining participants were still matched on age, gender and education (see results for statistics).

All participants, autistic and neurotypical, were right handed (specified as inclusion criteria due to the scanner set-up, where participants needed to use their right hand to make responses), fluent English speakers, none had a history of past head injury or organic brain disorders, cognitive impairment or a learning disability (general mental impairment); none had asthma/respiratory illnesses, epilepsy or evidence of psychotic experiences (i.e. none reported such co-morbid diagnoses or were currently taking anti-psychotic medication). Autistic participants were recruited from the Sussex Partnership (adult) Neurodevelopmental Service and through advertisements placed on social media and via leaflets and posters. All autistic participants had established diagnoses in accordance with DSM4-R criteria verified by consultant psychiatrist and multidisciplinary clinical team with expertise in evaluation and clinical management of neurodevelopmental

conditions. All autistic participants provided written informed consent with all procedures approved by the NHS Research Ethics Committee. Neurotypical participants were recruited from the University of Sussex and members of the local community. All neurotypical participants provided written informed consent with all procedures approved by the BSMS Research Governance Ethics Committee.

3.3.2 Experimental paradigm and procedure

The 'FearFaces' functional neuroimaging task was programmed in Matlab (MathWorks Inc., Natick, MA) (Garfinkel et al., 2014). Face stimuli, fear and neutral faces, with no graded intensities, were taken from the Karolinska Directed Emotional Faces (Lundqvist, Flykt, & Öhman, 1998) and the Ekman set (Ekman & Friesen, 1974), both of which have been well-validated/commonly utilised, indicating the face stimuli do reflect, and will likely be recognised as, their ascribed emotions (e.g. Goeleven, De Raedt, Leyman, & Verschuere, 2008). In brief, 20 faces (10 fear and 10 neutral), were presented over the period of peak ventricular systole and 20 faces (10 from each emotion) were presented at late diastole, resulting in a 2 x cardiac cycle (systole, diastole) x 2 emotion (fear, neutral) design. Face stimuli were presented for 100ms, to allow for precise cardiac timing. Trial types were randomised and the experiment was broken into two functional runs of 40 faces each. On each trial, the participant reported the perceived emotional intensity of the face stimulus (cue: 'How intense was the emotion on this face?'), from zero (0) to medium (50) to extreme (100) using an on-screen visual analogue scale (VAS) presented for 3 seconds. The cursor was controlled using a button box held in the right hand. Between trials, a fixation cross was presented for 5 seconds. See figure 3.1a/b for an overview of the experimental paradigm.

3.3.3 Cardiac timing

Throughout the experiment, real-time cardiac timing was obtained from a medical grade MRI-compatible pulse oximeter (8600FO; Nonin Medical Inc., MN, USA) attached to the participants left index finger and relayed as a waveform to CED hardware and software (Power 1401, Spike2 v7, Cambridge Electron Design Ltd, Cambridge). prior to the fMRI procedure, each participants pulse transit time (PTT) was calculated using an in-house script which utilised electrocardiography

and pulse oximetry data that was collected over a period of 60 seconds with the participant in supine position, following a rest period of 5 minutes to allow for stabilization of the participants heartbeat/blood pressure. The PTT gives a measure of how long it takes a pulse wave to travel between two arterial sites (here, the heart and fingertip). Accurate cardiac time-locking of stimuli presentation during the neuroimaging task was achieved by calculating the inter-beat interval (IBI), in the lead up to stimuli presentation, from three preceding pulse waves to predict the occurrence of the next pulse. Then, using each participant's own PTT, stimuli were presented either around late diastole, when arterial baroreceptors are quiescent (peak of the pulse wave time minus the PTT; which accounts for the temporal delay between heart activity and measurement of this activity at the fingertip, i.e. equivalent to the peak of an estimate timing of ECG R-wave and thus prior to myocardial depolarisation) and, alternatively, around maximal ventricular systole, when aortic and carotid baroreceptors maximally discharge (peak of the pulse wave time minus the PTT plus 300ms; equivalent to the estimate timing of ECG T-wave). Timings were validated prior to behavioural and imaging analyses; diastole trials that occurred $>$ 50ms and $<$ -200ms from the estimated R-wave time were excluded, and similarly, 'systole' trials that occurred $<$ 150ms and $>$ 400ms relative to the estimated R-wave time were also excluded (figure 3.1c).

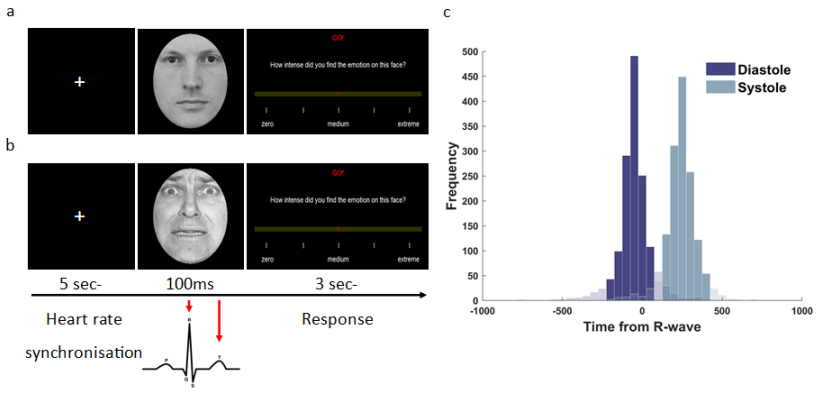


Figure 3.1. FearFaces paradigm: Experimental procedure and distribution of excluded trials.

Neutral face trials (a) and fear face trials (b) were time-locked to ventricular systole or diastole (20 trials per emotion/cardiac condition) and participants made subsequent intensity ratings. (c) Histogram of all stimuli (fear and neutral faces) presentation across the cardiac cycle for both groups. Faded bars represent trials excluded from timing analyses.

3.3.4 Questionnaires

All participants completed the trait section of the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001) and the Autism Quotient (AQ) (Baron-Cohen et al., 2001). Two participants (1 autistic and 1 neurotypical) did not complete the PHQ-9 and were excluded from any analysis involving this measure. Participants also provided demographic information including age, gender assigned at birth, and level of educational attainment.

3.3.5 fMRI data acquisition

Functional imaging datasets were acquired using a Siemens 3T Prisma MRI scanner with a 32-channel head coil. A multiband echo-planar imaging (EPI) sequence was used with multiband acceleration factor of 2 to acquire T2*-weighted images sensitive to blood oxygen level dependent (BOLD) contrast. Each functional volume consisted of 44 slices, acquired in an interleaved order. The following parameters were used: TR = 1500ms; TE = 30ms; flip angle = 70°; matrix =

94x94; FOV = 220mm; slice thickness = 3.0mm with a 25% gap. The total number of fMRI volumes acquired varied across participants depending on their heart rate and speed of response (mean 275 volumes).

3.3.6 fMRI pre-processing

fMRI data was pre-processed using SPM12 in Matlab R2017A (MathWorks, Inc., Natick, MA). For each participant, the first 5 volumes were removed to account for magnetization equilibrium. Remaining functional images were slice-time corrected to the first slice, realigned to the first volume and spatially normalised to a standard MNI EPI template (Calhoun et al., 2017). Normalised images were then smoothed using an 8mm Gaussian kernel (full width half maximum) and all images were visually inspected for artefacts.

3.4 Data analyses

3.4.1 Behavioural data analyses

Demographic information (age, baseline differences in anxiety and depression) was compared using independent sample t-tests. Education attainment was compared using Fisher's exact test. Between-group differences in behavioural data (mean intensity ratings) were examined using a 2 (group; autism vs neurotypical) x 2 (cardiac cycle; systole vs diastole) x 2 (emotion; fear vs neutral) repeated measured ANOVA. Additionally, based on the assumption that anxiety and depression are often co-morbid, particularly in autism, we entered depression and anxiety into an ANVCOVA model to test for main effects and interactions of affective symptomatology. We also tested for main effects and interaction of mean heart rate (calculated as the average heart rate across the task). Significant results were further explored using two-sided paired/independent sample t-tests and bivariate Pearson's correlations. All p values in the behavioural results are uncorrected.

3.4.2 fMRI data analyses

Within SPM 12, individual first level analytic models were constructed resulting in 4 single-regressor of interest; (1) fear at systole, (2) fear at diastole, (3) neutral at systole, (4) neutral at diastole. Six motion parameters (3 translation, 3 ro-

tations) calculated during realignment were included as confounding regressors. T-contrasts from regressors of interest were entered into a second level full-factorial model with group (autism/neurotypical) as an independent (between participant) factor and condition (emotion and cardiac cycle) as non-independent (repeated measures) factors. Resultant F-contrasts were generated to test for 1) all effects; 2) main effect of group; 3) main effect of cardiac cycle; 4) main effect of emotion; 5) specific interactions effects. The direction of significant main effects and interactions were explored using post-hoc t tests. Based on behavioural findings (described below), we generated a first-level contrast for fear systole>fear diastole to correlate associated brain activity across all participants with anxiety, controlling for depression, and with depression, controlling for anxiety. All t-contrasts tested for a positive interaction. Statistical maps were thresholded at cluster-forming threshold $p<0.001$ and False Discovery Rate (FDR) cluster-corrected at $p<0.05$ for multiple comparisons. Significant clusters were localized according to SPM's Anatomy toolbox (Eickhoff et al., 2005).

3.4.3 Psychophysiological interactions (PPI)

Based on previous work highlighting the role of the insula and amygdala in autonomic and emotion processing, particularly for fear (Garfinkel et al., 2014; Gray et al., 2009), and our findings from the second-level general linear model (GLM), we sought to better understand how these regions, when processing fear stimuli, modulated activity elsewhere in the brain. Thus, we undertook specific psychophysiological interaction (PPI) analyses; first extracting eigenvariate values (weighted mean of BOLD timeseries) from 10mm spheres at the peak coordinates of clusters identified from the t-contrast of fear>neutral, namely left (x-26, y-4, z-22) and right (x22, y-4, z-16) amygdala, and left (x-36, y10, z-8) and right (x34, y12, z-14) insula.

Additionally, we undertook a further PPI analysis to understand the mechanism, through functional connectivity (FC), that may underscore atypical interoceptive signaling in autistic individuals. Thus, we extracted eigenvariate values at the peak coordinate from our GLM finding of the t-contrast neurotypical systole>autism systole, that showed greater activation in right insula (x36, y-8, z16).

Thus for each participant, an interaction regressor was computed for the fear>neutral contrast with BOLD time series data from (1) left amygdala, (2) right amygdala, (3) left insula and (4) right insula. Additionally, the PPI regressor was computed for (5) the interaction between the contrast for systole only and the right insula time series data. In separate analyses, the PPI regressor term was entered into a first-level model with regressors representing the regional BOLD activity (PPI.Y) and task effect (PPI.P). As the data were acquired across two functional runs, for the PPI analysis, the two runs were concatenated with a 'block transition' regressor modelling the transition from the end of one block to the start of the next. The six movement regressors calculated during realignment were also included as confounds. T-contrasts were generated for the PPI term and entered into second-level models. F-contrast tested for task effects (four one-sample t-test models, for fear>neutral) or group effects and t-contrasts examined the direction of significant effects (two-sample t-test, for neurotypical systole>autism systole). As in univariate analyses, statistical maps were thresholded at cluster-forming threshold $p<0.001$ and False Discovery Rate (FDR) cluster-corrected at $p<0.05$ for multiple comparisons. Significant clusters were localized according to SPMs Anatomy toolbox (Eickhoff et al., 2005).

3.5 Results

3.5.1 Demographics

Autistic and neurotypical participants were matched for gender and did not significantly differ on age (mean autism 32.40, SD 12.14; mean neurotypical 30.37, SD 12.97; $t(68) = 0.676$, $p = 0.502$) or education (mean autism 3.42, SD 1.33; mean neurotypical 3.80, SD 1.12; fisher's exact, $p = 0.573$). There was also no difference in mean heart rate (mean autism 71.25, mean neurotypical 70.46; $t(67) = 0.282$, $p = 0.779$) but the autistic group did show elevated baseline levels of trait anxiety (STAI mean autism 59.43; mean neurotypical 39.94; $t(68) = 7.258$, $p<0.001$), depression (PHQ mean autism 14.85; mean neurotypical 4.97; $t(66) = 6.733$, $p<0.001$) and autistic traits (AQ mean autism 38.16; mean neurotypical 16.29; $t(68) = 14.412$, $p<0.001$).

3.5.2 Between group differences in cardiac modulation of emotion intensity

No main effect of group was observed ($F(1, 68) = 1.064$, $p = 0.306$) indicating that intensity ratings did not reliably differ between autistic versus neurotypical participants across all trial types. Additionally, no significant interactions were observed between cardiac cycle and group ($F(1, 68) = 0.017$, $p = 0.896$), nor between emotion and group ($F(1, 68) = 2.812$, $p = 0.098$), and there was no significant three way interaction between group, cardiac cycle and emotion ($F(1, 68) = 1.184$, $p = 0.280$), suggesting intensity ratings did not reliably differ between the two groups across emotion categories nor emerge as a function of cardiac timing (figure 3.2).

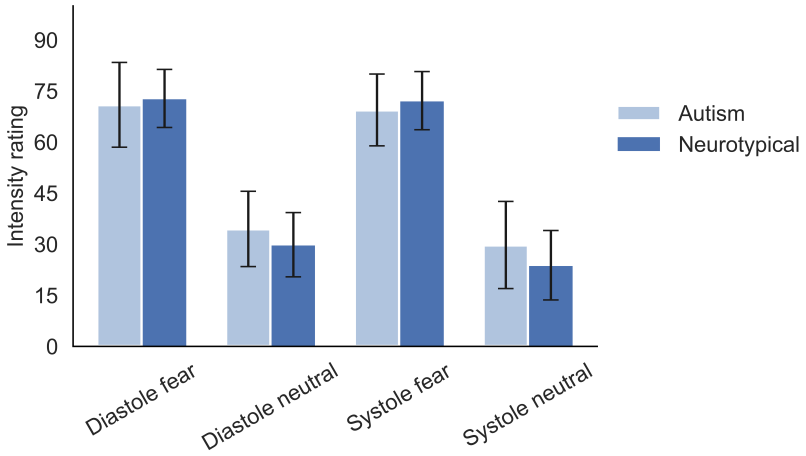


Figure 3.2. Summary of face intensity ratings for autistic and neurotypical participants.

Effects of cardiac signals on intensity ratings across fear and neutral emotion categories for autistic and neurotypical participants. Bars represent standard deviation.

There was a main effect of emotion ($F(1, 68) = 521.213$, $p<0.001$) indicating that, across all participants, fear faces were rated as markedly more intense (mean, 71.71; SD, 9.74) than neutral faces (mean, 29.42; SD, 11.17; $t(69) = 22.890$, $p<0.001$), irrespective of cardiac cycle. A main effect of cardiac cycle ($F(1, 68) = 36.438$, $p<0.001$) indicated that across all participants, face stimuli presented at diastole (mean, 52.26; SD, 7.77) were rated as more intense than at systole (mean, 48.87; SD, 7.28; $t(69) = -5.929$, $p<0.001$). However, cardiac cycle also significantly interacted with emotion ($F(1, 68) = 17.534$, $p<0.001$), reflecting the

propensity for all participants to rate neutral faces as more intense at diastole (mean, 32.31; SD, 10.70) compared to systole (mean, 26.83; SD, 12.25; t(69) = 8.269, p<0.001) (figure 3.3), while fear faces were impervious to the inhibitory effect of the heart, with no significant difference in intensity ratings for fear faces presented at diastole (mean, 72.18; SD, 10.88) versus systole (mean, 70.77; SD, 9.98; t(69) = 1.718, p = 0.090).

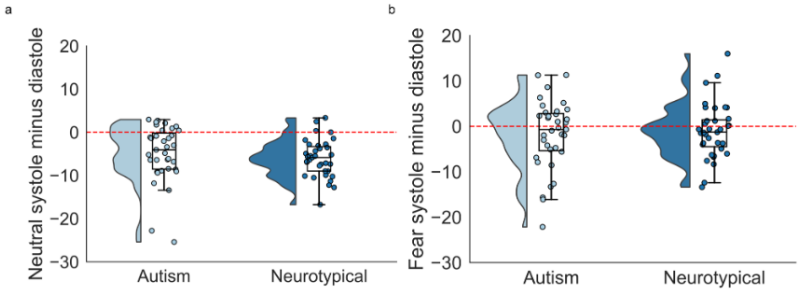


Figure 3.3. Fear and neutral face processing at systole relative to diastole.

All participants showed an inhibitory effect of neutral faces presented at systole (a), an effect that was not maintained for fear faces (b). Group distribution displayed as individual data points (horizontally jittered), violin plots (probability density functions), boxplots showing upper/lower quartiles and the median value, and whiskers showing the minimum and maximum values.

3.5.3 Anxiety, depression and mean heart rate

A main effect of depression (F(1, 64) = 7.831, p = 0.007) indicated that depression dampened overall intensity ratings across all participants. A significant three-way interaction between cardiac cycle, emotion and anxiety (F(1, 64) = 6.767, p = 0.012) revealed that individuals with elevated levels of trait anxiety provided greater intensity ratings toward fear faces at systole relative to fear faces at diastole (r = 0.296, p = 0.015). This relationship was not observed for neutral faces (r = -0.044, p = 0.721) (Figure 3.4).

No significant main effect of heart rate was observed (F(1, 66) = 0.79, p = 0.403) and no significant interaction between heart rate and emotion (F(1, 66) = 0.016) p = 0.899), heart rate and cardiac cycle (F(1 ,66) = 1.115, p = 0.295) nor between heart rate, cardiac cycle and emotion (F(1, 66) = 0.733, p = 0.395) suggesting mean heart rate did not influence intensity ratings.

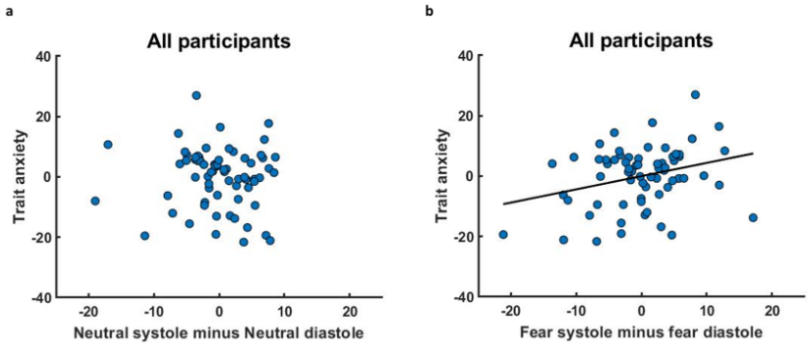


Figure 3.4. Fear processing and anxiety.

(a) Across participants, results revealed a significant partial correlation, controlling for depression (r =0.296, p = 0.015), between trait anxiety and fear ratings (fear systole minus fear diastole). (b) No such relationship was found for neutral faces (r = -0.44, p = 0.721).

3.5.4 Between group differences in cardiac modulation of emotion intensity: fMRI results

Main effect of group: Differential brain activation between autistic and neurotypical participants was observed within a set of specific brain regions as signified by a main effect of group (regardless of emotion or cardiac cycle; for neurotypical>autistic, no significant autistic>neurotypical activations were observed). In particular, bilateral insula, cingulate cortex and precuneus showed greater activation in neurotypical participants (supplementary table 2.1).

Main effect of emotion: Across both groups, we observed differential peak brain activation between fear and neutral emotions as signalled by a significant main effect of emotion (regardless of group and cardiac cycle; for fear>neutral and neutral>fear). For the t-contrast of fear greater than neutral, we observed activation in bilateral amygdala (figure 3.5), bilateral insula, temporal lobe and superior parietal lobule. For the t-contrast of neutral>fear, we observed significant activation in precuneus, parietal lobule, angular gyrus, occipital gyrus, lingual gyrus, precentral gyrus, IFG and precentral gyrus (supplementary table 2.2).

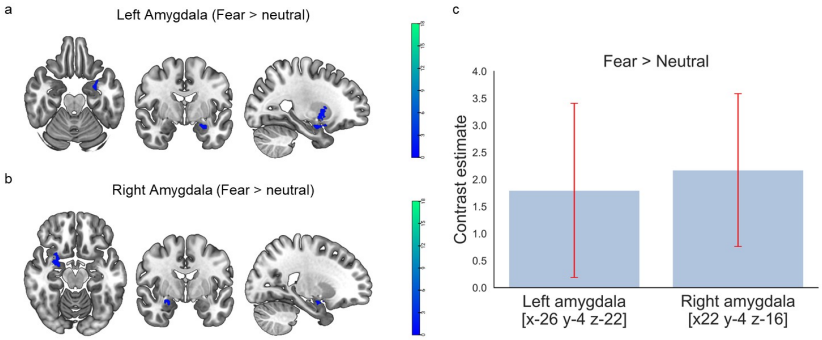


Figure 3.5. fMRI results: main effect of emotion.

A main effect of emotion (F-contrast) revealed significant activation differences when viewing fear versus neutral face stimuli across all participants. Post-hoc t-contrasts revealed greater activity in left (a) and right (b) amygdala. Contrast estimate effect size plot (c) show effect size in left and right amygdala. Red bars represent 90% confidence intervals.

Main effect of cardiac cycle: Across both groups, we observed a main effect of cardiac cycle reflecting greater thalamic and hippocampal activation for the t-contrast of systole>diastole (supplementary table 2.3).

Group and cardiac cycle interaction: We observed a significant group by cardiac cycle interaction (F-contrast). Post-hoc t-contrasts; neurotypical systole>autism systole, neurotypical diastole>autism diastole, autism systole>neurotypical systole and autism diastole>neurotypical diastole, revealed right insula and regions of cingulate cortex (mid and posterior) were enhanced at systole and diastole in neurotypical individuals, relative to autistic participants (figure 3.6; supplementary table 2.4). Autistic participants showed enhanced activation in left cuneus relative to neurotypical participants on systole trials but no significant activation on diastole trials.

3.5.5 Anxiety and depression

A second level model, examining the relationship between fear processing at systole (relative to diastole) and anxiety, controlling for depression, found no significant activation across all participants ($p<0.05$ cluster-wise FDR). For the same contrast, activation in cuneus, occipital gyrus and bilateral cerebellum correlated with depression scores, controlling for anxiety, across all participants (supplement-

ary table 2.6).

3.5.6 Functional connectivity: fear in amygdala and insula

Four second-level models tested for changes in the FC of left and right amygdala, and of left and right insula, as a function of emotion (processing fear relative to neutral faces). Across all participants, emotion-induced changes in FC were observed between left insula and the left precuneus and right cerebellum (VI). There were no significant PPI effects for bilateral amygdala nor right insula.

3.5.7 Functional connectivity: systole in right insula

One second-level model tested for group differences in the FC of right insula as a function of cardiac phase (systole). In right insula, we observed a significant group effect (F-contrast). A post-hoc t-contrast revealed that neurotypical compared to autistic participants had significantly greater FC on systole trials between right insula and parietal (angular gyrus, supramarginal gyrus, parietal lobule precuneus), occipital (occipital gyrus), cingulate and frontal cortices (figure 3.6; see supplementary table 2.7 for full PPI results).

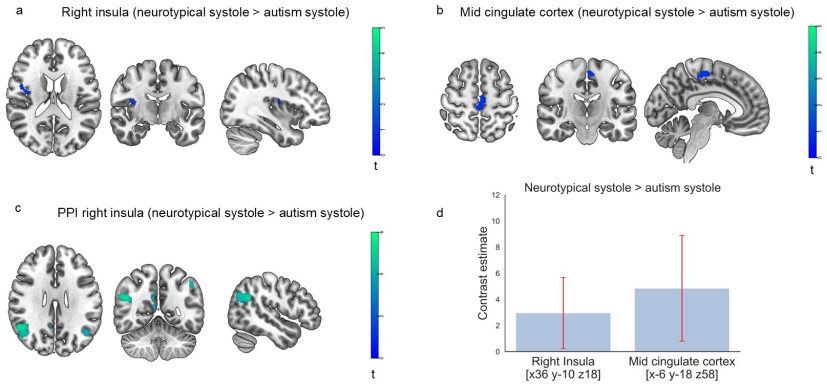


Figure 3.6. Mid cingulate cortex activity and right insula activity and functional connectivity.

A main effect of group (F-contrast) revealed significant activation differences between neurotypical and autistic participants on systole trials. Post-hoc t-contrasts revealed greater activity for neurotypical participants in right insula (a) and mid cingulate cortex (b). Psychophysiological interaction analyses revealed greater functional connectivity, in neurotypical participants on systole trials, compared to autistic participants, between right insula and regions including angular, frontal and supramarginal gyrus, mid and posterior cingulate cortex and precuneus (c). Contrast estimate effect size plot (d) show effect size in right insula and mid cingulate cortex for neurotypical > autism on systole trials. Red bars represent 90% confidence intervals.

3.6 Discussion

The processing and subjective evaluation of emotional stimuli are influenced by cardiac afferent signals. Behaviourally, with the noted limitation that uncorrected p values make our behavioural results preliminary, across autistic and neurotypical participants, subjective ratings of intensity of neutral faces were inhibited at systole, relative to diastole. In contrast, fearful faces were resistant to this inhibitory effect of the heart, and indeed, cardiac systole facilitated fear processing as a function of anxiety: increasing levels of anxiety across all participants, was predicted by increased enhancement of fear intensity at systole relative to diastole, when controlling for depression. Notably, the behavioural expression of cardiac effects on emotional processing did not differentiate autistic from neurotypical individuals. However, neural correlates of such cardiac effects on emotional processing did begin to show group differences in cardiac signalling: neurotypical

participants, compared to autistic participants, showed greater activity in right insula and mid cingulate cortices on systole trials. Crucially also, right insula showed reduced functional connectivity (FC) in autistic participants on systole trials. This suggests the presence of an aberrant interoceptive mechanism wherein signalling of cardiovascular arousal is less integrated with cognitive, affective and perceptual processing in autism.

Our findings provide further evidence that the impact of arterial baroreceptor activation (at ventricular systole) is nuanced, extending beyond a general inhibitory effect on sensory processing (Azevedo et al., 2017; Fiacconi et al., 2016; Gray et al., 2012; Makowski et al., 2020). Indeed, fear processing has previously been shown to be enhanced at systole (Garfinkel et al., 2014), along with other forms of perceived threat (Azevedo, Badoud, & Tsakiris, 2018). While we did not replicate the systolic enhancement of fear ratings at the group level, we demonstrate 1) a selective effect (fear systole>fear diastole) as a function of anxiety and show; 2) that fear processing at systole is resistant to the same inhibitory effect that is shown for neutral faces, a finding now consistently reported (Azevedo et al., 2018; Critchley et al., 2019; Garfinkel & Critchley, 2016; Garfinkel et al., 2014). Notably, our results are unique to fear and neutral faces as participants were asked to explicitly rate a face which they likely knew to be, based on validation studies (e.g. Goeleven et al., 2008), fearful or neutral and we would thus not expect to achieve similar results across other emotion categories. Indeed, prior work has shown these cardiac effects to be particularly sensitive to fear (Garfinkel et al., 2014), with some effects for disgust (Gray et al., 2012). Importantly, this is the first work to investigate cardiac phase fear processing in autistic individuals, who often show atypical patterns of emotional processing (Hill et al., 2004; Mulcahy, Davies, et al., 2019). However, our findings indicate that differences in this mechanism in autism are rather subtle.

In response to fear faces (relative to neutral faces), we observed activation across all participants (autistic and neurotypical) in bilateral insula and amygdala. The amygdala is considered a critical hub for fear processing (Adolphs, Tranel, Damasio, & Damasio, 1995; LeDoux, 2003) and is implicated in interoception and autonomic control (Critchley, 2005; Critchley et al., 2005), potentially integrating psychological distress with bodily arousal (Critchley et al., 2002; Gianaros et al.,

2008). In autism, amygdala responses when processing social-emotion information may be functioning atypically (Critchley et al., 2000; Schultz, 2005), including response to fear stimuli (Top Jr et al., 2016). However, our results here do not necessarily indicate atypical amygdala activation in autism, since both groups, autistic and neurotypical, recruited the amygdala when processing fearful face stimuli.

Nevertheless, the insula was one region that showed group, emotion-related and cardiac effects. Across all task conditions, bilateral insular activation was lower in autistic participants. While across all participants fearful faces generally evoked a greater responses than neutral faces, neurotypical but not autistic participants, showed increased insular activation when stimuli were presented at systole. Moreover, right insula showed increased activation and FC on systole trials in neurotypical participants. The insula, particularly right, is consistently implicated in interoceptive representation and autonomic control, as a 'hub' for the integration of afferent interoceptive signals (Craig, 2002; Critchley & Harrison, 2013; Gu et al., 2013). Thus, insula dysfunction may underscore aberrant interoceptive signalling and subsequent deficits in emotional processing (Singer et al., 2009). Here, we provide novel evidence to suggest that insular activation is dampened in autism, yet the region is still recruited when processing fearful stimuli. Importantly, we also show that, in autism, right insular activation is reduced to stimuli processed at systole, i.e. during the signalling of cardiovascular arousal. Additionally, autistic participants did not show the same increase in FC between right insula and parietal, cingulate and frontal regions observed in neurotypical individuals. Thus, we speculate that the region's reactivity and connectivity is atypical in autism and may lead to aberrant integration of interoceptive signals (Elwin et al., 2012; Fiene & Brownlow, 2015; Garfinkel, Tiley, et al., 2016; Palser et al., 2018). This, in turn may contribute to the increased vulnerability and manifestation of anxiety disorders in autistic individuals (Garfinkel, Tiley, et al., 2016). Indeed, previous work has demonstrated reduced activation and FC of the insula in autism (Ebisch et al., 2011; Odriozola et al., 2016; Silani et al., 2008) and, perhaps differences in insular activation can predict autistic social difficulties in the absence of differences at the groups level (Failla et al., 2020). However, more work is needed to characterise this relationship, for example by em-

ploying alternative interoceptive paradigms during fMRI (Critchley et al., 2004), to quantify the integrity of neural substrates of distinct facets of interoception (Garfinkel et al., 2015). We also acknowledge that the behavioural data (i.e. no group differences) does not reflect our neuroimaging data, which show clear group differences in regions involved in emotion processing and autonomic/interoceptive processes. Thus the task employed may not adequately capture emotion processing differences and future work should look to improve the study design (e.g. more naturalistic faces; Barrett, Adolphs, Marsella, Martinez, & Pollak, 2019). The inclusion of EEG would also benefit this work, i.e. simultaneous temporally sensitivity (millisecond) EEG and spatially sensitivity (millimetre) fMRI.

With regards to affective symptomatology, behaviourally, we found no group differences in the relationship between anxiety and cardiac contingent fear processing between autistic and neurotypical participants, despite the increased prevalence of anxiety in autistic individuals (Hollocks et al., 2019). We did however find anxiety and depression paralleled cardiac-contingent fear processing across all participants; controlling for depression, fear intensity ratings at systole, relative to diastole, increased with greater levels of anxiety. Anxiety and depression present distinct yet somewhat similar autonomic profiles; anxiety is typically associated with heightened tonic cardiovascular arousal and perhaps reactivity, e.g. increased heart-rate, blood pressure, cortical arousal and reduced cardiovagal tone (lower heart-rate variability) while depression can encompass a hypo-responsive autonomic profile, still including both sympathetic and cardiovagal withdrawal, although results are not always consistent (Dunn, Dalgleish, Ogilvie, & Lawrence, 2007; Friedman & Thayer, 1998; Mulcahy, Larsson, et al., 2019). Thus, as in our data, depressive symptoms and accompanying autonomic indices, may counter anxiety-evoked effects on fear processing. An inhibitory effect of depression was also evident at the neural level. In brain, controlling for anxiety, activation in cuneus, occipital gyrus and bilateral cerebellum correlated with depression scores. However, as in our behavioural results, we show no group differences between fear processing at systole, relative to diastole, that were related to baseline levels of anxiety and/or depression. Thus, interestingly, whatever inhibitory role is played by the cerebellum during the processing of fearful stimuli at systole, the effect is related to depressive symptoms not autism diagnosis, an interesting extension of

work implicating cerebellar involvement in both autonomic and emotional control (Barrett, 2017; Schutter & Van Honk, 2005). Our findings illustrate how broadly central interoceptive processing interacts with affective symptomatology, potentially causally influencing the maintenance of affective symptoms.

This is the first study to investigate cardiac effects on emotion processing in autistic adults. We show that, behaviourally, autistic individuals do not differ from neurotypical individuals when processing fearful or neutral stimuli presented at different phases of the cardiac cycle. We replicated previous work showing an inhibitory effect of cardiac systole for neutral stimuli, with systolic enhancement of fear processing emerging as a function of anxiety symptomatology. Thus, increased arousal, via afferent baroreceptor signalling, relatively enhances fear processing in neurotypical and autistic participants, pointing toward a targetable transdiagnostic mechanism for the treatment for anxiety symptoms (Watson et al., 2019). Finally, beyond fear processing, we found autistic individuals manifest reduced activation and FC in the 'interoceptive' right insula cortex suggesting a mechanism that may underscore expressions of aberrant interoceptive integration in this population.

Chapter 4

Neural correlates of cardiac interoception in autistic adults: an fMRI investigation of interoceptive attention and interoceptive training

4.1 Abstract

Interoception, the sensing and signaling of internal bodily sensations, influences behaviour and emotional experience. In autistic individuals, a disrupted interoceptive system may contribute to sensory difficulties and causally influence anxiety symptomatology. As such, targeted interoceptive training, aimed at better aligning interoceptive signals, may influence emotional experience and thus reduce anxiety. The aim of the current study was thus two-fold; to better characterize the behavioural and neural profile of interoception in autistic adults and to implement a novel interoceptive training paradigm to reduce anxiety in autistic adults. In study 1, we employed cardiac interoception tasks (heartbeat tracking and discrimination), along with subjective measures of affective symptoms, and quantified neural signatures of interoception during functional brain scanning. In study 2, we employed a novel interoception training paradigm where, over the course of 6 training sessions, autistic adults were trained to better perceive and understand their heartbeats. Participants undertook interoceptive tasks and functional scanning pre and post interoceptive training to quantify neural and behavioural markers of change. Results revealed comparable levels of interoceptive accuracy yet a heightened belief about sensitivity to interoceptive sensations in autistic adults. In brain, functional connectivity of right and left insula revealed group differences across interoceptive dimensions (accuracy and insight). Following interoceptive training, all participants were better able to perceive their heartbeat yet no significant reduction in anxiety was observed at the group level, although we were likely underpowered to detect such an effect. However, functional connectivity of right and left insula significantly increased suggesting interoceptive training can increase neural communication with regions involved in emotional and autonomic control which has broad implications for mitigating emotion difficulties in diverse populations.

4.2 Introduction

The representation of the internal viscera is modulated by a set of cortical and subcortical regions; namely the insular cortices, amygdala and the anterior cingulate cortices (Craig, 2002; Critchley et al., 2005; Critchley & Harrison, 2013). Ascending afferent information from interoceptive axis (e.g. cardiac, respiratory, gustatory) provides a moment by moment mapping to the brain of internal bodily state which can guide behaviour (Barrett & Simmons, 2015; Critchley & Harrison, 2013), decision making (Dunn et al., 2010; Kirk, Downar, & Montague, 2011) and emotional experience (Lange et al., 1967). Concurrently, the accuracy, cohesion and interpretation of interoceptive signals can optimally influence emotional processing whilst interoceptive disparity can contribute to the development and presentation of affective symptomatology, namely anxiety (Garfinkel, Tiley, et al., 2016).

The relationship between interoception and emotion is nuanced; interoceptive signals can operate unconsciously, guiding behaviour in the absence of perception or attention (Azevedo et al., 2017; Garfinkel et al., 2014; Gray et al., 2009), or at the objective level, with attention, measured by objective performance on heartbeat detection paradigms (Katkin et al., 1983; Schandry, 1981; Whitehead et al., 1977), where anxiety has been associated with increased interoceptive accuracy (Dunn et al., 2010; Pollatos et al., 2007; Stevens et al., 2011), although results are not always consistent (Ehlers et al., 1988; De Pascalis et al., 1984). Heightened interoceptive sensibility is also commonly associated with anxiety (Anderson & Hope, 2009; Gregor & Zvolensky, 2008; Olatunji, Deacon, Abramowitz, & Valentiner, 2007) and, arguably, anxiety may manifest as a product of the discrepancy between objective and subjective dimensions of interoception, termed the interoception trait prediction error (ITPE) (Garfinkel, Tiley, et al., 2016) which has important clinical implications for the treatment of anxiety, particularly in patient populations where the prevalence of anxiety is significantly increased, such as autism spectrum conditions (Hollocks et al., 2019).

Autism spectrum conditions (ASCs) are a set of neurodevelopmental conditions characterized by social and emotional difficulties, restricted and repetitive interests and altered sensory processing. Current empirical findings detailing the nature of interoception in autism is mixed, where some demonstrate reduced in-

teroceptive accuracy in autistic children (Palser et al., 2018) and adults (Garfinkel, Tiley, et al., 2016), while other research indicates that interoceptive is not impaired in autism (e.g. Failla et al., 2020; Schauder et al., 2015; Shah et al., 2016). On a subjective level, autistic adults have been shown to display increased interoceptive sensibility compared to controls (Garfinkel, Tiley, et al., 2016; Mul et al., 2018). Evidence also suggests autistic participants may report a hyper sensitivity to external stimuli yet a blunted, hypo-sensitivity to internal sensations (Elwin et al., 2012; Fiene & Brownlow, 2015), although it should be noted that it is possible autistic individuals experience both a hyper and hypo sensitivity to external stimuli, both across and within sensory modalities (e.g. Robertson & Simmons, 2015). Conversely, some argue that interoceptive impairments are not a core feature of autism (Nicholson et al., 2018; Schauder et al., 2015; Shah et al., 2016) and provide evidence to support the contribution of other factors that may explain different or null findings; including a different developmental trend in autism (Mash et al., 2017; Nicholson, Williams, Carpenter, & Kallitsounaki, 2019), the impact of cognitive ability (Mash et al., 2017), the severity of core autistic symptoms (Palser, Fotopoulou, Pellicano, & Kilner, 2020) or the presence of alexithymia (Bird et al., 2010; Cook et al., 2013; Shah et al., 2016), described as difficulty identifying and describing one's own emotional feelings (Apfel & Sifneos, 1979).

In brain, despite the perspective that the insula represents the interoceptive 'hub' involved in interoceptive/social/emotional integration (Craig, 2002; Craig & Craig, 2009; Critchley et al., 2004), and the evidence of altered activation and connectivity of the insula in in autism (e.g. Ebisch et al., 2011; Francis et al., 2019; Odriozola et al., 2016), only one study has directly examined cardiac interoception during fMRI in autistic individuals (Failla et al., 2020), utilizing the heartbeat tracking task (Schandry, 1981), one of the dominant heartbeat detection methods. In this study, insular response interacted with group to predict autistic symptoms yet no objective accuracy differences between autistic and control participants and no group differences in insula activation were observed. However, altered interoceptive sensitivity in autism in other domains, i.e. insight, sensibility or trait prediction, may present with distinct neural functional architecture and are yet to be investigated. The aim of study one was thus to better characterize

the neural underpinnings of interoceptive attention in autism using the heartbeat discrimination paradigm (Katkin et al., 1983; Whitehead et al., 1977) and to explore how neural activation relates to subjective and metacognitive indices of interoception.

Additionally, based on the findings that individuals prone to anxiety may manifest an altered interoceptive prediction signal (Paulus & Stein, 2006, 2010), particularly in autism (Garfinkel, Tiley, et al., 2016; Palser et al., 2018), we employed a novel interoceptive training paradigm to improve interoceptive precision and thus reduce objective/subjective mismatch to reduce anxiety. Previous work has targeted processes associated with interoception as a mechanism to reduce anxiety with success, for example using mindfulness with a body scan component (Farb, Segal, & Anderson, 2013; Serpa, Taylor, & Tillisch, 2014; Spek, Van Ham, & Nyklíček, 2013) or by targeting breath control (Holtz, Hamm, & Pané-Farré, 2019), whilst others have focused on improving distinct aspects of interoception, namely accuracy, through the use of feedback (Ainley, Tajadura-Jiménez, Fotopoulou, & Tsakiris, 2012; Ainley, Maister, Brokfeld, Farmer, & Tsakiris, 2013; Canales-Johnson et al., 2015; Schaefer, Egloff, Gerlach, & Witthöft, 2014) and exercise (Kirk et al., 2011; Montgomery, Jones, & Hollandsworth Jr, 1984), which increases the strength and rate of heartbeats, providing an accessible and usable mechanism for enhancing interoceptive signals. Thus, the aim of study 2 was to employ a novel interoceptive training paradigm, utilizing both feedback and exercise, to improve objective and subjective facets of interoception (i.e. a greater interoceptive alignment), and investigate training effects on anxiety symptomatology. We also quantify neural signatures of interoceptive pre versus post training as a further, higher level, index of interoception.

We hypothesize that, at baseline, autistic participants relative to non-autistic participants will show reduced interoceptive accuracy, across tracking and discrimination tasks, yet increased interoceptive sensibility. This divergence will correspond to an increased ITPE in autistic adults which will correlate with increased levels of trait anxiety. In brain, insula response will be blunted and functional connectivity (FC) will be significantly reduced in autistic adults. We also hypothesise that this reduction will correspond with behavioural scores of accuracy, insight and sensibility. Following training, we predict all participants

will show a significant increase in interoceptive accuracy scores which will better align with subjective perceptual judgements of interoceptive ability, i.e. a reduced ITPE. This reduction will correlate with a significant reduction in trait anxiety. In brain, insula response and connectivity will be significantly increased and change in interoceptive scores (accuracy, insight and sensibility) will correspond to altered neural networks involved in interoceptive and autonomic processes.

4.3 Methodology

4.3.1 Participants

For study 1, 40 participants with a confirmed ASC diagnosis and 40 non-autistic controls were recruited. However, due to scanning abnormalities (2 incidental findings, 4 incomplete data, 6 excessive movement), 12 participants (6 autistic, 6 neurotypical) were excluded from the study, resulting in a final sample of 34 autistic (17 male, 17 female as assigned at birth; mean age 32.94yrs, range 18-64yrs) and 34 non-autistic neurotypical participants (17 male, 17 female as assigned at birth; mean age 30.20yrs, range 18-63yrs). Remaining participants were still matched on age, gender and education (see results for statistics). For study 2, of the 40 autistic adults initially recruited, 24 completed the second scan and were thus used in this analyses (14 dropped out, 2 excessive movement). Remaining participants included 13 males and 11 females as assigned at birth; mean age 35.89yrs, range 18-64yrs.

All participants, autistic and neurotypical, were right handed, fluent English speakers, none had a history of past head injury or organic brain disorders, cognitive impairment or a learning disability (general mental impairment); none had asthma/respiratory illnesses, epilepsy or evidence of psychotic experiences (i.e. none reported such co-morbid diagnoses or were currently taking antipsychotic medication). Autistic participants were recruited from the Sussex Partnership (adult) Neurodevelopmental Service and through advertisements placed on social media and via leaflets and posters. All autistic participants had established diagnoses in accordance with DSM4-R criteria verified by consultant psychiatrist and multidisciplinary clinical team with expertise in evaluation and clinical management of neurodevelopmental conditions. All autistic participants provided written

informed consent with all procedures approved by the NHS Research Ethics Committee. Neurotypical participants were recruited from the University of Sussex and members of the local community. All neurotypical participants provided written informed consent with all procedures approved by the BSMS research Governance Ethics Committee.

4.3.2 Offline interoceptive assessments

During fMRI scanning, participants completed the heartbeat discrimination task, however, due to a cardiac timing error (described below in section 4.3.3), interoceptive accuracy, insight and confidence scores calculated from the scanner task were invalid. Thus, interoceptive assessments that were completed outside of the scanner, prior to the scanning taking place, are included here. Two measures were used to determine measures of interoceptive ability; the heartbeat-tracking task (Schandry, 1981) and the heartbeat discrimination task (Katkin et al., 1983; Whitehead et al., 1977). Participants' heartbeat was measured at rest using a medical-grade pulse oximeter (Nonin4600 pulse oximeter, Nonin Medical Inc. Plymouth MN USA) fitted with soft finger cuff (not tension / spring-loaded).

Participants first completed the heartbeat-tracking task, and were required to concentrate on their heartbeat and without physically checking, silently count how many heartbeats they felt in their body from the time they heard "start" to when they heard "stop". Six durations, presented in a random order, of 25, 30, 35, 40, 45 and 50s were used. Heartbeat tracking accuracy was thus calculated based on the ratio of actual to perceived heartbeats: 1-(|nbeatsreal – nhbeatsreported|/((nbeatsreal + nbeatsreported)/2) (Garfinkel et al., 2015). After each trial, participants completed a visual analogue scale (VAS), with a scale of 0–10, to signal confidence of their decision.

Participants next completed the heartbeat discrimination task which involved the presentation of a periodic external stimulus and participants were tasked with identifying whether the tones were presented synchronous or asynchronous with their own heartbeat. Participants were presented with 10 auditory tones, 20 times to form 20 trials. Tones were presented at 440Hz with a 100ms duration. In the heartbeat discrimination task, tones were triggered at the rising edge of the pulse pressure wave, representing mid ventricular systole, on synchronous trials. On

the delayed trials, tones were triggered 300ms after the rise of the pulse pressure wave, representing early diastole. Adjusting for an average pulse transit time of 250ms, these tone timings corresponded to 250ms or 550ms after the ECG R-wave, putatively the time of peak perceptual differentiation. At the end of each trial, participants reported whether the tone was synchronous or asynchronous with their heartbeats, and then provided a confidence rating using the VAS scale. The auditory tones were always presented at the participant's own heart rate, hence the participant was unable to use the tempo of tones or knowledge about their own heart rate to inform their response (Garfinkel et al., 2015).

Thus, interoceptive ability was quantified using: interoceptive accuracy, objective performance on the heartbeat tracking and heartbeat discrimination tasks, interoceptive sensibility, as measured by the Body perception questionnaire (BPQ) (Porges, 1993), the Multidimensional Assessment of Interoceptive Awareness (MAIA) (Mehling et al., 2012) and trial-by-trial confidence judgements, and, finally, interoceptive insight, quantified using receiver operating characteristics (ROC) curve analysis (Green et al., 1966) for confidence-accuracy correspondence on the heartbeat discrimination task. One participant from the neurotypical group and 2 participants from the autistic group, post-training, did not complete the interoception task and were thus not included in any analyses involving this measure.

In the current study, we also employed a time-control task for comparison against the heartbeat tracking task, based on the work linking performance on the heartbeat counting task to time-estimation (Murphy, Millgate, et al., 2018). In this task, participants were required to 'count how many seconds they think has passed between the words start and stop'. Mirroring the design of the heartbeat tracking task, participants completed 6 trials each of duration 25, 30, 35, 40, 45 and 50 seconds. Accuracy was then computed on a trial-by-trial basis based upon the ratio of perceived to actual seconds 1-(|nsecondsreal – nsecondsreported|/((nsecondsreal + nsecondsreported)/2) and these were then averaged to form a mean time tracking score.

4.3.3 MRI experimental paradigm

The Interoception paradigm was programmed in Matlab R2013A (The MathWorks, Inc., Natick, MA) which utilized Cogent2000 for stimuli presentation.

Auditory tones were played through MRI safe in-ear headphones and participants made their responses on a MRI compatible button box (2 buttons; left and right). Auditory tones were generated using 'Wavplay', a built in Matlab function. The task comprised 40 trials, 20 interoceptive attention and 20 exteroceptive attention trials. On half of all trials (10 interoception, 10 exteroception) one note was subtly different in pitch (randomized across trials) to the other 9 notes (800Hz was changed to 785Hz). The presence or absence of note modulation was irrelevant on heart trials. The experiment thus employed a 2 (group) x 2 (attention) repeated measures design.

Upon commencement of the task, participants were presented with onscreen instructions; 'If you see the word 'HEART', concentrate on your heartbeat. Are the beeps ON your heartbeat (<) or OFF your heartbeat (>)?', 'If you see the word 'NOTE', concentrate on the beeps. Is there an ODD ONE OUT (<) or are the beeps all the SAME (>)?', 'You will have 6 seconds to make your response.', 'Use the slider to say how sure you were about your answer.', 'You will have 3 seconds to make your response'. Each trial began with the presentation of the word 'Heart' or 'Note', displayed for 2 seconds. This was followed by a fixation cross (displayed for the duration of the 10 beeps) and 10 auditory beeps (100ms duration each). After each trial, participants indicated their response (left or right button; 'ODD ONE OUT < > all beeps the SAME?' or 'ON heartbeat < > OFF heartbeat?'), displayed for 5 seconds, and rated how confident they were in their response using avisual analogue scale (VAS), displayed for 3 seconds; 'How sure are you of your answer?', with a scale of 0 (guess) to 100 (sure). 'HEART' trials served as a measure of interoception, i.e. focus towards the heartbeat, whilst 'NOTE' trials served as a contrasting measure of exteroception, where the note modulation seeks to ensure participants attend to the sound of the beeps, rather than their heartbeat (Critchley et al., 2004).

Throughout the experiment, real time cardiac timing was obtained from a medical grade MRI-compatible pulse oximeter (8600FO; Nonin Medical Inc., MN, USA) attached to the participants left index finger and was available as a waveform in Spike software (power 1401, Spike2 v7 software, CED ltd). Here, the rise of the pulse wave represents T-wave (cardiac systole, when baroreceptors fire). However, post-experiment analyses revealed an error in cardiac timing which was

thus inaccurate. Systole trials, where aortic and carotid baroreceptors discharge (250ms after R-wave), were occurring in late diastole (>450ms after R-wave). Thus, subsequent analyses could not analyse cardiac timing effects, task-based interoceptive accuracy or insight scores. Instead, we utilize offline interoceptive scores.

4.3.4 Interoceptive training

All participants completed 6 interoceptive training sessions (mean number of days to complete training 82.29). Each training session followed an identical procedure; all participants started by completing the heartbeat tracking task (see section 4.3.2) comprising 6 trials. All participants started training with the duration of the first heartbeat tracking trial as 10 seconds. Participants informed the experimenter how many heartbeats they counted over the temporal window who then gave feedback in the form of 'that is correct', for exact reporting of actual number of heartbeats (measured via pulse-oximeter), or ' that is incorrect, your actual number of heartbeats were n'. If participants were accurate in counting their heartbeats (+/- 2 heartbeats) the next trial progressed in length, increasing in increments of 5 seconds, up to a maximum of 50 seconds. Similarly, if participants were inaccurate, > +/-3, the trial length decreased in increments of 5 seconds. Upon completion of heartbeat tracking, participants completed 20 heartbeat discrimination trials. As in heartbeat tracking, participants informed the experimenter whether the auditory tones were 'in synch' or 'out of synch' with their own heartbeat. The experimenter then provided feedback; 'That is correct' or 'That is incorrect, that was actually in/out of synch'.

After completion of the heartbeat discrimination task, participants were asked to perform exercise for a period of 2-3 minutes, until their heartbeat where noticeably elevated. The purpose of the exercise component was to enhance heartbeat sensation and to simulate a state of heightened arousal. Form of exercise were decided by the participants but included, running on the spot, start jumps or the use of an exercise bike. Following completion of exercise, participants re-completed heartbeat tracking and discrimination with experimenter feedback, as described above. See figure 4.1 for graphical view of interoceptive training paradigm.

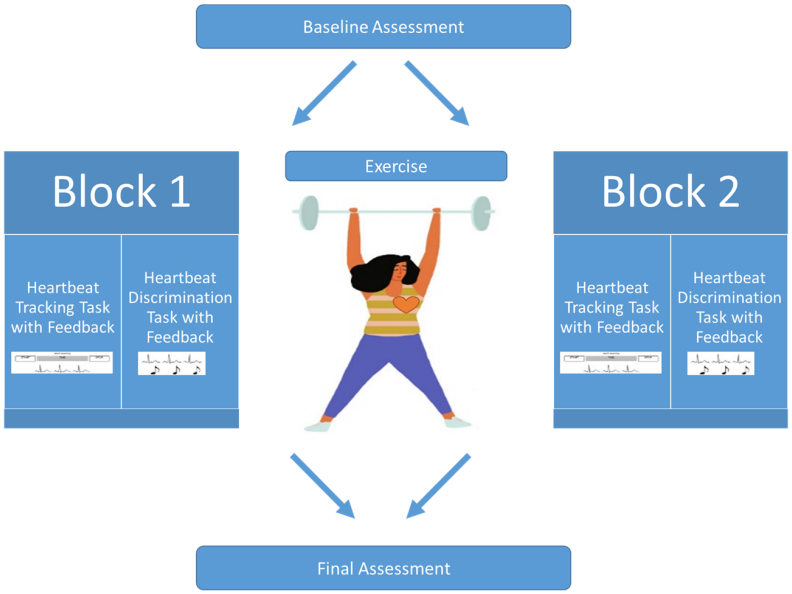


Figure 4.1. Interoception training paradigm.

All participants completed a baseline assessment followed by 6 separate interoceptive training sessions where they completed the heartbeat tracking and heartbeat discrimination task with feedback, pre and post exercise, before finishing with a final assessment.

4.3.5 Questionnaires

Before and after interoceptive training, participants completed the trait section of the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Patient Health Questionnaire (PHQ-9)(Kroenke et al., 2001), the Autism Quotient (AQ)(Baron-Cohen et al., 2001), the Toronto Alexithymia Scale (TAS-20) (Bagby et al., 1994), the Body perception questionnaire (BPQ) (Porges, 1993) and the Multi-Dimensional Assessment of Interoceptive Awareness (MAIA) (Mehling et al., 2012). For each questionnaire, the total score was computed and used in the analysis. Participants also provided demographic information including age, gender assigned at birth, and level of educational attainment. One participant in the neurotypical group did not complete the PHQ-9 or the TAS-20 and one participant from the autistic group did not complete the PHQ-9 and, post-training, one participant did not complete any questionnaires, and one other did not complete the PHQ-9, STAI or MAIA, and were thus not included in any analyses involving these measures.

4.3.6 MRI data acquisition

Functional imaging datasets were acquired using a Siemens 3T Prisma MRI scanner with a 32-channel head coil. A multiband echo-planar imaging (EPI) sequence was used with multiband acceleration factor of 2 to acquire T2*-weighted images sensitive to blood oxygen level dependent (BOLD) contrast. Each functional volume consisted of 44 slices, acquired in an interleaved order. The following parameters were used: TR = 1500ms; TE = 30ms; flip angle = 70°; matrix = 94x94; FOV = 220mm; slice thickness = 3.0mm with a 25% gap. The total number of fMRI volumes acquired varied depending on the participants heart rate/speed of response (mean number of volumes 548).

4.3.7 fMRI pre-processing

fMRI data was pre-processed using SPM12 in Matlab R2017A (MathWorks, Inc., Natick, MA). For each subject, the first 5 volumes were removed to account for magnetization equilibrium. All remaining functional images were slice-time corrected to the first slice, realigned to the first volume and spatially normalised to a standard MNI EPI template, a method that has been shown to reduce variability across subjects(Calhoun et al., 2017). The normalised images were then smoothed using an 8mm Gaussian kernel (full width half maximum) and all images were visually inspected for artefacts. The 6 motion parameters (3 translation, 3 rotations) calculated during realignment were included as regressors in the first-level model.

4.4 Data analyses

4.4.1 Behavioural data analyses

For study one, demographic information (age, baseline differences in anxiety, depression, autistic and alexithymic traits) was compared using independent sample t-tests. Education attainment was compared using Fisher's exact test. Between group differences in interoceptive and time estimation ability were also explored using independent sample t-tests and the relationship between interoception and affective symptomatology was explored using Pearson's correlations. In a replication regression analysis (Garfinkel, Tiley, et al., 2016) we tested which variable

best predicted trait anxiety by performing multiple linear regression with trait anxiety as the dependent variable and heartbeat tracking accuracy, interoceptive sensibility, discrimination ITPE, autism severity, group status, age and gender as predictor variables. As in (Garfinkel, Tiley, et al., 2016), we also switched tracking accuracy for discrimination accuracy and discrimination ITPE for tracking ITPE.

For study 2, in the smaller sample of autistic adults (n=24) who completed interoceptive training, we first tested the impact of time between scanning sessions by modeling time as a covariate in a repeated measures ANCOVA for each variable of interest. No significant effects of time (main effect or interactions) were found, all p's < 0.05, suggesting the time between scanning sessions did not impact the results. Thus we ran and report paired-sample t-tests for interoceptive scores (accuracy, insight, sensibility), time estimation ability (accuracy) and affective symptomatology (anxiety, depression, alexithymia, autistic traits) pre versus post interoceptive training. Significant changes in interoceptive ability were further explored using Pearson's correlation to establish if change in interoceptive ability correlated with change in behavioural dimensions. As we only used a subset of the full sample in these analyses, to make behavioural and neuroimaging results comparable, I also report preliminary results for the primary outcome measures from the full sample (which will be written and reported elsewhere); namely pre versus post scores in interoceptive accuracy, interoceptive trait prediction error and trait anxiety, analysed using paired-sample t-tests.

In the analyses of difference scores, we also report Bayesian statistics, using default priors in JASP software (JASP, 2020), to complement the frequentist statistics. Bayesian statistics are reported here as the data included is preliminary, the final data set/analysis will be reported elsewhere, and thus Bayesian statistics serve to aid in understanding the extent to which we can draw meaningful conclusions from this incomplete data set. Here, a Bayes factor10 (BF10) > 3 provides moderate evidence for the alternate hypothesis, a BF10 > 10 provides strong evidence in favor of the alternate hypothesis, a BF10 $< 1/3$ provides moderate evidence in favor of the null hypothesis, a BF10 $< 1/10$ provides strong evidence for the null hypothesis whilst a BF10 < 3 and BF10 $> 1/3$ suggests insufficient evidence to draw conclusions for or against either hypothesis (Jeffreys, 1998; Keysers, Gazzola, & Wagenmakers, 2020). In cases where BF10 was $< 1/3$

(i.e. evidence in favor of the null hypothesis), we also report BF01, where BF10 = 1/BF01, which provides and index for evidence of an absent effect (e.g. if BF01 = 4.5 then the data are 4.5 times more likely under the null than the alternative hypothesis). All p values in the behavioural results are uncorrected.

4.4.2 fMRI data analyses

For experiment one, using SPM12 in Matlab, individual first level models were constructed for each group individually resulting in 2 single-regressor t-contrasts; (1) heart, (2) note. Six motion parameters (3 translation, 3 rotations) calculated during realignment were included as nuisance regressors. The two t-contrasts were entered into a second level full-factorial model with group (autism/neurotypical) as an independent (between-subjects) factor, and condition (heart vs note) as a non-independent (repeated measures) factor. Resulting F-contrasts were generated to test for 1) all effects; 2) main effect of group; 3) main effect of condition; 4) group x condition interaction. Significant main effects were explored using post-hoc t-tests. In order to establish the relationship between activation and behavioural scores (interoception measures) and questionnaire data, a single t-contrast was constructed for heart > note. Within each group, one-sample t-tests tested for correlation with covariates and, where individual level (i.e. autistic or neurotypical alone) significant correlations where found, two-sample t-tests examined group differences (i.e. to examine if the slope of correlation, between heart > note activation and the covariate of interest, differed between the two groups).

For experiment 2, a single first-level contrast was constructed for ((post-heart > post-note) > (pre-heart > pre-note)) and entered into a one-sample t-test to test for change in brain activation toward heart over note trials, post interoceptive training. Finally, the series of change scores that prevailed as significant at the behavioural level (i.e. pre > post or post > pre) were entered against the contrast as a covariate to investigate the relationship between change scores and change in brain activation. Throughout all analyses of experiment 2, the time between scanning sessions in days was modeled as a covariate of no interest. statistical maps were thresholded at cluster-forming threshold $p < 0.001$ and False Discovery Rate (FDR) cluster-corrected at $p < 0.05$ for multiple comparisons. Significant clusters were localized according to SPMs Anatomy toolbox (Eickhoff et al., 2005).

4.4.3 Psychophysiological interaction (PPI)

Based on the large literature implicating the insula as a focal region involved in integrating interoceptive bodily feelings to inform emotional experience (Critchley & Garfinkel, 2017), with implications for affective disorders, particularly autism (Garfinkel, Tiley, et al., 2016), we sought to understand how the insula, when processing interoceptive signals, modulated activity elsewhere in the brain. We also sought to investigate if FC of left and right insula was altered as a result of interoceptive training.

Thus, we undertook specific generalized psychophysiological interactions (gPPI) analyses using the CONN toolbox. For experiment one, the GLM comprised regressors for condition (heart/note) and group (autism/neurotypical). The 6 realignment parameters (3 translations/3 rotations) were modeled as nuisance regressors. For experiment two, the GLM comprised regressors for condition (heart/note) and time (pre/post training) as well as nuisance regressors, including the 6 motion parameters and the time in days between scanning sessions (mean centered). For all FC analyses, the data was denoised by regressing out signal from white matter (WM), cerebral spinal fluid (CSF) and from each individual condition. The seed regions for all FC analyses were defined as the peak cluster identified from the GLM in left (x-32, y2, z12) and right (x36, y8, z10) insula. Both ROIs were defined as 10mm spheres using the MarsBaR toolbox.

For each participant, the psychophysiological interaction term was calculated according to the t-contrast of heart > note and the time series of (1) left insula and (2) right insula. For experiment one, F-contrast tested for task and group effects and t-contrasts examined the direction of significant effects; for task (two one-sample t-test models for heart > note) and for group effects (two two-sample t-tests, for ((neurotypical heart > note) > (autistic heart > note)). Additionally, interoceptive dimensions, including accuracy, insight, sensibility and ITPE, as well as affective symptomatology, including anxiety and depression, and alexithymia and autistic traits, were modeled as covariates to investigate their relationship with functional connectivity of insula cortices. Where individual group effects where found, two-sample t-tests examined group differences in the slope of correlation between FC and the covariate of interest. For experiment 2, a single t-contrast tested for training effects; within-sample t-tests, for ((post heart > note)

> pre (heart > note)). As in univariate analyses, statistical maps were thresholded at cluster-forming threshold $p < 0.001$ and False Discovery Rate (FDR) cluster-corrected at $p < 0.05$ for multiple comparisons.

4.5 Results

4.5.1 Study 1: Behavioural results

4.5.1.1 Demographics

Autistic and neurotypical participants were matched for gender and did not significantly differ on age ($t(66) = 0.894$, $p = 0.375$) or education (fisher's exact, $p = 0.782$). Significant differences were however identified in levels of anxiety ($t(66) = 8.105$, $p < 0.001$, BF10 = 3.689e+8), depression ($t(51.476) = 7.021$, $p < 0.001$, BF10 = 4.828e+6), autistic traits ($t(66) = 14.404$, $p < 0.001$, BF10 = 5.330e+18) and alexithymia; for total score ($t(65) = 7.811$, $p < 0.001$, BF10 = 1.072e+8), and the difficulty describing feelings ($t(65) = 6.213$, $p < 0.001$, BF10 = 250013.822), difficulty identifying feelings ($t(65) = 12.150$, $p > 0.001$, BF10 = 1.478e+8) and externally oriented thinking ($t(65) = 2.987$, $p = 0.004$, BF10 = 9.767) subscales (see table 4.1 for mean scores).

	Age	Education	Trait anxiety	depression	Autistic traits	Alexithymia total score
Autistic	32.94	3.59	**61.18**	**15.48**	**38.09**	**64.44**
	12.01	*1.28*	*11.34*	*7.37*	*5.20*	*10.55*
Neurotypical	30.02	4.74	**40.06**	**5.06**	**16.12**	**43.06**
	13.20	*1.08*	*10.11*	*4.29*	*7.21*	*11.83*

Table 4.1. Demographic information of autistic and neurotypical participants.Values show mean scores, where those in bold are statistically different between the two groups, and *standard deviation*.

4.5.1.2 Between group differences in interoceptive ability

Interoceptive accuracy: No significant difference in heartbeat tracking ($t(65) = -0.121$, $p = 0.904$, BF10 = 0.252, BF01 = 3.966) nor discrimination ($t(65) =$

0.773, p = 0.442, BF10 = 0.323, BF01 = 3.094) accuracy was observed between the ASC and neurotypical group.

Interoceptive insight: No significant group difference in heartbeat discrimination insight (t(65) = 1.994, p = 0.333, BF10 = 1.115) was observed.

Interoceptive trait prediction error: No significant difference in ITPE tracking (t(64) = 0.306, p = 0.760, BF10 = 0.263, BF01 = 3.808) nor discrimination (t(64) = -0.140, p = 0.889, BF10 = 0.254, BF01 = 3.931) was observed. See table 4.2 for all mean scores.

	HBT accuracy	HBD accuracy	HBD insight	ITPE tracking	ITPE discrimination
Autistic	0.53	0.57	0.65	-0.50	-0.05
	0.33	*0.16*	*0.11*	*1.43*	*1.43*
Neurotypical	0.54	0.54	0.51	-0.16	0.05
	0.56	*0.13*	*0.13*	*1.42*	*1.23*

Table 4.2. Summary of scores for interoceptive accuracy, insight and the interoceptive trait prediction error (ITPE). Values show mean scores and *standard deviation*.

Interoceptive sensibility: For the MAIA total score, autistic participants scored significantly lower than neurotypical participants (mean autism 16.83, SD 5.34, mean neurotypical 21.78, SD 4.46; t(65) = -4.120, p ¡ 0.001, BF10 = 205.696). Autistic participants also scored significantly higher on the BPQ compared to neurotypical participants (mean autism 128.44, SD 35.18, mean neurotypical 92.70, SD 29.33; t(65) = 4.510, p < 0.001, BF10 = 684.881). No significant difference in mean confidence on the tracking (mean autism 4.12, SD 2.32, mean neurotypical 3.97, SD 2.29; t(65) = 0.799, p = 0.799, BF10 = 0.258, BF01 = 3.880) nor discrimination (mean autism 4.78, SD 2.53, mean neurotypical 4.84, SD 2.08; t(65) = -0.104, p = 0.917, BF10 = 0.252, BF01 = 3.972) task was observed.

Time control task: No difference in performance (accuracy) on the time control task between autistic and neurotypical participants was observed (mean autism 0.60, SD 0.37, mean neurotypical 0.74, SD 0.18; t(41.709) = -1.855, p = 0.071). Accuracy on the time control task did however significantly correlate with performance on the heartbeat tracking task (r = 0.442, p < 0.001) across all

participants and in each group individual, for autistic (r = 0.538, p = 0.002) and neurotypical (r = 0.355, p = 0.042) participants.

4.5.1.3 Relationship between interoception and affective symptomatology

Interoceptive accuracy: Across all participants, heartbeat discrimination accuracy significantly correlated with trait anxiety (r = 0.266, p = 0.029, BF10 = 1.553). This relationship was significant in the autistic group (r = 0.346, p = 0.045, BF10 = 1.481) but not in the neurotypical group (r = 0.188, p = 0.294, BF10 = 0.367), although the correlations did not differ significantly (p = 0.251; figure 4.2a). No other significant correlations between interoceptive accuracy (tracking or discrimination) and affective variables were found, all p's > 0.05.

Interoceptive insight: No significant correlations between heartbeat discrimination insight and affective variables were found, all p's > 0.05.

Interoceptive trait prediction error: Across all participants, no correlation between trait anxiety and heartbeat tracking ITPE (r = 0.014, p = 0.913, BF10 = 0.155) or discrimination ITPE (r = -0.168, p = 0.179, BF10 = 0.372) emerged. In neurotypical participants, trait anxiety significantly correlated with heartbeat tracking ITPE (r = -0.385, p = 0.030, BF10 = 2.107) but not heartbeat discrimination ITPE (r = -0.325, p = 0.070, BF10 = 1.062). No relationships were found in the autistic group between trait anxiety and ITPE tracking (r = 0.296, p = 0.090, BF10 = 0.851) or ITPE discrimination (r = -0.136, p = 0.443, BF10 = 0.283). The correlations in each group between trait anxiety and ITPE tracking differed significantly (p = 0.005). The relationship with depression revealed only a significant correlation in the autistic groups with the heartbeat tracking ITPE (r = 0.364, p = 0.037, BF10 = 1.724), but not the neurotypical group (r = -0.113, p = .547, BF10 = 0.266) and the two correlations did not differ (p = 0.051). No other significant correlations or group differences were found, all p's > 0.05.

Interoceptive sensibility: Across all participants, BPQ scores significantly correlated with trait anxiety (r = 0.363, p = 0.003, BF10 = 13.108; figure 4.2b), TAS total scores (r = 0.306, p = 0.012, BF10 = 3.387), the TAS DIF sub-scale (r = 0.422, p < 0.001, BF10 = 74.030), autistic traits (r = 0.406, p = 0.001, BF10 = 45.225) and depression (r = 0.469, p < 0.001, BF10 = 308.346). In each

group individually, only depression significantly correlated with BPQ scores in the autistic (r = 0.400, p = 0.021, BF10 = 2.756), but not the neurotypical group (r = -0.107, p = 0.558, BF10 = 0.259), and the correlations in each group differed significantly (p = 0.02; figure 4.2c). No other significant correlations or group differences were found.

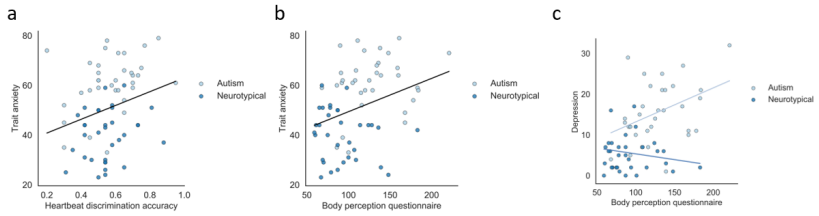


Figure 4.2. Relationship between interoception and anxiety and depression.

Correlation plots showing the relationship between heartbeat discrimination accuracy and trait anxiety (a), BPQ scores and trait anxiety (b) and BPQ scores and depression (c) in autistic and neurotypical participants.

Regression analysis: In order to detect the relative contribution of interoceptive accuracy, sensibility, ITPE, autism severity, group status, age and gender to anxiety, all variables were entered into a multiple regression analysis which prevailed as significant ($F(7, 65) = 11.241$, $p < 0.001$, $R^2 = 0.576$), with discrimination ITPE the largest predictor of anxiety (table 4.3). We then switched tracking accuracy for discrimination accuracy and discrimination ITPE for tracking ITPE and the regression again prevailed as significant ($F(7, 65) = 10.931$, $p < 0.001$, $R^2 = 0.569$) with heartbeat discrimination accuracy and group status the largest predictors of anxiety (table 4.4).

	β	*t*	*p*
Interoceptive accuracy (HBT)	-0.41	-0.468	0.641
Interoceptive sensibility	0.240	1.813	0.075
Discrimination ITPE	**-0.298**	**-2.644**	**0.011**
Autistic traits (AQ)	0.272	1.560	0.124
Group	-0.337	-1.789	0.079
Age	-0.098	-1.127	0.265
Gender	-0.079	-0.919	0.362

Table 4.3. Regression analysis investigating the relative contribution of interoception (heartbeat tracking accuracy and heartbeat discrimination ITPE) to trait anxiety.

	β	*t*	*p*
Interoceptive accuracy (HBD)	**0.211**	**2.374**	**0.021**
Interoceptive sensibility	0.032	0.195	0.846
Tracking ITPE	-0.042	-0.300	0.766
Autistic traits (AQ)	0.284	1.618	0.111
Group	**-0.425**	**-2.141**	**0.036**
Age	-0.100	01.133	0.262
Gender	-0.076	-0.875	0.385

Table 4.4. Regression analysis investigating the relative contribution of interoception (heartbeat discrimination accuracy and heartbeat tracking ITPE) to trait anxiety.

4.5.2 Study 1: fMRI results

Main effect of group: No significant differential brain activation was observed between autistic and neurotypical participants across all task (interoceptive and exteroceptive) conditions.

Main effect of condition: Across both groups, we observed differential peak brain activation between heart and note conditions as signaled by a significant main effect of condition (F-contrast). For the t-contrast of heart > note, we observed activation in bilateral insula, mid and anterior cingulate cortex, cerebellum, supramarginal gyrus, middle frontal gyrus and precuneus (figure 4.3, supplementary table 3.1). For the reverse t-contrast of note > heart we observed increased activation in paracentral lobule, precentral gyrus and postcentral gyrus.

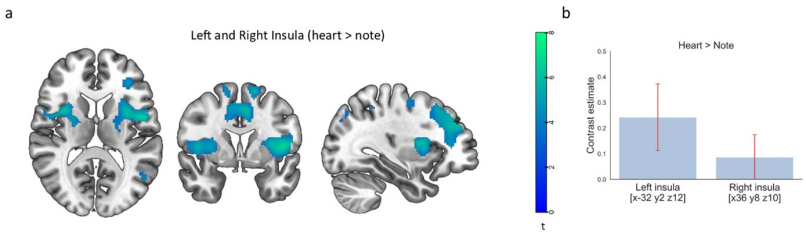


Figure 4.3. Main effect of condition.

A main effect of condition revealed significant activation differences when performing the heart versus note task. Significant activation was notably observed in (a) bilateral insula and cingulate cortex for heart > note. Contrast estimate plots (b) show effect size in left and right insula for heart > note. Red bars represent 90% confidence intervals.

Group and condition interaction: No significant differential brain activation was observed between autistic and neurotypical participants for the group by condition interaction suggesting similar levels of activation when processing interoceptive (heart) versus exteroceptive (note) stimuli, in either direction, across the two groups.

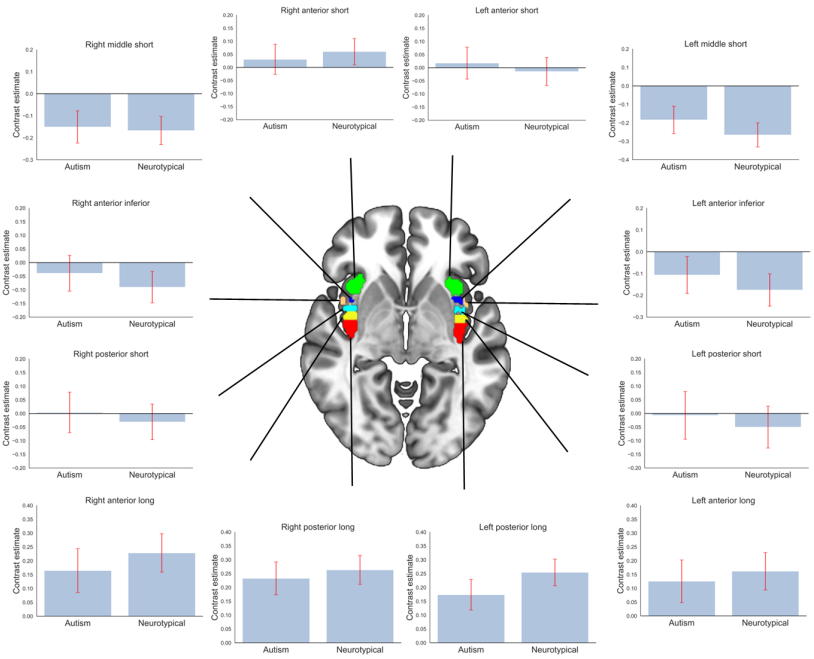


Figure 4.4. Contrast estimates of insula subdivisions during heart attention across autistic and neurotypical participants.

Overall, autistic participants show reduced contrast estimates compared to neurotypical individuals, although none are statistically significant. Insula sub-divisions were divided macro-anatomically (Faillenot et al., 2017) into left and right anterior short, middle short, anterior inferior, posterior short, anterior long and posterior long.

4.5.2.1 Functional connectivity

Two second level models tested for FC of left and right insula as a function of condition (heart > note). In right insula, we observed no significant group effect (F-contrast) but did observe a significant effect of task (F-contrast) indicating greater FC between right insula and regions including supramarginal, precentral, angular, lingual and middle frontal gyrus, opercular cortex, and occipital pole. Post-hoc t-contrasts revealed greater FC between right insula and postcentral gyrus and supramarginal gyrus for heart > note, and between right insula and angular gyrus for note > heart (supplementary table 3.2).

In left insula, we also observed no significant group effect (F-contrast) but did observe a significant effect of task (F-contrast) indicating greater FC between left insula and regions including postcentral, precentral, angular and middle frontal gyrus, occipital and temporal cortex and occipital pole. Post-hoc t-contrasts

revealed greater FC between left insula and superior parietal lobule, postcentral, precentral gyrus, left superior/middle frontal gyrus and operculum cortex for heart > note, and between left insula and temporal gyrus, occipital cortex, occipital fusiform gyrus and occipital pole, for note > heart (supplementary table 3.3).

4.5.2.2 Relationship with interoception - fMRI results

Interoceptive accuracy for heart > note: No significant activation was associated with heartbeat tracking or discrimination accuracy across all participants (autistic and neurotypical), nor between each group individually.

Interoceptive insight for heart >note: No significant activation was associated with heartbeat discrimination insight across all participants (autistic and neurotypical), nor between each group individually.

Interoceptive trait prediction error for heart > note: Activation in temporal gyrus was significantly associated with ITPE tracking in neurotypical participants only (supplementary table 3.4). The slope of correlation did not differ between the two groups and no other significant correlations were found for ITPE tracking or discrimination across neurotypical or autistic participants or all participants combined.

Interoceptive sensibility for heart>note: Significant activation correlated with BPQ scores in neurotypical participants in left and right ACC, superior medial gyrus, mid orbital gyrus and middle frontal gyrus (supplementary table 3.4). No significant activation correlated with BPQ scores in autistic participants alone, nor all participants combined, and there was no significant difference in the slope of correlation between the two groups. No relationship was found with the MAIA or confidence scores on the tracking or discrimination task.

4.5.2.3 Relationship with variables of interest - fMRI results

Anxiety and depression: No significant brain activation was associated with anxiety or depression in each group individual, nor across all participants (autism and neurotypical) combined.

Alexithymia and autistic traits: No Significant brain activation was associated with alexithymia or autistic traits in each group individual, nor across all participants (autism and neurotypical) combined.

4.5.2.4 Relationship with interoception - functional connectivity

Interoceptive accuracy in PPI (supplementary table 3.5)

All participants: No significant FC was associated with heartbeat tracking or discrimination accuracy across all participants (autistic and neurotypical).

Individual groups: In neurotypical participants only, FC of right insula with middle frontal gyrus, supplementary motor cortex and putamen correlated with heartbeat tracking accuracy. FC of left insula with middle frontal gyrus significantly correlated with tracking accuracy in neurotypical participants only.

Group differences: The correlation between FC of right insula with heartbeat tracking accuracy differed significantly between the two groups showing greater FC with better tracking accuracy in neurotypical participants, above the autistic group, of right insula with putamen, middle frontal gyrus, frontal pole and pallidum. The same analysis in left insula revealed neurotypical participants with better tracking accuracy, above autistic participants, showed greater FC of left insula with putamen, pallidum, amygdala, frontal orbital cortex and frontal pole.

Interoceptive insight in PPI (supplementary table 3.6)

All participants: FC of right insula with temporal gyrus and FC of left insula with cerebellum, parietal operculum, post central and temporal gyrus, thalamus and brainstem, significantly correlated with heartbeat discrimination insight across all participants.

Individual groups: In autistic participants only, FC of right insula with paracingulate and temporal gyrus and FC of left insula with right insula, temporal gyrus, thalamus, opercular cortex and putamen, significantly correlated with discrimination insight. In neurotypical participants only, FC of right insula with frontal pole and FC of left insula with cerebellum, precentral and postcentral gyrus, temporal cortex, Parahippocampal and lingual gyrus, correlated with discrimination insight.

Group differences: The correlation between FC of left insula, with occipital fusiform gyrus, precuneus, occipital cortex, parietal lobule, occipital pole and cerebellum, and heartbeat discrimination insight was greater in neurotypical participants. No other group differences were found.

Interoceptive sensibiltiy in PPI (supplementary table 3.7)

FC of left insula with cerebellum and lingual gyrus significantly correlated with

BPQ scores across all participants. In neurotypical participants only, FC of left insula with temporal gyrus and occipital cortex significantly correlated with BPQ scores. No other significant relationships or group differences were found for the MAIA or mean confidence.

Interoceptive trait prediction error (ITPE) in PPI (supplementary table 3.8)

Across all participants, no association between FC of left and right insula and tracking ITPE nor discrimination ITPE was found. In neurotypical participants only, FC of right insula with orbital cortex and frontal pole significantly correlated with heartbeat tracking ITPE and FC of left insula with parahippocampal gyrus and hippocampus significantly correlated with heartbeat discrimination ITPE. In autistic participants only, FC of left insula with cerebellum significantly correlated with heartbeat tracking ITPE. No significant group differences were found.

4.5.2.5 Relationship with variables of interest - functional connectivity

Relationship with anxiety and depression (supplementary table 3.9)

FC of right insula with right cerebellum (crus i and crus ii) significantly correlated with trait anxiety across all participants. In neurotypical participants only, FC of left insula with brain stem and cerebellum significantly correlated with trait anxiety. No other relationships or group differences with trait anxiety were found. No significant relationship with depression were found across all participants nor in each group individually.

Relationship with alexithymia and autistic traits (supplementary tables 3.10 and 3.11)

No significant relationship between autistic traits and FC of left and right insula was found across all participants. In neurotypical participants only, FC of right insula with occipital pole and occipital cortex and FC of left insula with occipital pole, lateral occipital cortex and brain stem, significantly correlated with autistic traits. No other significant relationships with autistic traits were found. Across all participants, FC of left insula with cerebellum significantly correlated with alexithymia scores. Additionally, in autistic participants only, FC of right insula

with occipital cortex, frontal pole and frontal medial cortex significantly correlated with alexithymia scores. This significant correlation between right insula FC and alexithymia scores was significant in the autistic group, above the neurotypical group, in operculum cortex, frontal pole and frontal medial cortex. No other significant relationships were found.

4.5.3 Study 2: Behavioural results

4.5.3.1 Change in interoceptive ability

Interoceptive accuracy: As a result of interoceptive training, heartbeat tracking ($t(21) = -4.451$, $p < 0.001$, $BF10 = 270.753$) and discrimination ($t(21) = -5.184$, $p = < 0.001$, $BF10 = 1282.164$) accuracy significantly increased. See table 4.5 for summary mean scores pre and post training. In the full sample, reported elsewhere, an increase in heartbeat tracking (mean pre 0.48, SD 0.42, mean post 0.76, SD 0.18; $t(43) = -4.421$, $p < 0.001$, $BF10 = 348.546$) and discrimination (mean pre 0.53, SD 0.17, mean post 0.66, SD 0.18; $t(42) = -4.982$, $p < 0.001$, $BF10 = 1768.688$) accuracy was also observed.

Interoceptive insight: No significant difference in heartbeat discrimination insight, pre versus post training, was observed ($t(21) = 0.818$, $p = 0.423$, $BF10 = 0.307$).

Interoceptive sensibility: No significant change in MAIA total score was observed (mean pre 17.47, mean post 19.35; $t(20) = -2.084$, $p = 0.050$, $BF10 = 1.361$). There was also no significant change in BPQ scores (mean pre 110.836, SD 36.882, mean post 122.000, SD 38.190; $t(22) = 1.767$, $p = 0.091$, $BF10 = 0.832$) or mean confidence, for the tracking (mean pre 4.045, SD 2.292, mean post 4.931, SD 2.302; $t(21) = -0.675$, $p = 0.507$, $BF10 = 0.274$, $BF01 = 3.652$) or discrimination (mean pre 4.813, SD 2.300, mean post 5.312, SD 2.347; $t(21) = -1.420$, $p = 0.170$, $BF10 = 0.537$) tasks.

Interoceptive trait prediction error: Heartbeat discrimination ITPE significantly reduced after training ($t(21) = 3.395$, $p = 0.003$, $BF10 = 29.890$). No significant change in heartbeat tracking ITPE was observed ($t(21) = 1.979$, $p = 0.061$, $BF10 = 2.213$). In the full sample, reported elsehwere, we observed a significant reduction in heartbeat discrimination ITPE (mean pre 0.19, SD 1.40,

mean post -0.40, SD 1.44; t(41) = 2..907, p = 0.006, BF10 = 6.346) and heartbeat tracking ITPE (mean pre -0.07, SD 1.53, mean post -0.54, SD 1.26; t(42) = 2.028, p = 0.049, BF10 = 1.057).

	HBT accuracy	HBD accuracy	HBD insight	ITPE tracking	ITPE discrimination
Pre-training	**0.54** *0.33*	**0.56** *0.15*	0.54 *0.12*	-0.10 *1.41*	**0.02** *1.31*
Post-training	**0.80** *0.14*	**0.73** *0.15*	0.54 *0.16*	-0.51 *1.34*	**-0.66** *1.49*

Table 4.5. Summary of pre and post training interoceptive scores. Scores indicate mean values, where those in bold are statistically different pre versus post training, and *standard deviation*.

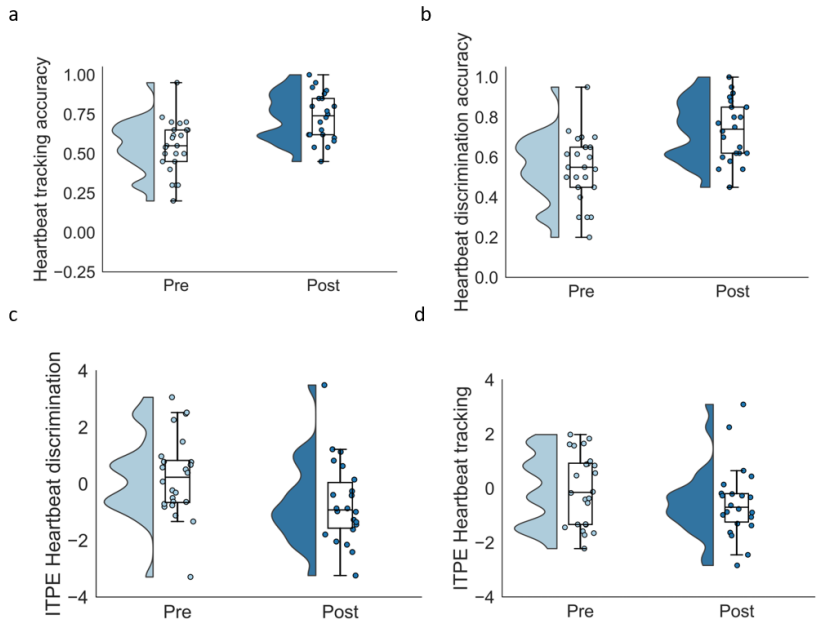


Figure 4.5. Interoceptive change scores following interoceptive training.

Significant increases in heartbeat tracking accuracy (a), heartbeat discrimination accuracy (b) and a significant reduction in ITPE on the heartbeat discrimination task (c) was observed. No significant change in heartbeat tracking ITPE was observed (d). Group distribution displayed as individual data points (horizontally jittered), violin plots (probability density functions), boxplots showing upper/lower quartiles and the median value, and whiskers showing the minimum and maximum values.

Time control task: No significant difference in accuracy on the time control task was observed pre versus post interoceptive training (mean pre 0.64, SD 0.27, mean post 0.74, SD 0.15; t(17) = -1.519, p = 0.147) was observed.

4.5.3.2 Change in affective symptomatology

Significant reductions in scores following interoceptive training were observed for TAS total scores (t(22) = 3.082, p = 0.005, BF10 = 8.194) and the difficulty identify feelings subscale of the TAS (t(22) = 3.222, p = 0.004, BF10 = 10.839). No significant change in trait anxiety (t(21) = 1.311, p = 0.204, BF10 = 0.473), depression (t(21) = 1.740, p = 0.097, BF10 = 0.810), autistic traits (t(22) = 1.824, p = 0.082, BF10 = 0.902), the difficulty describing feelings sub-scale of the TAS (t(22) = 1.626, p = 0.118, BF10 = 0.685) or the externally oriented thinking subscale of the TAS (t(22) = 0.857, p = 0.401, BF10 = 0.304, BF01 = 3.288) was

observed. See table 4.6 for mean scores. Change in affective symptomatology, across all variables, was not associated with change in interoceptive scores across any dimensions, all p's > 0.05. In the full sample, reported elsewhere, a significant reduction in trait anxiety was observed (mean pre 59.00, SD 10.51, mean post 54.28, SD 11.26; t(45) = 6.539, p < 0.001, BF10 = 280956.185).

	Trait anxiety	Depression	Autistic traits	TAS total score	TAS DDF	TAS DIF	TAS EOT
Pre-training	58.50 *15.06*	13.82 *7.96*	37.83 *12.71*	**61.65** *15.48*	17.74 *5.24*	**24.87** *8.77*	19.04 *4.36*
Post-training	56.50 *10.23*	12.32 *6.56*	36.52 *6.51*	**57.61** *11.73*	16.48 *4.38*	**22.57** *5.41*	18.57 *4.95*

Table 4.6. Summary of pre and post training affective symptomatology scores. Scores indicate mean values, where those in bold are statistically different pre versus post training, and *standard deviation.*

4.5.4 Study 2: fMRI results

For the contrast of ((pre heart > pre note) < (post heart > post note)), we observed no significant brain activation suggesting activation patterns did not change as a result of interoceptive training.

Additionally, for the series of change scores that prevailed as significant at the behavioural level, we observed no correlation between brain activation for the contrast ((pre heart > pre note) < (post heart > post note)) and any of these variables; change in heartbeat tracking accuracy, heartbeat discrimination accuracy, heartbeat discrimination ITPE, empathy, TAS total or TAS difficulty identifying feelings.

4.5.4.1 Functional connectivity

For the contrast of ((pre heart > pre note) < (post heart > post note)), we observed significant FC between left insula and middle temporal gyrus, temporal pole and inferior temporal gyrus suggesting increased connectivity following training when attending to one's heart. No significant FC was observed for right insula (supplementary table 3.12).

4.5.4.2 Relationship with interoception - functional connectivity

Interoceptive accuracy in PPI (supplementary table 3.13): Change in heartbeat tracking accuracy correlated with FC of right insula with left insula suggesting greater FC following training. No relationship was found between change in tracking accuracy and FC of left insula. Change in heartbeat discrimination accuracy correlated with increased FC of left insula with anterior cingulate gyrus and with FC of right insula with frontal medial cortex and paracingulate gyrus, following interoceptive training.

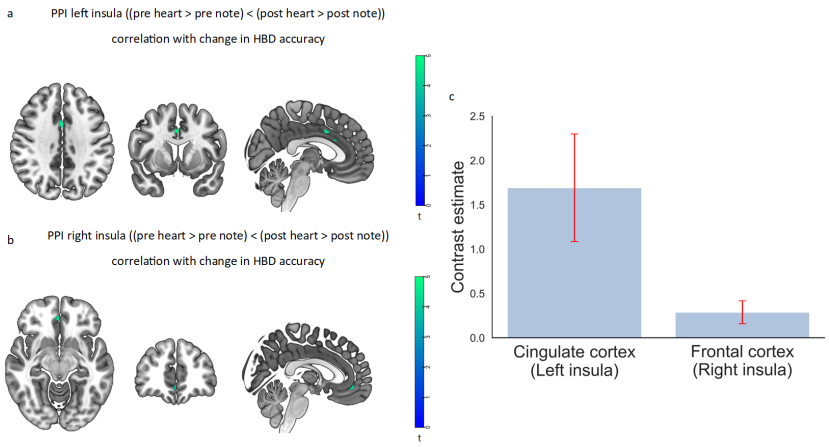


Figure 4.6. Functional connectivity of right and left insula correlated with change in heartbeat discrimination accuracy.

Functional connectivity of left insula with cingulate cortex (a) and right insula with frontal cortex (b). (c) Contrast estimates represent global maximum contrast in cingulate cortex (for PPI with left insula) and frontal cortex (for PPI with right insula). Red bars represent 90% confidence intervals.

Interoceptive sensibility in PPI (supplementary table 3.14): No correlation of left and right insula FC with the MAIA total score was found.

Interoceptive trait prediction error in PPI (supplementary table 3.15): Increased FC of right insula with frontal gyrus and frontal pole significantly correlated with change in discrimination ITPE scores.

4.5.4.3 Relationship with variables of interest - functional connectivity

Change in total scores from the TAS significantly correlated with increased FC of left insula with Right middle frontal gyrus, parietal lobule, angular gyrus and occipital cortex. No significant correlation with FC of right insula was found. No relationship with the TAS difficulty identifying feeling subscale in left or right insula was found. Despite no significant behavioural change in depression, change in depression scores did significantly correlate with increased FC of left insula with paracingulate gyrus and superior frontal gyrus. No such relationship was observed for FC of right insula and no relationship with change in FC and change in anxiety was found (supplementary table 3.16).

4.6 Discussion

In study 1, we employed offline heartbeat tracking and discrimination tasks and a modified heartbeat attention paradigm during functional MRI scanning. Behavioural results, with the noted limitation that uncorrected p values make our behavioural results preliminary, revealed no difference in interoceptive accuracy, insight or trait prediction error (ITPE) yet elevated interoceptive sensibility in autistic adults suggesting greater subjective sensitivity to general internal bodily sensations. The degree of perceived sensitivity to internal bodily sensations (interoceptive sensibility) positively correlated with depressive symptomatology in autistic but not in non-autistic adults. We also observed a subtle relationship between heartbeat discrimination accuracy and trait anxiety in autistic participants suggesting the ability to perceive interoceptive signals could relate to anxiety symptomatology. In brain, we showed strong activation in insula cortices when attending to heart versus note trials across all participants, but this activation did not differ as a function of group. Functional connectivity (FC) of left and right insula revealed no group differences when processing heart versus note trials yet, when correlated with interoceptive tracking accuracy, neurotypical participants, above autistic participants, showed greater FC of right and left insula with regions including amygdala, putamen and frontal regions. Interestingly, FC of left insula, correlated with heartbeat discrimination insight, was greater

in neurotypical participants. Whilst subtle relationships with anxiety in brain did emerge, no significant group differences in activation or FC of left and right insula, correlated with anxiety, prevailed as significant. Our results thus paint a complicated picture of domain specific interoceptive differences between autistic and neurotypical participants.

In study 2, we employed a novel interoceptive training paradigm which aimed to reduce anxiety and quantify neural signatures of interoceptive and affective symptomatology change. Behavioural results, again with the noted limitation that uncorrected p values make our behavioural results preliminary, revealed significant improvements in heartbeat tracking and discrimination accuracy yet no changes in interoceptive insight. We observed subtle changes in interoceptive sensibility suggesting greater perception of general internal bodily sensations following training. The heartbeat discrimination ITPE significantly reduced following training yet no change in trait anxiety or depression was observed in this small sample. We did, however, find a subtle enhancement of emotional sensitivity, indexed by a reduction in alexithymic traits. In brain, following training, no change in activation when attending to heart versus note trials was observed but FC of left insula significantly increased. Additionally, FC of right and left insula significantly correlated with change in interoceptive accuracy, change in discrimination ITPE scores and change in alexithymic traits. Thus, despite no change in activation, interoceptive training significantly altered FC of right and left insula, the primary interoceptive hubs, which may have clinical implications for alleviating sensory difficulties in autistic adults.

Previous work employing heartbeat tracking and discrimination paradigms has found mixed result regarding the performance ability of autistic adults, compared to neurotypicals, with some showing significant differences (Garfinkel, Tiley, et al., 2016; Mul et al., 2018; Palser et al., 2018), whilst others report none, both behaviorally (Nicholson et al., 2018; Schauder et al., 2015; Shah et al., 2016) and at the neural level (Failla et al., 2020). We, in part, support these null findings as we show no group differences in objective or metacognitive indices of interoception and no accompanying brain activation differences between autistic and neurotypical participants, who both recruited regions well known to be involved in interoceptive processes, namely insula cortices, at baseline. Notably, there was

subtle evidence to suggest possible reduced activation in subdivisions of insula in autistic individuals (figure 4.4), although no threshold significant differences were found. We did, however, observe group difference in subjective indices of interoception, namely greater scores in autistic participants on the BPQ, which indexes general self-report sensitivity to internal bodily sensations, and greater scores on the MAIA in neurotypical participants. Both the BPQ and MAIA focus on more general sensitivity to interoceptive signals (i.e. cross modality) rather than the specificity of the objective measures employed here which focus on the cardiac domain. Thus, our data suggest autistic individuals do differ in the way they perceive interoceptive signals and it is plausible that group differences may emerge in other interoceptive domains or using other tests within the cardiac domain. Indeed, cardiac and gastric sensations may align (Herbert, Muth, Pollatos, & Herbert, 2012; Van Dyck et al., 2016), yet cardiac and respiratory sensations may not (Garfinkel, Manassei, et al., 2016) suggesting future work should look to compare objective, subjective and metacognitive performance across interoceptive domains in autistic adults.

We also observed group differences in the FC of primary interoceptive regions; left and right insula. With interoceptive attention, we observed no differences in FC of left and right insula on heart trials with all participants showing FC with regions including postcentral, precentral, supramarginal and frontal gyrus, suggesting intact integration of somatosensory information across insula cortices. However, when correlated with heartbeat tracking accuracy, neurotypical participants, above autistic participants, show greater FC of left and right insula with regions including putamen, pallidum, amygdala and frontal regions (frontal pole and orbital cortex). Thus, we show that, despite no group differences at the behavioural level, neurotypical participants show greater connectivity between regions involved in interoceptive, emotional and autonomic processes underlying heartbeat accuracy. Whilst we can draw no firm conclusions regarding the impact of this finding, given our null behavioural findings, we speculate this may provide evidence of an altered system responsible for the veridical perception and integration of sensory (interoceptive) information which may contribute to emotional and cross-modal sensory difficulties often reported in autism (Schauder & Bennetto, 2016).

Given the noted dissociation between interoceptive accuracy and insight (Garfinkel et al., 2015), we also observed differences in FC related to interoceptive insight. Neurotypical participants showed a positive correlation between FC of left insula, with occipital fusiform gyrus, precuneus, occipital cortex, parietal lobule, occipital pole and cerebellum, and heartbeat discrimination insight. Such a finding is consistent with the work showing atypical integration of interoceptive and exteroceptive information (Noel et al., 2018), although at a metacognitive level, and also hints at a potential neural marker underscoring the relationship between interoceptive insight and affective prosody recognition (Mulcahy, Davies, et al., 2019), which remains an important avenue for future work.

Despite previous work linking interoceptive ability to anxiety, particularly in autism (Garfinkel, Tiley, et al., 2016), we found only a subtle relationship with anxiety. Autistic adults showed greater levels of anxiety with increasing heartbeat discrimination accuracy and all participants, regardless of autism status, who scored higher on the BPQ showed increased levels of trait anxiety. These findings are consistent with work linking heartbeat perception (Dunn et al., 2010; Pollatos et al., 2007), and general sensitivity to internal bodily sensations (Anderson & Hope, 2009; Gregor & Zvolensky, 2008; Olatunji et al., 2007), with anxiety. In brain, no activation during heartbeat perception correlated with anxiety and no group differences related to anxiety emerged. We did, however, observe a significant correlation between anxiety and FC of right insula with right cerebellum across all participants, consistent with the work implicating cerebellar involvement in autonomic and emotional control (Barrett, 2017; Schutter & Van Honk, 2005).

Regarding the findings from previous work linking the mismatch between objective and subjective indices of interoception to anxiety in autism (Garfinkel, Tiley, et al., 2016; Palser et al., 2018), we found no evidence to suggest the trait prediction error predicted anxiety specifically in autistic individuals. We did however show that trait anxiety was linked with tracking ITPE in neurotypical participants and, in a series of regression analyses, we found heartbeat discrimination ITPE to be the only significant predictor of trait anxiety across all participants. Interestingly, the relationship was the reverse of that observed by (Garfinkel, Tiley, et al., 2016), such that as tracking ITPE decreases, trait anxiety increases.

Thus, in our sample, the propensity for all individuals (autistic and neurotypical) to under-estimate their interoceptive ability was associated with heightened anxiety. When we included heartbeat tracking ITPE in the model, interestingly, group status and heartbeat discrimination accuracy best predicted anxiety. Thus, the presence of an autism diagnoses and increased heartbeat discrimination accuracy was associated with greater anxiety. The discrepancy between the two trait prediction error findings likely results from the different mechanisms the two tasks target, i.e. sustained attention versus interoceptive/exteroceptive integration (Hickman, Seyedsalehi, Cook, Bird, & Murphy, 2020). Nonetheless, our findings are an interesting extension linking objective and subjective indices to anxiety symptomatology and serve to illustrate the heterogeneity of the contribution of interoceptive signals to anxiety, across populations.

Relatedly, in study 2, based on the work by (Garfinkel, Tiley, et al., 2016), we employed a novel interoceptive training paradigm which trained autistic adults to perceive and understand their heartbeat, to better align interoceptive signals (i.e. ITPE) and thus reduce anxiety. As the ITPE is designed in such a way that scores greater than 0 represent an overestimation of interoceptive ability whilst scores below 0 represent an underestimation, we expected improved alignment between objective and subjective indices and thus scores that center roughly around 0. In fact, in our small sample, we found a significant reduction in heartbeat discrimination ITPE suggesting participants had a greater tendency to under-estimate their own interoceptive ability following training. This finding may highlight a crucial design limitation in the attempted mitigation of interoceptive disparity. This limitation is twofold, participants were trained specifically on heartbeat perception (i.e. cardiac interoception), which our results show significantly improved on objective measures, whilst the BPQ, used as the sensibility measure to calculate ITPE, assesses general sensitivity to bodily sensations. Thus, the alignment between objective performance and subjective belief about sensitivity to cardiac perception may have improved yet not been adequately captured by the BPQ and thus inadequately represented by the ITPE score. Future work should look to either assess interoceptive sensibility using a measure of cardiac perception or look to train individuals across modalities (i.e. respiratory, gastric and cardiac) which may better align with the BPQ. This conclusion also supports the

lack of a significant change on the heartbeat tracking ITPE score. Relatedly, as we predicted better alignment of interoceptive signals would reduce anxiety, and our results did not improve alignment, we observe no significant change in trait anxiety. Interestingly, we did observe a significant reduction in alexithymic traits, specifically for total scores and the difficulty identifying feelings sub-scale, which is consistent with the work showing a relationship between interoceptive ability and emotional experience (Critchley & Garfinkel, 2017). Additionally, in brain, FC of left insula increased and correlated with the change/reduction in alexithymic traits suggesting greater communication between regions known to underscore emotion processing difficulties in alexithymic individuals, notably left insula (Bird et al., 2010) and parietal lobe regions (Reker et al., 2010; van der Velde et al., 2015). Thus, Interoceptive training may have validity in mitigating emotional difficulties in other clinical populations by altering neural communication networks.

In further support of this claim, we observed significant increases in FC of insula cortices following training when attending to ones heartbeat. As we have established, the insula cortex is often considered the primary interoceptive hub (Craig, 2002, 2008; Critchley et al., 2004) involved in integrating bodily signals to inform emotional experience (Critchley & Garfinkel, 2017), where interoceptive information is integrated in posterior insula (Craig, 2014) whilst social and emotional processing involves anterior insula (Garfinkel & Critchley, 2013; Terasawa et al., 2013). Notably, whilst most work typically focuses on right insula, we observed differential FC of left and right insula. It has been argued that right insula is predominantly involved in sympathetic activity (i.e. arousal and survival emotions) whilst left insula has greater involvement in parasympathetic activity (i.e. affect and group orientated emotions) (Craig, 2008). For example, right insula shows increased activation during interoception about physical and emotional state (Critchley et al., 2004; Zaki et al., 2012), whilst left insula increases in activation during the perception of others experiencing emotion (Caria, Sitaram, Veit, Begliomini, & Birbaumer, 2010; Singer et al., 2004). In this work, we observed increased FC of left insula when attending to one's heart, with regions including temporal gyrus and temporal pole, following training. Additionally, FC of right insula, with left insula, cingulate and frontal cortex, and of left insula, with anterior cingulate, correlated with change in interoceptive accuracy suggest-

ing greater inter-hemispheric communication for the facilitation of interoceptive processing following training, and greater communication with regions involved in processing autonomic and emotional information (Craig, 2002; Critchley et al., 2004; Critchley & Harrison, 2013), supported by the evidence positing a relationship between interoceptive accuracy and emotional experience (Wiens et al., 2000). Likewise, change in heartbeat discrimination ITPE correlated with FC of right insula, with frontal regions, which is consistent with the implication that the ITPE may provide insight into anxiety mechanisms (Garfinkel, Tiley, et al., 2016; Paulus & Stein, 2006), where autonomic dysregulation and heightened sympathetic activity is common (Mulcahy, Larsson, et al., 2019; Hoehn-Saric & McLeod, 1988), which may thus more heavily rely on right insula involvement.

Although our results show convergence between interoceptive ability and neural markers of regions well established to be involved in interoceptive processes, there are a few noteworthy limitations regarding the validity and reliability of both the heartbeat tracking and discrimination tasks. Firstly, the two task rely on fundamentally different processes; sustained attention, and likely working memory, is required to complete the heartbeat tracking task whilst the heartbeat discrimination task relies on multisensory integration. Indeed, accuracy measures across these two tasks show little convergence (Hickman et al., 2020) yet remain interchangeably employed throughout the literature. Furthermore, the heartbeat tracking task is arguably influenced by higher-order knowledge (Brener & Ring, 2016; Ring et al., 2015; Ring & Brener, 1996) and time estimations (Murphy, Millgate, et al., 2018) of heartbeats and the heartbeat discrimination tasks relies on the assumption that all individuals experience heartbeat sensations at the same temporal location relative to R-wave (Brener & Ring, 2016), when individual variation may exist. One important point to note is we only examined heartbeat discrimination insight, despite work showing interoceptive insight calculated from the heartbeat counting and tracking tasks do not align, likely due to the varying number of trials and different methodology used to calculate insight, Pearson's correlation versus ROC (Hickman et al., 2020), suggesting they may tap different underlying processes. We chose not to include heartbeat tracking insight due to the low number of trials used to calculate the measure in the current study (n=6) which may be heavily influence by outliers and thus calls into question the reli-

ability of tracking insight scores. Nonetheless, with these limitations in mind, we still identify neural markers of interoceptive processes that show differences at the group level. We also show that interoceptive accuracy across both tasks, and thus attention and multisensory integration, can be enhanced following interoceptive training in autistic adults.

Whilst we did show differential brain activation for interoceptive versus exteroceptive (heart versus note) processes, due to a programming error we were unable to investigate cardiac contingent effects of interoceptive processing (i.e. neural activation related to processing heart signals during systole versus diastole). Indeed, in chapter 3, we showed reduced activation and FC of right insula during systolic processing in autistic adults suggesting a potential mechanism underlying altered interoceptive processes in this population. This would thus have been an interesting avenue for investigation in the current study. Likewise, the inaccurate cardiac timing during scanning meant we had to rely on offline interoceptive measures to relate brain activity to interoceptive dimensions which may have compromised our findings. Finally, in addition to reporting frequentist statistics, we also reported Bayesian statistics. In many of our analyses, notably our investigation group differences of interoceptive ability and of change in anxiety following interoceptive training (our primary outcome measure), Bayesian statistics indicated we had insufficient evidence to draw meaningful conclusions regarding the outcome of the analyses. Our samples sizes were low, and thus insufficient power could have contributed to non-significant findings. Future work should ensure the recruitment of a suitable sample size to draw meaningful conclusions, particularly when implementing longitudinal training paradigms.

In conclusion, our results demonstrate dimension specific dissociations in interoceptive ability in autistic adults, notably comparable accuracy yet a heightened sensibility to interoceptive sensations. In brain, autistic and neurotypical participants recruit similar regions, namely insula cortices, when attending to their own heartbeat. However, FC of insula cortices revealed dissociable connections between the two groups across different interoceptive dimensions (accuracy and insight) which may provide insight into higher level mechanisms responsible for sensory difficulties seen in autism. In autistic individuals only, the ability to objective perceive heartbeats is associated with anxiety yet interoceptive training,

aimed at improving interoceptive understanding and alignment, did not impact anxiety symptomatology, despite significant increases in interoceptive accuracy. Interoceptive training also significantly enhanced FC of insula cortices which subtly reduced emotion difficulties. This work has broad clinical implications for improving sensory regulation and provides the first evidence that targeted interoceptive training in autistic adults can increase neural communication which may causally alleviate interoceptive perception and emotion difficulties in this population.

Chapter 5

Cardiac-contingent fear processing in autism: the effect of interoceptive training

5.1 Abstract

Interoceptive signalling of physiological arousal can enhance sensitivity to emotional stimuli. In chapter 3, we showed systolic enhancement of fear processing in autistic and neurotypical adults with increasing levels of anxiety. In autistic adults only, we observed a pattern of blunted reactivity and reduced functional connectivity of autonomic regions, including amygdala and insula, involved in emotion processing. In the current study, we employ a novel interoceptive training paradigm and quantify emotion processing effects, as a function of cardiac-cycle, pre versus post interoceptive training. In all participants, interoceptive training subtly increased intensity ratings towards all emotional stimuli and functional connectivity of insula and amygdala cortices was increased. A pattern of atypical processing in individuals with high levels of co-morbid anxiety and depression highlights the need to tailor interoceptive training paradigms towards individual differences in co-morbid affective symptomatology.

5.2 Introduction

In chapter 3, we demonstrated that cardiac signals unconsciously influence emotion perception for both fear and neutral faces. We showed that when stimuli were presented at cardiac systole (when baroreceptors fire), relative to diastole (when baroreceptors are quiescent), intensity ratings of neutral, but not fear, faces was reduced. Additionally, individuals who scored high in trait anxiety showed heightened levels of fear processing at systole relative to diastole. In brain, amygdala and insula were recruited in autistic and neurotypical participants when processing fear faces, however, at cardiac systole, autistic participants showed reduced activation and functional connectivity (FC) of right insula, relative to neurotypical participants, suggesting a potentially aberrant interoceptive system in this population.

In chapter 4, we employed a novel interoceptive training paradigm which aimed to reduce anxiety in autistic adults. We found a significant increase in interoceptive accuracy post versus pre-training. In brain, we found evidence of increased FC of left and right insula, with regions including cingulate and frontal cortex, correlated with change in heartbeat discrimination (Katkin et al., 1983; Whitehead et al., 1977) and heartbeat tracking accuracy (Schandry, 1981). Thus, we argued that interoceptive training significantly improved interoceptive ability, both at a behavioural level and at the neural level, by boosting connectivity of the interoceptive pathway in autistic adults.

In addition to completing the fMRI interoceptive paradigm post-interoceptive training, autistic participants also completed the 'FearFaces' paradigm (Garfinkel et al., 2014) both pre and post interoceptive training. Based on the literature demonstrating a relationship between interoceptive ability and emotional experience (Critchley & Garfinkel, 2017), particularly in autism (Garfinkel, Tiley, et al., 2016), and our findings of an altered interoceptive system in autistic adults, the aim of this chapter was to investigate interoceptive training effects on emotion perception, specifically for fearful and neutral faces.

Based on our findings of improved interoceptive ability, both behaviourally and at the neural level (chapter 4), we hypothesise that the behavioural effects typically observed as a function of cardiac cycle, i.e. systolic inhibition of intensity ratings toward neutral faces but an increase in intensity ratings toward fearful

faces, would be enhanced following interoceptive training. We also hypothesise that the noted enhancement of fear processing at systole in highly anxious autistic adults would be reduced post interoceptive training as a result of improved interoceptive signalling and a subsequent reduction in trait anxiety. In brain, we predict, post interoceptive training, we will observe increased activation of amygdala and insula cortices when viewing fearful stimuli and increased FC of right insula during systole. These brain effects would be modulated by change in interoceptive accuracy and anxiety symptomatology.

5.3 Methodology

5.3.1 Participants

As in chapter 4, 40 participants were initially recruited however only 22 participants completed the 'FearFaces' paradigm post-interoceptive training (described in chapter 4, section 4.3.4). Thus the final sample used in this analyses comprised 22 autistic adults, 12 male and 10 female as assigned at birth. Participant details are described in chapter 3, section 3.3. All participants provided written informed consent with all procedures approved by the BSMS research Governance Ethics Committee.

5.3.2 Experimental paradigm and procedure

As described in chapter 3, section 3.3.2, 20 faces (10 fearful and 10 neutral) were presented over the period of peak ventricular systole and 20 faces (10 from each emotion) were presented at late diastole, resulting in a 2 x cardiac cycle (systole, diastole) x 2 emotion (fear, neutral) design. Face stimuli were presented for 100ms, to allow for precise cardiac timing. Trial types were randomised and the experiment was broken into two functional runs of 40 faces each. On each trial, the participant reported the perceived emotional intensity of the face stimulus (cue: 'How intense was the emotion on this face?'), from zero (0) to medium (50) to extreme (100) using an on-screen visual analogue scale (VAS) presented for 3 seconds. The cursor was controlled using a button box held in the right hand. Between trials, a fixation cross was presented for 5 seconds. As in chapter 3, real time cardiac timing ensured stimuli were displayed at cardiac systole or

diastole and post-hoc analyses excluded inaccurately timed trials: diastole trials that occurred > 50ms and < -200ms from the estimated R-wave time were excluded, and similarly, 'systole' trials that occurred < 150ms and > 400ms relative to the estimated R-wave time were also excluded (see figure 5.1). Participants completed the fear faces paradigm pre and post interoceptive training (for details of the training procedure, see chapter 4, section 4.3.4).

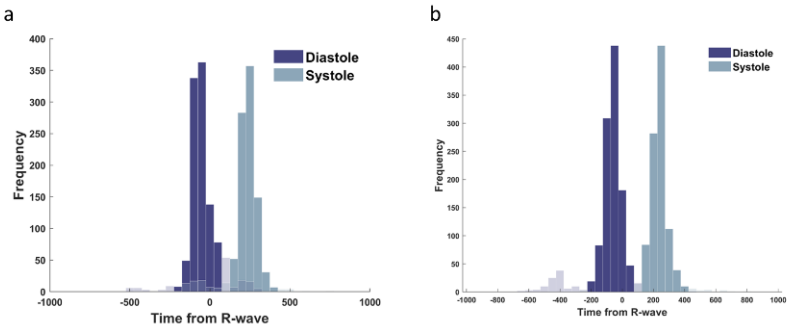


Figure 5.1. FearFaces paradigm: distribution of included/excluded trials pre and post interoceptive training.

Neutral face trials and fear face trials were time-locked to ventricular systole or diastole (20 trials per emotion/cardiac condition) and participants made subsequent intensity ratings. Histograms represent cardiac timing of stimuli presentation for participants pre-training (a) and post training (b).

5.3.3 Questionnaires

As in chapter 3, all participants completed the trait section of the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001) and the Autism Quotient (AQ) (Baron-Cohen et al., 2001). Participants also provided demographic information including age, gender assigned at birth, and level of educational attainment. Post-training, 2 participants completed no questionnaires and were thus excluded from analyses involving this measure.

5.3.4 Interoceptive accuracy

Based on our findings from chapter 4 showing significant increases in interoceptive accuracy across both heartbeat tracking and discrimination tasks, which were

replicated in the sample in this chapter, we also considered the impact of change in heartbeat tracking accuracy and heartbeat discrimination accuracy on face intensity ratings and the subsequent neural response. Details of both interoceptive tasks are described in chapter 4, section 4.3.2. Post-training, 3 participants did not complete the interoceptive tasks and were thus not included in the analyses involving this measure.

5.3.5 fMRI data acquisition

Functional imaging datasets were acquired using a Siemens 3T Prisma MRI scanner with a 32-channel head coil. A multiband echo-planar imaging (EPI) sequence was used with multiband acceleration factor of 2 to acquire T2*-weighted images sensitive to blood oxygen level dependent (BOLD) contrast. Each functional volume consisted of 44 slices, acquired in an interleaved order. The following parameters were used: TR = 1500ms; TE = 30ms; flip angle = 70°; matrix = 94x94; FOV = 220mm; slice thickness = 3.0mm with a 25% gap.

5.3.6 fMRI pre-processing

fMRI data was pre-processed using SPM12 in Matlab R2017A (MathWorks, Inc., Natick, MA). For each participant, the first 5 volumes were removed to account for magnetization equilibrium. Remaining functional images were slice-time corrected to the first slice, realigned to the first volume and spatially normalised to a standard MNI EPI template (Calhoun et al., 2017). Normalised images were then smoothed using an 8mm Gaussian kernel (full width half maximum) and all images were visually inspected for artefacts.

5.4 Data analyses

5.4.1 Behavioural data analyses

Behavioural data (mean intensity rating as a function of cardiac cycle and emotion) was extracted using a custom script in Matlab R2017a. Pre versus post interoceptive training differences on intensity ratings were examined using a 2 (time; pre vs post interoceptive training) x 2 (cardiac cycle; systole vs diastole) x 2 (emotion; fear vs neutral) repeated measures ANOVA. Time between scanning

sessions was modelled as a covariate to test for interaction effects. Significant results were further explored using paired-sample t-tests.

Next, based on our findings showing a relationship between cardiac-contingent fear processing with anxiety and depression (see chapter 3), we included change in anxiety and depression scores in the 2 x 2 x 2 ANCOVA model to investigate individual differences in sensitivity to interoceptive training as a function of change in anxiety and depression. We also report the impact of change in interoceptive accuracy (for both tracking and discrimination tasks), as an index of interoceptive training effects, by entering both separately into the ANCOVA model as covariates. Finally, we report group level differences in pre-training versus post training changes in interoception accuracy, anxiety and depression using paired-sample t-tests. All p values in the behavioural results are uncorrected.

5.4.2 fMRI data analyses

Using SPM in Matlab, individual first level models were constructed resulting in 8 single-regressor T-contrasts; (1) pre-training fear at systole, (2) pre-training fear at diastole, (3) pre-training neutral at systole, (4) pre-training neutral at diastole, (5) post-training fear at systole, (6) post-training fear at diastole, (7) post-training neutral at systole, (8) post-training neutral at diastole. These were entered into a second level full-factorial model with session (pre/post interoceptive training) and condition (emotion and cardiac cycle) as non-independent (repeated measures) factors.

Resultant F-contrasts were generated to test for 1) all effects; 2) main effect of session; 3) main effect of cardiac cycle; 4) main effect of emotion; 5) specific interactions, i.e. session x cardiac cycle, session x emotion, emotion x cardiac cycle and session x cardiac cycle x emotion. Significant main effects and interactions were explored using post-hoc t-tests.

Based on our findings from chapter 3 linking anxiety and depression to fear processing at systole relative to diastole, we generated a contrast ((fear systole post-training > fear diastole post-training) > (fear systole pre-training > fear diastole pre-training)) to test if interoceptive training impacted this anxiety mechanism. Thus, using this contrast, we correlated associated brain activity with change in anxiety and change in depression scores. We also investigated the impact of

change in heartbeat tracking and discrimination accuracy scores to test if training induced improvement on heartbeat perception influenced emotional perception. Statistical maps were thresholded at cluster-forming threshold $p < 0.001$ and False Discovery Rate (FDR) cluster-corrected at $p < 0.05$ for multiple comparisons. It is worth noting that results reported from these analyses are likely under-powered due to the small sample size ($n = 22$) and number of predictor variables included in the model.

5.4.3 Psychophysiological interactions (PPI)

Based on our work in chapter 3 showing increased activation in left and right amygdala and right and left insula for the contrast fear > neutral, we sought to investigate whether interoceptive training would increase brain sensitivity to emotional stimuli (i.e. heightened reactivity to fearful faces). Thus, using the CONN toolbox, we undertook a specific generalized psychophysiological interaction (gPPI) analyses for the contrast ((post-training fear > post-training neutral) > (pre-training fear > pre-training neutral)). Seed regions were identical to the regions showing significant activation in chapter 3: left amygdala (x-26, y-4, z-22), right amygdala (x22, y-4, z-16), left insula (x-36, y-10, z18) and right insula (x34, y12, z-14).

Additionally, in chapter 3, we showed neurotypical participants, compared to autistic participants, had increased FC of right insula when processing faces during systole. Thus in another gPPI analyses, we sought to investigate whether interoceptive training would increase brain connectivity sensitivity to stimuli presented during systole using the contrast post-training systole > pre-training systole. The seed region was again identical to the region identified in chapter 3: right insula (x36, -10, 18). In the CONN toolbox, the GLM comprised regressors for condition (fear/neutral/systole/diastole) and session (pre/post-training) as well as nuisance regressors, including the 6 motion parameters (3 translations/3 rotations) and the time in days between scanning sessions (mean cantered). For all FC analyses, the data was denoised by regressing out signal from white matter (WM), cerebral spinal fluid (CSF) and from each individual condition.

For each participant, the psychophysiological interaction term was calculated according to the contrast of ((post-training fear > post-training neutral) > (pre-

training fear > pre-training neutral)) and the time series of (1) left amygdala, (2) right amygdala, (3) left insula and (4) right insula, as well as the psychophysiological interaction term form the contrast (post-training systole > pre-training systole) for the time series of (1) right insula. Within-sample t-tests tested for significant FC with each individual seed-region. Finally, based on our work from chapter 3 linking anxiety and depression symptomatology to fear processing, as well a reduction in anxiety being our primary outcome measure from the clinical trial, we investigated how change in anxiety and depression impacted brain connectivity. As in the activation analyses, we also investigated how training induced improvement in heart perception influenced FC. Thus, change in anxiety, depression, heartbeat tracking accuracy and heartbeat discrimination accuracy scores were correlated against all PPI regions noted above. As in univariate analyses, statistical maps were thresholded at cluster-forming threshold $p < 0.001$ and False Discovery Rate (FDR) cluster-corrected at $p < 0.05$ for multiple comparisons.

5.5 Results

5.5.1 Training effects on cardiac modulation of emotion intensity

Results revealed a significant main effect of session ($F(1,21) = 5.536$, $p = 0.029$) indicating that overall intensity ratings in both groups significantly increased following interoceptive training ($t(21) = -2.351$, $p = 0.029$). We also observed a significant main effect of cardiac cycle ($F(1, 21) = 6.348$, $p = 0.020$) indicating that, averaged across time points and emotion categories, faces presented at diastole were rated as more intense than faces presented at systole ($t(21) = -2.519$, $p = 0.020$). A significant main effect of emotion ($F(1, 21) = 96.306$, $p < 0.001$) demonstrated that, averaged across time and cardiac categories, fear faces were rated as significantly more intense than neutral faces ($t(21) = 9.814$, $p < 0.001$), see figure 5.2 for summary accuracy across emotion categories.

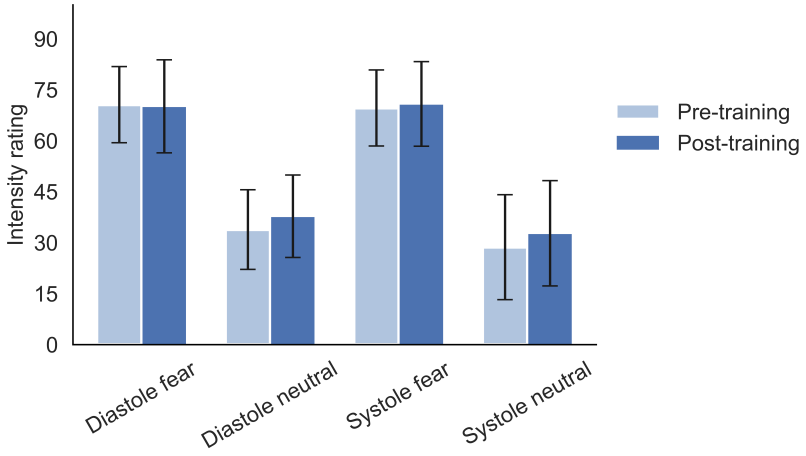


Figure 5.2. FearFaces paradigm: Summary of intensity ratings pre and post interoceptive training.

Effects of cardiac signals on intensity ratings across fear and neutral categories for autistic participant (n = 22) pre and post interoceptive training. Bars represent standard deviation.

A significant interaction between cardiac cycle and emotion ($F(1, 21) = 18.340$, $p < 0.001$) reflected the propensity for participants, averaged across time, to rate neutral faces as more intense at diastole relative to systole ($t(21) = -4.303$, $p < 0.001$) whilst fear faces were impervious to the inhibitory effect showing no significant difference between intensity ratings for faces presented at systole versus diastole ($t(21) = -0.111$, $p = 0.913$), see figure 5.3. No session by cardiac cycle interaction was observed ($F(1, 21) = 1.064$, $p = 0.314$) indicating intensity ratings at systole or diastole did not differ pre versus post training. Similarly, no significant interaction between session and emotion was found ($F(1,21) = 3.196$, $p = 0.088$) suggesting intensity ratings across fear and neutral faces did not differ pre versus post training. Finally, no significant three-way interaction between session, emotion and cardiac cycle was observed ($F(1, 21) = 0.475$, $p = 0.498$) indicating intensity ratings did not differ pre versus post training as a function of cardiac and emotion categories.

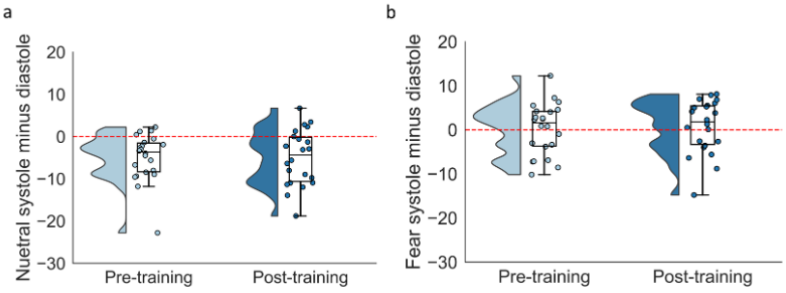


Figure 5.3. FearFaces paradigm: Fear and neutral processing at systole relative to diastole, pre and post interoceptive training.

Participants, independent of time, showed an inhibitory effect of neutral faces presented at systole (a), an effect that was not maintained for fear faces (b). Group distribution displayed as individual data points (horizontally jittered), violin plots (probability density functions), boxplots showing upper/lower quartiles and the median value, and whiskers showing the minimum and maximum values.

Regarding our covariate to control for the time between scanning sessions, this did not have an effect on intensity ratings ($F(1, 20) = 0.414$, $p = 0.527$), and did not interact with our main session variable of interest (pre versus post-training) $F(1, 20) = 0.135$, $p = 0.717$). In addition, there was no three-way interaction between session, cardiac cycle and time between scans ($F(1, 20) = 1.007$) $p = 0.328$) nor between session, emotion and time between scans ($F(1, 20) = 1.249$, $p = 0.277$) and, finally, no four-way interaction between session, cardiac cycle, emotion and time between scans ($F(1, 20) = 1.120$, $p = 0.303$). Thus, the duration of time between scanning sessions did not impact intensity ratings.

5.5.2 Interoceptive accuracy

No significant main effect and no significant interactions with change in heartbeat tracking or discrimination accuracy were found, all p's > 0.05. Thus, change in tracking or discrimination accuracy did not influence emotional intensity ratings overall nor across emotion and/or cardiac categories, and change in accuracy did not influence emotional intensity ratings between sessions (pre versus post interoceptive training).

5.5.3 Anxiety and depression

A significant interaction between session and change in trait anxiety ($F(1, 15) = 9.849$, $p = 0.007$) revealed that participants with greater levels of anxiety at final provided reduced intensity ratings at final, compared to baseline ($r = -0.630$, $p = 0.007$). We also observed a significant three-way interaction between session, cardiac cycle and change in trait anxiety ($F(1, 15) = 7.180$, $p = 0.017$) suggesting that individuals with greater anxiety at final provided reduced intensity ratings on diastole trials, i.e. for post-training diastole minus pre-training diastole, correlated with post-training minus pre-training trait anxiety ($r = -0.763$, $p < 0.001$) whilst no relationship was found with systole trials ($r = -0.337$, $p = 0.186$). Finally, we observed a significant 4-way interaction between session, cardiac cycle, emotion and change in trait anxiety ($F(1, 15) = 6.313$, $p = 0.024$). Thus, broken down by emotion, for fear faces only, we observed a significant 3-way interaction between session, cardiac cycle and anxiety ($F(1, 15) = 11.007$, $p = 0.005$), whilst, for neutral faces only, no significant interaction between time, cardiac cycle and anxiety ($F(1, 15) = 0.386$, $p = 0.544$) was observed. Therefore, participants who rated fear faces as more intense during diastole post-training had reduced levels of anxiety post-training, i.e. for fear diastole post-training minus fear diastole pre-training, correlated with post-training minus pre-training trait anxiety ($r = -0.560$, $p = 0.019$), whilst no effect of change in anxiety was observed for change in intensity ratings on fear systole trials ($r = -0.141$, $p = 0.590$). No significant main effect and no significant interactions with change in depression were found, all p's > 0.05, suggesting change in depression scores did not influence responses.

5.5.4 Change in anxiety, depression and interoceptive accuracy

In the analyses of pre-training versus post-training scores for trait anxiety and depression, we observed no significant change in anxiety (mean pre-training 57.55, SD 11.65, mean post-training 55.37, SD 10.27; $t(18) = 0.773$, $p = 0.450$) or depression (mean pre-training 13.41, SD 6.59, mean post-training 11.11, SD 6.19; $t(18) = 1.632$, $p = 0.120$) scores at the group level. We did however observe a significant increase in heartbeat tracking (mean pre-training 0.58, SD 0.23, mean post-training 0.81, SD 0.14; $t(18) = -4.480$, $p < 0.001$) and discrimination (mean pre-training 0.54, SD 0.18, mean post-training 0.73, SD 0.16; $t(18) = -4.476$, $p <$

0.001) accuracy. Change in heartbeat tracking accuracy or heartbeat discrimination accuracy was not associated with change in anxiety or depression, all p's $<$ 0.05.

5.5.5 Training effects on cardiac modulation of emotion intensity: fMRI results

Main effect of session: No significant main effect of session was identified suggesting overall brain activation did not differ pre versus post interoceptive training.

Main effect of cardiac cycle: No significant main effect of cardiac cycle was identified suggesting, averaged across sessions and valence, brain activation did not differ when faces were presented at systole versus diastole.

Main effect of emotion: A significant main effect of emotion (F-contrast) revealed, across sessions, brain activation differed towards fear and neutral stimuli. For the contrast fear $>$ neutral, activation was observed in bilateral amygdala, lingual gyrus, cuneus, occipital gyrus, precuneus and temporal gyrus (supplementary table 4.1). For the contrast of neutral $>$ fear, significant activation was observed in lingual gyrus, precentral and frontal gyrus (supplementary table 4.1).

Session and cardiac cycle interaction: We observed a significant session x cardiac cycle interaction (F-contrast) in regions including mid/anterior cingulate cortex, frontal gyrus and right insula (supplementary table 4.2). Therefore, brain activation at systole was significantly modulated by session (i.e. interoceptive training); for the contrasts pre-training systole $>$ post-training systole in left and right precuneus. No significant activation was identified for pre-training diastole $>$ post-training diastole, post-training systole $>$ pre-training systole nor post-training diastole $>$ pre-training diastole.

Emotion and cardiac cycle interaction: No significant interaction was identified suggesting, averaged across sessions, brain activation as a function of cardiac and emotion categories did not differ.

Session, emotion and cardiac cycle interaction: No significant three-way interaction was identified, suggesting brain activation in emotion and cardiac categories did not differ pre versus post interoceptive training.

Relationship with change in interoceptive accuracy: For the con-

trast ((fear systole post-training > fear diastole post-training) > (fear systole pre-training > fear diastole pre-training)), we observed significant activation in parahippocampal gyrus and hippocampus (supplementary table 4.3) correlated with change in heartbeat discrimination accuracy suggesting interoceptive training altered emotional processing regions during cardiac systole, as indexed by heartbeat discrimination accuracy. No significant activation was associated with change in heartbeat tracking accuracy.

relationship with change in anxiety and depression: For the contrast ((fear systole post-training > fear diastole post-training) > (fear systole pre-training > fear diastole pre-training)), no significant activation was associated with change in anxiety or change in depression scores.

5.5.6 Functional connectivity

Amygdala and insula connectivity with emotion: For the contrast ((post-training fear > post-training neutral) > (pre-training fear > pre-training neutral)), we observed significant FC of right amygdala with precentral gyrus and of left insula with vermis (supplementary table 4.4). For left amygdala and right insula, no differences in FC with interoceptive training was observed.

Right insula connectivity with cardiac cycle: For the contrast post-training systole > pre-training systole, no significant FC of right insula was observed.

Relationship with change in interoceptive accuracy: For the contrast ((post-training fear > post-training neutral) > (pre-training fear > pre-training neutral)), FC of right amygdala, with parahippocampal gyrus, occipital and lingual gyrus, and FC of left insula, with frontal gyrus and frontal pole, significantly correlated with change in heartbeat discrimination accuracy (supplementary table 4.5). Similarly, FC of left amygdala, with bilateral insula, frontal gyrus, precentral gyrus, occipital fusiform cortex and operculum cortex, and FC of right amygdala, with left insula, frontal gyrus and right amygdala, and FC of right insula, with temporal pole, frontal gyrus and temporal occipital fusiform cortex, positively correlated with change in heartbeat tracking accuracy (supplementary table 4.5). Thus, increased interoceptive accuracy, as a result of interoceptive training, significantly increased FC of regions involved in fear processing. For the contrast

post-training systole > pre-training systole, no relationship with change in tracking or discrimination accuracy was found.

Relationship with change in anxiety and depression: For the contrast ((post-training fear > post-training neutral) > (pre-training fear > pre-training neutral)), FC of left amygdala, with cerebellum and frontal gyrus, positively correlated with change in trait anxiety (i.e. greater anxiety at final was associated with increased connectivity during fear processing post-training). Similarly, FC of left insula, with Occipital cortex, angular gyrus, temporal gyrus and cerebellum, and FC of right insula, with cerebellum, temporal gyrus, postcentral gyrus and parietal lobule (supplementary table 4.6), significantly correlated with change in anxiety. No relationship with change in depression scores was found.

For the contrast post-training systole > pre-training systole, FC of right insula, with supramarginal and postcentral gyrus, precuneus, occipital cortex and cingulate gyrus (supplementary table 4.7), positively correlated with change in trait anxiety. No significant FC was associated with change in depression scores.

5.6 Discussion

In the current study, we employed a novel interoceptive training paradigm and quantified emotion perception (intensity ratings toward fear and neutral faces) as a function of cardiac cycle (systole and diastole) pre versus post training. Our behavioural results, with the noted limitation that uncorrected p values make our behavioural results preliminary, found face stimuli, regardless of the emotional content, were rated as more intense post interoceptive training versus pre-training. Rather than interoceptive training serving to alter the cardiac modulation of emotion at the behavioural level, we in fact, replicated our finding from chapter 3, across both sessions; neutral stimuli presented during systole, relative to diastole, showed an inhibition of intensity ratings whilst fear faces were impervious to this effect, showing no inhibitory effect of systolic signalling. In brain, we found no evidence of enhanced activation post interoceptive training but we did observe a subtle increase in functional connectivity (FC) of right amygdala and left insula with frontal and sub-cortical regions and a more profound effect of increased FC of amygdala and insula cortices when coupled with objective heartbeat perception performance, consistent with the work linking interoceptive accuracy with

increased emotion intensity ratings (Wiens et al., 2000). Finally, while we found no significant reduction in anxiety following interoceptive training, we observed a pattern of altered emotion processing coupled with change in trait anxiety at the individual level. Individuals who were the most susceptible to interoceptive training, indexed through reduced anxiety, showed increased intensity ratings towards face stimuli whilst individuals for which interoceptive training increased anxiety, they showed a pattern of reduced intensity ratings towards face stimuli coupled with a hyper-connectivity pattern in brain for regions involved in emotion and autonomic control.

Although we found no enhanced facilitation of stimuli processing at systole, we did again replicate the consistently reported inhibitory properties of systole toward neutral faces and the 'breakthrough' of this inhibition for fear faces (Garfinkel & Critchley, 2016; Garfinkel et al., 2014; Gray et al., 2009) across sessions. At the neural level, we found no difference in brain activation for the relationship between cardiac cycle and emotion. However, replicating our finding from chapter 3, we did show enhanced amygdala activation when processing fear over neutral faces, a finding consistently reported (Adolphs et al., 1995; LeDoux, 2003), however this effect operated independent of sessions and thus interoceptive training did not, at the neural activation level, impact fear processing. However, when we investigated the impact of objective heartbeat discrimination performance, neural activation of hippocampus and parahippocampal gyrus during fear processing at systole, above diastole, following training, was significantly enhanced. Thus, interoceptive training altered heartbeat perception performance which increased brain activations in regions involved in facilitating emotion processing during cardiac systole. Interestingly, the only other activation result to emerge as a function of interoceptive training was in the opposite direction to that expected; systolic signalling was greater pre-training, relative to post-training, in the precuneus. This finding was in direct opposition to our hypothesis that, as a result of increased interoceptive ability and signalling, seen in chapter 4, we would observe increased activation in regions involved in autonomic and baroreceptor signalling following training. The small sample included in this study make these findings hard to interpret however, speculatively, we propose that interoceptive training may have altered brain activation in autonomic regions which may have resulted in a suppression

of activation in some regions (i.e. precuneus) and a subtle increase in others, although this increase was evidently not strong enough to prevail as significant.

In accordance with our hypothesis, and supporting our prediction that brain connectivity would be altered following interoceptive training, was the finding that FC of right amygdala with precentral gyrus and left insula with vermis was greater when processing fear over neutral faces post, relative to pre-training. The amygdala is implicated in the processing of salience and fear whilst the precentral gyrus is primarily involved in motor control (Banker & Tadi, 2019). The recruitment of a motor region may be linked to a state of motor readiness in response to fear, i.e. a 'fight or flight' response (Butler et al., 2007), however there is also some evidence of altered precentral gyrus signalling in autism which may contribute to atypical findings (Nebel, Eloyan, Barber, & Mostofsky, 2014). Nonetheless, our findings suggest that interoceptive training altered FC in areas implicated in autonomic arousal. Concurrently, the finding of increased FC between left insula and vermis, which plays an integral role in cardiovascular autonomic control (Baker & Kimpinski, 2020), may also represent central increased autonomic signalling to facilitate emotional processing.

In further support of this, we identified an association between improved interoceptive accuracy, across both tracking and discrimination tasks, and increased FC of bilateral amygdala and insula. Interestingly, these effects emerge only when interoceptive accuracy is considered suggesting these mechanisms were altered to facilitate conscious perception of one's heartbeat as well as for the perception of emotional (fearful) faces. We have clearly established that both insula and amygdala cortices are involved in emotion perception and we now show that the regions with which they show increased FC, including these regions themselves (i.e. increased FC between amygdala and insula cortices), as well as other regions, including frontal regions, precentral gyrus, temporal pole and occipital cortex, are directly coupled to heartbeat perception and fear processing. Thus, Interoceptive training can significantly alter both the behavioural and neural response to interoceptive and emotion processing in autistic adults. This finding has important clinical implications; interoceptive training can alter both interoceptive ability, which may impact, or potential alleviate difficulties associated with, sensory sensitivity in autism, and emotion processing, which may be directly couple to affective

symptomatology (Garfinkel et al., 2014), as we have shown in chapter 3.

In this regard, we observed an interesting relationship with anxiety; the degree of change in trait anxiety modulated intensity ratings across sessions and across emotion and cardiac categories. For individuals who scored lower in anxiety at final, they provided greater intensity ratings at final, relative to baseline. Likewise, for individuals who scored higher in anxiety at final they provided reduced intensity ratings at final. Thus, for some, following interoceptive training, anxiety was increased and emotion sensitivity was reduced, whilst for others, following training, anxiety was reduced and emotion sensitivity was increased. Interestingly, this effect emerge specifically for diastole trials towards fear faces which we propose may reflect the clarity of diastolic signalling and the noisy impact of systolic signalling during the processing of fear faces. The typical systolic facilitation of fear faces, which is not observed on diastole trials, perturbs our ability to detect any relationship with fear faces through this channel whereas during cardiac diastole no inhibitory or facilitation effect of fear faces is typically observed and thus the increase in anxiety and subsequent reduction in emotion sensitivity is only reflected through this diastolic channel. These results serve to highlight the contribution of individual differences; interoceptive signals can be beneficial for some, by reducing anxiety and improving emotion sensitivity, whilst for others, interoceptive signals may be 'noisy' thus leading to increased anxiety and reduced emotion sensitivity. This claim is supported by our neuroimaging data whereby increased anxiety post training was associated with increased FC of left amygdala and left and right insula with regions involved in emotion and autonomic control, e.g. cerebellum, angular, temporal and postcentral gyrus, when processing fearful faces post interoceptive training. Similarly, greater anxiety post training was associated with increased FC of right insula during systolic signalling, consistent with the work linking anxiety to atypical emotional processing during cardiac systole (Garfinkel et al., 2014). Thus, when anxiety is increased following interoceptive training, we observe a pattern of hyper-connectivity of regions involved in autonomic control and emotion processes which may increase noise leading to a subsequent reduction in emotion sensitivity. It is important to note however that we found no direct association between change in interoceptive ability and change in anxiety in this sample. Thus, interoceptive training did not alter anxi-

ety and anxiety may have increased/decreased as a result of other factors not measured here. We nonetheless still highlight important mechanisms responsible for processing emotional and interoceptive information that can be influenced by anxiety.

This study has a few noteworthy limitations. First, we were significantly limited and likely under-powered in our analyses as a result of the high drop-out rate in the autistic interoceptive training group. Indeed, effects present in the larger sample (chapter 3) were not seen in the smaller sample at baseline here, despite them being taken from the same group at the same time-point. Thus, we may also inadequately capture interoceptive training effects on cardiac-contingent emotion processing. Additionally, as we highlight in chapter 4, the pre-post training component of this study, particularly the neuroimaging data, is confounded by time. As we had no control group who also completed pre and post training scanning sessions, we are unable to state that any effects observed did not emerge simply as a function of participants being scanned on a different day. Future work should look to employ the interoceptive training paradigm with a control group who are also scanned pre and post training to be able to more reliable make inferences about specific training effects on brain activation and connectivity. Nonetheless, this work still has validity in providing the first evidence of employing a novel interoceptive training paradigm and quantifying emotion processing ability, as a function of cardiac cycle, pre versus post training at a neural and behavioural level, in autistic adults. We provide the first tentative evidence that interoceptive training influences emotional experience in autistic adults and we highlight the importance of considering co-morbid affective symptomatology when designing and implementing training paradigms, that target emotion processing and autonomic signalling, in this population.

Chapter 6

Affective prosody recognition is enhanced in autistic adults following the implementation of a novel Affective Prosody Training Protocol (APTP)

6.1 Abstract

Affective prosody refers to the non-linguistic features of speech that are used to convey emotional information. In autism, the production and recognition of affective prosody is impaired which has negative implications for social functioning. In the current study we propose a novel Affective Prosody Training Protocol (APTP) and test its utility as a tool to improve affective prosody recognition in a sample of autistic adults. Over the course of 6 training session, 39 autistic adults were trained to recognise positive and negative, complex and basic emotions from, content neutral, affective spoken sentences. Additionally, participants were trained to pair affective prosody with facial expressions to increase training effects and generalisation in social situations. Results revealed significant enhancement of affective prosody recognition across emotional categories, across all assessment trial types (face only, face with text and text only choices). APTP training therefore has implications for improving emotion recognition and social functioning, and thus quality of life, in autistic adults.

6.2 Introduction

Emotions play a fundamental role in human experience and serve to optimise social functioning. Emotions can be adaptive, facilitating allostasis (the brains ability to regulate necessity in an optimum way thus maintaining homeostasis), or maladaptive (incorrect allocation of resources within the body resulting in the manifestation of affective disorders). Emotional experience is arguably constructed based on prior knowledge (Barrett, 2017) and influenced by contextual information, including interoceptive (Critchley & Garfinkel, 2017), environmental (Somerville, Jones, & Casey, 2010) and social cues (Tang, Chen, Falkmer, Blte, & Girdler, 2019).

As we have established in chapter 2, one important stream of emotional information is auditory information which can be divided into semantic information, the content of a sentence, and prosodic information, the intonation, pitch and volume of the spoken sentence (Wang & Tsao, 2015). Prosody can be further partitioned; affective prosody refers to the use non-linguistic features of speech to convey emotional information (Hubbard et al., 2017; Shriberg et al., 2001) which is distinct from pragmatic prosody, the accenting of words or syllables to convey meaning, and syntactic prosody, the use of boundary markers or pauses or the segmentation of utterances (Peppé et al., 2011).

In previous work, we (Mulcahy, Davies, et al., 2019), see chapter 2, along with many others (Golan et al., 2006; Lindner & Rosén, 2006; Rosenblau et al., 2017), have demonstrated that autistic individuals find the recognition of affective prosody more challenging than non-autistic populations. Consistent with these findings, autism spectrum conditions often show patterns of difficulty in emotional processing (e.g. Black et al., 2017; Cai, Richdale, Dissanayake, & Uljarević, 2019; Cibralic, Kohlhoff, Wallace, McMahon, & Eapen, 2019; Hill et al., 2004; Uljarevic & Hamilton, 2013; Zantinge, van Rijn, Stockmann, & Swaab, 2019) and social interaction and communication (e.g. Latinus et al., 2019; Neuhaus, Webb, & Bernier, 2019; Patriquin, Hartwig, Friedman, Porges, & Scarpa, 2019). Indeed, such characteristics are currently defined in the diagnostic criteria (American Psychiatric Association, 2013). Of course, there is work showing no difference in performance on affective prosody recognition paradigms between autistic and non-autistic populations (Brennand et al., 2011; Grossman et al., 2010; Le Sourn-

Bissaoui et al., 2013) however many of these paradigms suffer from several limitations, which we addressed in the current and our previous work (Mulcahy, Davies, et al., 2019), including small sample sizes, the use of 'basic' emotions only; happy, sad, disgusted, surprised, angry, afraid (Ekman, 1992), as well as confounding assessments with semantic information (Globerson et al., 2015; Grossman et al., 2010; Wang & Tsao, 2015). We thus argue that autistic individuals do have difficulty recognising emotions from voices and this effect may be related to interoceptive ability (specifically interoceptive insight) where ones metacognitive ability to understand interoceptive signals facilitates affective prosody recognition (Mulcahy, Davies, et al., 2019). This provides two potentially targetable treatment mechanisms; an interoceptive training paradigm aiming to improve interoceptive insight and, as is the focus of the current paper, an affective prosody training protocol aimed at improving one's ability to understand emotion from voices, independent of semantic information.

Training paradigms developed for autistic individuals have been successfully implemented to improve, for example, emotion regulation (for a review see Reyes, Pickard, & Reaven, 2019), facial emotion recognition (Wieckowski & White, 2020), social cognition (Didehbani, Allen, Kandalaft, Krawczyk, & Chapman, 2016), social skills (Becker, Rogers, & Burrows, 2017; Ke & Moon, 2018; Radley, McHugh, Taber, Battaglia, & Ford, 2017) and emotion understanding (Junek, 2007; Petrovska & Trajkovski, 2019). In the prosodic domain, some work has shown utility both in training receptive (Lacava, Golan, Baron-Cohen, & Smith Myles, 2007; Lacava, Rankin, Mahlios, Cook, & Simpson, 2010; Matsuda & Yamamoto, 2013; Peppé et al., 2007; Rothstein, 2013) and, more commonly, expressive prosody (Akbari & Davis, 2019; Bellon-Harn, Harn, & Watson, 2007; Dunn et al., 2007; Hutchison, 2015; Simmons, Paul, & Shic, 2016; Wan et al., 2011; Wilson, Steinbrenner, Kalandadze, & Handler, 2019). Of the current affective prosody recognition training protocols available, certain limitations, including confounding stimuli with semantic information (PEP-C; Peppé et al., 2007), the use of basic emotions only (Matsuda & Yamamoto, 2013) and no training in facial-audio emotion pairing (Lacava et al., 2007, 2010), hinder what has the potential to significantly improve social function and well-being in autistic populations. Thus, in the current study, building on these limitations and the work from (Golan et al., 2006; Lacava et

al., 2007; Matsuda & Yamamoto, 2013), we propose a novel Affective Prosody Training Protocol (APTP) for adults. We demonstrate the utility of this training protocol in a sample of autistic individuals who, arguably, serve to benefit most from improved affective prosody recognition to facilitate social functioning.

Given our previous work relating prosody recognition to interoceptive insight, previously termed interoceptive awareness (Mulcahy, Davies, et al., 2019), we also quantify interoceptive ability pre and post prosody training using the commonly employed heartbeat discrimination task (Katkin et al., 1983; Whitehead et al., 1977). Additionally, we consider alexithymia, defined as difficulty identifying and describing one's own emotions (Apfel & Sifneos, 1979), which may play a pertinent role in emotion difficulties often seen in autism (Foulkes, Bird, Gökçen, McCrory, & Viding, 2015; Mul et al., 2018) and thus the recognition of affective prosody. Finally, we measure affective symptomatology, anxiety and depression, both of which have high a prevalence rate in this population (Bird et al., 2010; Bird & Cook, 2013; Cook et al., 2013; Hollocks et al., 2019; Liss, Mailloux, & Erchull, 2008) and, arguably, may arise, or be exaggerated, by social communication and interaction difficulties often observed in autism and thus may be mitigated by APTP training.

We therefore hypothesise that, following APTP training, all participants will show significant improvement in the recognition of affective prosody across trial types and emotion categories. This improvement will correspond with improvement in subjective states of anxiety, depression and with a reduction in self-reported alexithymia traits. Finally, based on our prior work (Mulcahy, Davies, et al., 2019), we tested whether improved prosody recognition would correspond with increased interoceptive insight.

6.3 Methodology

6.3.1 Participants

one hundred and twenty participants with a confirmed Autism Spectrum Condition (ASC) diagnosis were recruited for the ADIE study. Sixty participants were randomly allocated to the prosody training group whilst 60 participants were allocated to the interoception training group (see chapter 4). In the prosody group,

21 participants dropped out mid-way through training and were thus excluded from the analyses. The final prosody group consisted of 39 participants (18 male, 21 female, mean age 37.03; range 19 – 59 years). In the interoception training group, 23 participants dropped out/did not completed the prosody protocol during the final assessment and were thus excluded from the analyses. The final interoception group thus consisted of 37 participants (22 male, 15 female, mean age 35.81; range 18 – 64 years). All autistic participants were fluent English speakers and none of the autistic participants had a history of past head injury or organic brain disorders, cognitive impairment or a learning disability (general mental impairment); none had asthma/respiratory illnesses, epilepsy or evidence of psychotic experiences. All participants provided written informed consent with all procedures approved by the NHS Research Ethics Committee.

6.3.2 Prosody paradigm: pre and post training assessment

During baseline (pre-training) and final (post-training), the affective prosody protocol employed in (Mulcahy, Davies, et al., 2019), chapter 2, was administered. In brief, the paradigm comprised 507 audio files and 166 photographs depicting 21 different emotions. Emotions included the 6 basic emotions; happy, sad, disgusted, surprised, angry, afraid (Ekman, 1992) and 13 complex emotions; bored, kind, jealous, unfriendly, hurt, disappointed, interested, joking, ashamed, proud, excited, frustrated and worried. Three different trial types were utilised; matching voices to faces (face-only), matching voices to emotion descriptors (text-only) and matching voices to faces and emotion descriptors combined (face with text). Each domain was further divided into positive and negative valence. In total 114 trials were completed (38 face-only, 38, text-only and 38 face with text). Each of the 19 verbally expressed emotions were presented twice for each domain but remained novel. The presentations were randomised and no trials were repeated. Out of 114 trials, 72 were of a negative valence (24 out of each trial type). In order to establish if improvement in prosody performance was a learning effect (i.e. audio-visual stimuli pairings) or a generalisability effect (i.e. enhanced prosody recognition toward novel stimuli) 18 participants re-completed the baseline assessment at final whilst 21 participants were presented with novel auditory stimuli that had not been heard during the baseline assessment or any training sessions.

6.3.3 Prosody training procedure

The Affective Prosody Training protocol (APTP) comprises 6, progressively more difficult, individual training sessions (mean number of days to complete training 62.41). The rationale for this approach was to gradually develop the participants' sensitivity to expressed affective prosody, e.g. by gradually introducing same/opposing valence choices, graded emotion intensities (e.g. happy vs happy mild) and the inclusion of child voices. Similar to baseline and final assessment sessions, participants heard audio clips of different content neutral phrases and were instructed to 'focus on the tone of voice as much as possible' and were then presented with different emotions options from which they had to choose the one that best matched the tone of voice in the audio clip they had just heard. Unlike the assessment sessions, one trial type, face with text, was employed throughout training and all trials were completed twice to reinforce learning. Additionally, and the most important aspect of training, after participants made their choice, they received feedback regarding their choice; 'That's correct, well done!' or 'Incorrect, the correct answer was ...'.

Session 1: The first session comprised four randomized training blocks totalling 100 trials. The first 2 blocks utilised only basic emotions (24 trials in each block, 4 trials for each of the 6 basic emotions) and the remaining 2 blocks utilised only complex emotions (26 trials in each block, 2 trials for each of the 13 complex emotions). Throughout session 1, a two-choice training paradigm was employed with emotion options of opposing valance as outcome choices (e.g. happy vs sad) which makes emotions easier to distinguish and thus may enhance sensitivity to emotional tone. Each of the four blocks ended with trials pairing same valence emotions in order increase the task difficulty and implement a gradual enhancement of participants sensitivity to tonal differences.

Session 2: The second session comprised 2 blocks of 38 trials and again utilised two-choice training. Each block included basic and complex emotions (6 basic emotions, 2 trials of each; 13 complex emotions, 2 trials of each) but the first block employed stimuli of opposing valence whilst the second block utilised same valence stimuli.

Session 3: The third session comprised 2 blocks of 48 and 50 trials respectively. The first block again utilised two-choice training whilst the second block

implemented three-choice training. Importantly, this session introduced graded intensities (i.e. happy vs happy mild) to further increase the difficulty of the task and develop prosodic sensitivity. Thus, the first block employed basic emotions only (8 trials for each of the 6 basic emotions) with two choices of the same, but graded intensity, emotion. The second block integrated basic and complex emotions across different intensities (4 trials per basic emotion, 2 trials per complex emotion) with three outcome choices following the structure of 1) the target emotion 2) the opposing valence to the target emotion and 3) the same valence as the target emotion.

Session 4: The fourth session comprised 2 blocks of 50 (4 trials per basic emotion, 2 trials per complex emotion) and 38 trials (2 trials per basic emotion, 2 trials per complex emotion) respectively and three-choice training was employed throughout. The three choices offered in the first block followed the same structure of the second block in session 3 (i.e. the three choices were 1) the target emotion 2) the opposing valence to the target emotion and 3) the same valence as the target emotion). During the second block, all 3 choices were the same valence as the target emotion.

Session 5: The fifth session comprised 2 blocks of 50 trials (4 trials per basic emotion, 2 trials per complex emotion) and utilised four-choice training. To develop sensitivity to affective prosody across age ranges, the first block utilised adult voices only and the second block utilised child voices only. The target emotion was presented alongside 2 emotions of the same valence and one of the opposing valence.

Session 6: The final training session comprised 88 trials in one complete block (6 trials per basic emotion, 4 trials per complex emotion) and replicated session 5 except for the inclusion of a mixture child and adult voices across the two blocks (44 trials each). The final session thus targeted age, valence and intensity of emotional stimuli, i.e. a combination of all aspects implemented throughout training.

6.3.4 Heartbeat discrimination task

Based on our previous work showing a relationship between heartbeat discrimination insight (Mulcahy, Davies, et al., 2019), we employed the heartbeat discrim-

ination task (see chapter 2, section 3.3) to investigate the relationship between prosody and heartbeat discrimination accuracy and insight. 3 participants did not complete the interoception task post training and were thus not included in any analyses involving this measure.

6.3.5 Questionnaires

In addition to completing the awareness sub-scale of the Body Perception Questionnaire (BPQ) and the Multidimensional Assessment of Interoceptive Awareness (MAIA), all participants completed the Autism Quotient (AQ) (Baron-Cohen et al., 2001), the State-Trait Anxiety Inventory (STAI) (Spielberger, 2010), the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001) and the Toronto Alexithymia Scale (TAS-20) (Bagby et al., 1994). For each questionnaire, the total score was computed and used in the analysis. Five participants did not complete the MAIA or BPQ pre or post training and 1 other participants completed no questionnaires post training and, thus, these participants were not included in any analyses involving these measures.

6.4 Data analyses

Demographic information (age and education) across the two training groups and, within the prosody training group, across the repeated/novel groups was compared using independent sample t-tests. Performance on the prosody task was measured by percent correct across all trials and broken down by trial type (face, face with text and text only), emotional valence (positive and negative) and emotional complexity (basic and complex). We first examined the effectiveness of prosody training by comparing pre and post-performance across groups, broken down by trial type, emotional valence (positive and negative) and emotional complexity (basic and complex). Thus, we first computed a 3 (trial type) x 2 (session) x 2 (group) mixed ANOVA, with group as the between-subjects factor and trial type and type as within-subject factors. Next, we tested for emotional valence and complexity effects by computing 2, 3 (trial type) x 2 (session) x 2 (group) x 2(valence/complexity) mixed ANOVAs, with group as the between-subjects factor and session, trial type and emotional complexity or valence as within-subject

factors.

We then sought to better understand the impact of affective prosody training in the prosody training group alone. We thus computed a 3 (trial type) x 2 (session) repeated measures ANOVA. We also tested for effects of emotional valence and emotional complexity by computing 2, 3 (trial type) x 2 (session) x2 (valence/complexity) repeated measures ANOVAs. Throughout each analysis we also tested for the specific generalisability/learned effect by including group (novel versus repeated stimuli) as a between subjects-factor to test for significant group by session interactions (i.e. did one group perform better or worse pre versus post training across trial types and emotional valence/complexity categories). Significant main effects and interactions were further explored using paired/independent sample-t tests. We also tested for the impact of time between the pre and post training sessions (i.e. duration to complete training) by including days between sessions as a covariate. No significant effects of time (main effect or interactions) were found, all p's < 0.05, suggesting the time between sessions did not impact the results.

Finally, change in interoceptive accuracy, insight and affective symptomatology was examined using paired-sample t-tests. Where significant differences were identified, the relationship between change in affective prosody (final – baseline) and change in prosody performance (final – baseline) was explored using Pearson's correlations. All p values in the results section are uncorrected.

6.5 Results

6.5.1 Demographic information

The prosody and interoceptive training groups did not significantly differ on age ($t(74) = 0.406$, $p = 0.686$) or education (fishers exact, $p = 0.520$). Within the prosody group, no difference in age ($t(37) = -0.701$, $p = 0.488$) nor education (fishers exact, $p = 0.293$) between the novel and repeated groups was observed.

6.5.2 Between group differences in affective prosody recognition

All trial types: The affective prosody training and interoceptive training groups significantly differed in overall prosody recognition performance following their re-

spective training protocols, as signified by a significant session x group interaction ($F(1, 74) = 35.371$, $p < 0.001$). Participants in the prosody group significantly improved following prosody training ($t(38) = -6.925$, $p < 0.001$), whilst the interoception training group showed no difference between pre and post training scores in prosody recognition ($t(36) = 0.106$, $p = 0.916$). We also observed a significant session x trial type x group interaction ($F(2, 148) = 4.587$, $p = 0.012$) indicating the interaction between session and trial type differed between the two groups. Indeed, in the prosody group, no significant interaction between session and trial type was identified ($F(2, 76) = 1.116$, $p = 0.333$) whilst the interaction was significant in the interoception group ($F(2, 72) = 5.246$, $p = 0.007$) indicating significant improvement on face only trials ($t(36) = -2.487$, $p = 0.018$), a significant reduction in performance on text only trials ($t(36) = 2.213$, $p = 0.033$) and no difference in performance on face with text trials ($t(36) = 0.662$, $p = 0.512$).

Emotional valence: We observed no significant session x valence x group interaction ($F(1, 74) = 0.033$, $p = 0.855$) indicating the two groups did not significantly differ in change in accuracy toward positive or negative emotions following training. We also observed no significant session x valence x trial type x group interaction ($F(2, 148) = 1.810$, $p = 0.167$) indicating the two groups did not significantly differ in change in accuracy toward positive or negative emotions broken down by trial type.

Emotional complexity: We observed no significant session x complexity x group interaction ($F(1, 74) = 0.262$, $p = 0.610$) indicating the two groups did not significantly differ in change in accuracy toward basic or complex emotions. We also observed no significant session x complexity x trial type x group interaction ($F(2, 148) = 2.720$, $p = 0.069$) indicating the two groups did not differ in change in accuracy toward positive or negative emotions across the three trial types.

6.5.3 Prosody training

All trial types: As indicated above, performance on the affective prosody paradigm was significantly improved following prosody training as signified by a main effect of session ($F(1, 38) = 47.957$, $p < 0.001$). Thus, relative to baseline,

participants at final were significantly more accurate at recognising emotion from voices across all trial types ($t(38) = -6.925$, $p < 0.001$). See figure 6.1. We also observed a main effect of trial type ($F(2, 76) = 41.991$, $p < 0.001$) indicating that, independent of session, participants were more accurate on face and text trials relative to face only trials ($t(38) = -8.29$, $p < 0.001$), text trials relative to face only trials ($t(38) = -5.88$, $p < 0.001$) and face and text trials relative to text only trials ($t(38) = 3.166$, $p = 0.003$). We did not however find a significant interaction between session and trial type ($F(2, 76) = 1.116$, $p = 0.333$) indicating that, despite improvement across all trial types, the magnitude of improvement did not differ across the three trial types (figure 6.2). In the test for the effect of novel stimuli, we observed a significant group by session interaction ($F(1, 37) = 10.599$, $p = 0.002$) indicating greater improvement in the repeated group ($t(17) = -8.102$, $p < 0.001$), although we still observed significant improvement in the novel group ($t(17) = -3.327$, $p = 0.003$). No significant three-way interaction between group, session and trial type was found ($F(2, 74) = 1.106$, $p = 0.304$).

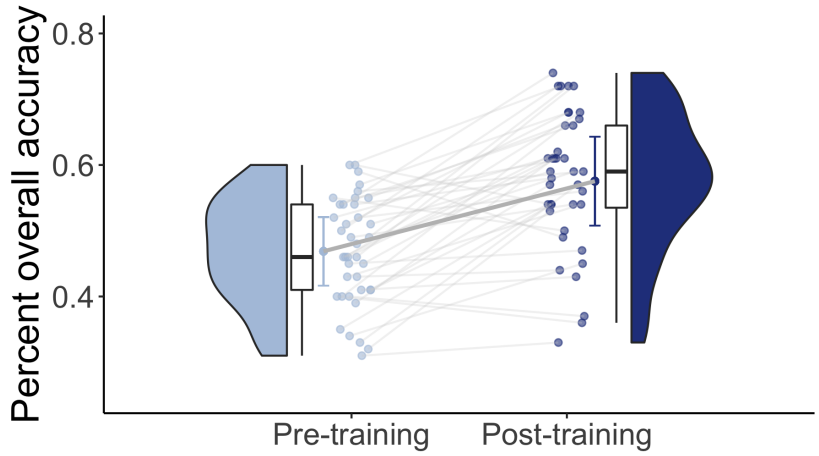


Figure 6.1. Prosody training: change in overall accuracy.

Summary of prosody training effects: pre versus post training (x-axis) against overall accuracy (y-axis). Participants were significantly more accurate at recognising emotions from voices following prosody training. Group distribution displayed as individual data points (horizontally jittered), violin plots (probability density functions), boxplots showing upper/lower quartiles and the median value, and whiskers showing the minimum and maximum values.

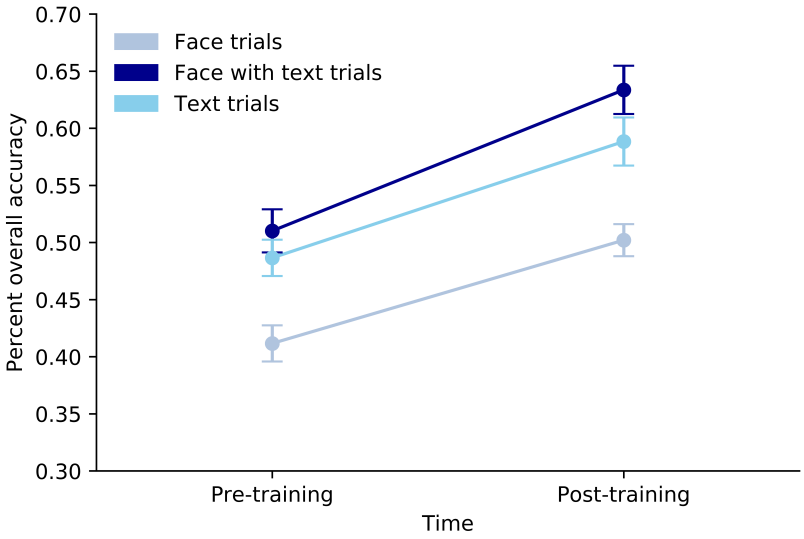


Figure 6.2. Prosody training: change in accuracy across trial types.

Following prosody training, participants were significantly more accurate at identifying emotion from voices. The magnitude of improvement was consistent across all trial types as signified by no interactions (no crossed lines). Bars represent standard error of the mean.

Emotional valence: A significant main effect of emotional valence ($F(1, 38) = 12.618$, $p = 0.001$) revealed, independent of session and trial type, participants were significantly more accurate at recognising negative rather than positive emotions ($t(38) = -3.552$, $p = 0.001$). We also observed a significant session by valence interaction ($F(1, 38) = 4.731$, $p = .036$) indicating significant improvement following training for both positive ($t(38) = -3.297$, $p = 0.002$) and negative emotions ($t(38) = -7.745$, $p < 0.001$) and the magnitude of change was greater towards negative emotions ($t(38) = -2.175$, $p = 0.036$). We did not observe a trial type by valence interaction ($F(2, 76) = 2.546$, $p = 0.085$) but we did observe a significant three way interaction between session, trial type and valence ($F(2, 76) = 4.485$, $p = .014$) indicating the interaction between trial type and valence varied as a function of session. Indeed, at baseline, there was no significant interaction between trial type and emotional valence ($F(2, 76) = 3.073$, $p = 0.052$) but there was a significant interaction post-training ($F(2, 76) = 3.417$, $p = .038$) indicating negative valence provided a recognition advantage on face with text trials ($t(38) = -4.687$, $p < 0.001$) but not on face only ($t(38) = -0.870$, $p = 0.390$) or text only

trials (t(38) = -0.895, p = 0.376). In the test for the effect of novel stimuli, no significant three-way interaction between group (novel versus repeated), session and valence was found (F(1, 37) = 0.359, p = 0.533) and no four-way interaction between group, session, valence and trial type was found (F(2, 74) = 2.638, p = 0.078). Thus, no difference in accuracy across trial types or valence categories at pre and post training was observed between the two (novel versus repeated) groups.

Emotional complexity: Participants were significantly more accurate at recognising basic compared to complex emotions (t(38) = 4.783, p < 0.001), independent of session and trial type, as indicated by the significant main effect of emotional complexity (F(1, 38) = 22.878, p < 0.001). There was also a significant trial type by complexity interaction (F(2, 76) = 24.394, p < 0.001) indicating, independent of session, responses toward basic emotions did not differ across trial types (F(2, 116) = 0.132, p = 0.887) but responses towards complex emotions did differ across trial types (F(2, 116) = 31.988, p < 0.001). Here, for complex emotions, independent of session, a recognition advantaged was conferred for face with text compared to face only trials (t(38) = -11.336, p < 0.001), text only trials compared to face only trials (t(38) = -7.530, p < 0.001) and for face with text compared to text only trials (t(38) = 3.084, p = 0.004). No significant session by complexity interaction was observed (F(1, 38) = 3.655, p = 0.063) indicating, despite improvement, participants did not differ in the magnitude of change in accuracy toward basic or complex emotions. Finally, we observed a significant three way interaction between session, trial type and emotional complexity (F(2, 76) = 8.241, p = 0.001) indicating that the interaction between trial type and emotional complexity varied as a function of session. Indeed, at baseline, a significant interaction between trial type and emotional complexity was identified (F(2, 76) = 6.507, p = .002) indicating basic emotions were more accurately recognised on face only (t(38) = 4.807, p < 0.001) and text only (t(38) = 4.577, p < 0.001) but not face with text trials (t(38) = 1.316, p = 0.196). Post-training, we also observed a significant interaction between trial type and emotional valence (F(2, 76) = 25.361, p < 0.001) indicating, following training, basic emotions only provided a recognition advantage for face only trials (t(38) = 6.142, p < 0.001), not for face with text (t(38) = -0.399, p = 0.692) or text only trials (t(38) =

-1.243, p = 0.222). In the test for the effect of novel stimuli, no significant three-way interaction between group, session and complexity was observed (F(1,37) = 3.599, p = 0.066) and no four-way interaction between group, session, complexity and trial-type was observed (F(1, 74) = 0.170, p = 0.844). Thus, as in the valence analyses, no group differences across sessions, between trial type and complexity categories was observed.

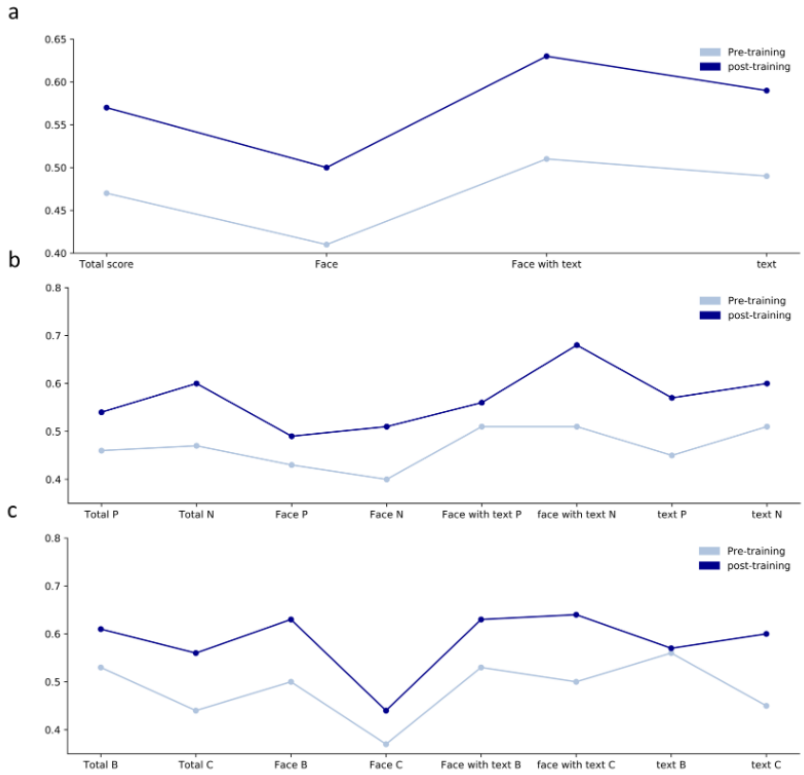


Figure 6.3. Prosody training: change in accuracy across trial types and valence/ complexity categories.

Summary of mean accuracy across all participants for final and baseline, broken down by trial type (a), emotional valence (b) and emotional complexity (c). In x-axis labels, P = positive, N = negative, B = basic, C = complex.

6.5.4 Change in interoception and affective symptomatology

No significant change in heartbeat discrimination accuracy (t(35) = 0.459, p = 0.586) or insight (t(34) = 1.585, p = 0.122) was observed. A significant reduction in trait anxiety was observed (t(36) = 2.092, p = 0.044) but no significant reduction in depression (t(31) = 0.876, p = 0.388) was observed. Additionally, no

significant reduction in autistic traits (t(37) = 1.073, p = 0.290) nor alexithymia; for total score (t(37) = 0.000, p = 1.00) the difficulty describing feelings (t(37) = -1.520, p 0 .137), difficulty identifying feelings (t(37) = 1.519, p = 0.137) and the externally oriented thinking (t(37) = -0.471, p = 0.641) subscales, was observed. The significant change in trait anxiety, nor any other change scores, were not associated with change in any of the prosody variables, all p's > 0.05.

6.6 Discussion

In the current study we implemented a novel affective prosody training protocol (APTP) as a comprehensive training tool to enhance affective prosody recognition in autistic adults. APTP training resulted in significant improvement in affective prosody recognition across emotion categories (basic, complex, positive and negative emotions) as measured across trial types (face only, face with text, text only). The design of ATPT training also facilitates direct emotional prosody and facial expression pairings. This training thus has important clinical implications for mitigating emotional social communication and interaction difficulties autistic individuals often face. It is however worth noting that uncorrected p values make our results preliminary.

When comparing across the two groups, we showed that individuals who received ATPT training significantly improved in affective prosody recognition compared to those who received interoception training. Additionally, participants in the APTP group improved across all trial types whilst those in the interoception group showed a more random pattern of change, dependent on trial type. This provides strong evidence for the utility of the APTP protocol as enhancement in accuracy was consistent across face, face with text and text only trials. This is particularly interesting given that the APTP training employed only face with text trial types through the 6 training sessions and thus the learning was strong enough and transferred to facilitate affective prosody recognition toward face only and text only trial types. Interestingly, we showed no difference in the level of change between the two groups across valence and complexity categories despite the significant improvement in accuracy observed. It may be that we were underpowered to investigate such a relationship a future work should look to increase the trial number and sample size.

Within our design, 21 participants were presented with novel auditory stimuli in the assessment post-training whilst 18 participants were presented with the same auditory stimuli they had heard in the initial pre-training assessment. Our results revealed that participants in the repeated group showed greater improvement in overall accuracy, however a significant improvement was still observed in the novel group demonstrating that prosody learning generalized to novel stimuli. Additionally, no difference in accuracy pre versus post training across trial types, valence and complexity categories was observed between the two groups. We therefore argue that the APTP training protocol can operate independent of a specific learning effect (i.e. audio-visual pairings) and can generalise to novel stimuli. This has important clinical implications for facilitating real-world social exchange when auditory and visual stimuli will be novel. Future work should look to employ a more ecologically valid assessment of affective prosody recognition (i.e. in a real social exchange) to establish the effectiveness of APTP training in this regard.

Despite our work showing a positive relationship between interoceptive insight and enhanced performance on the prosody paradigm (Mulcahy, Davies, et al., 2019), we found no evidence of enhanced interoceptive insight as a result of APTP training. It may be that more targeted interoceptive training is needed to enhance interoceptive ability and such training would have an impact on prosody performance. For example, a paradigm tailored to specifically enhance interoceptive insight which may then influence affective prosody recognition. It is thus likely that the relationship between affective prosody recognition and interoceptive insight operate with some degree of independence. Accurate affective prosody recognition may be influenced by higher order knowledge, which can be improved through targeted prosody training, as shown here, or by the sensing of bottom up interoceptive signals which may inform emotional recognition. We also found no evidence that improvement on the prosody paradigm mitigated subjective states of anxiety or depression. We did observe a subtle decrease in trait anxiety however this decrease was not related to change in affective prosody recognition. Arguably, levels of both anxiety and depression in autism are enhanced as a result of social communication difficulties in autism although the opposite could also be true, such that increased anxiety and/or depression may facilitate aberrant social

interaction (Factor, Ryan, Farley, Ollendick, & Scarpa, 2017; White & Roberson-Nay, 2009). While we found no evidence of either relationship, we suggest this may reflect affective symptomatology in a domain, i.e. social functioning, that is not adequately captured by the STAI (Spielberger, 2010) or the PHQ-9 (Kroenke et al., 2001). Indeed, the STAI focuses on how people generally feel and how they feel at the present moment, it does not ask how people feel in specific situations, i.e. during a social exchange. Equally, the PHQ-9 asks participants to report about general depression related symptoms they felt in the past 2 weeks, not specifically regarding social functioning. Thus, we predict that APTP training will reduce subjective states of anxiety and depression but in specific situations where the skills acquired from training are applicable and future work should thus use a domain specific measure to capture measures of anxiety and depression in social situations.

Interestingly, we found no relationship between alexithymia and prosody performance. Alexithymia, the inability to identify and describe one's emotions, is common in autism and arguably underscores interoceptive deficits often observed in this population (Shah et al., 2016) as well as emotion deficits (Bird et al., 2010; Cook et al., 2013). Despite this, following APTP training, we observed no significant reduction in alexithymic traits. This could be because APTP training focusing on the recognition of others emotion whilst alexithymia focuses more on the self and thus, it is possible that a more self-focused training protocol would likely lead to a reduction in alexithymia scores. We also observed no significant reduction in depression nor autistic traits although we did observe a subtle decrease in levels of trait anxiety. This reduction was not however related to change in any of the prosody variables and, as we have discussed, this may result from the application of an anxiety measure that does not specifically focus on social situations.

The current paradigm has a few noteworthy limitations. Opposing views argue that emotions have a distinct signature (a biological fingerprint); the classical view of emotion, or emotions are constructed based on the brains ability to make predictions about the world and, by drawing on incoming interoceptive (afferent signals from the internal milieu) and exteroceptive (information from outside the body) sensory information, reduce prediction errors to update and optimise

predictions to generate an appropriate response, i.e. emotional experience; the theory of constructed emotion (Barrett, 2017). Whilst the APTP is grounded in the theory that all emotions possess a unique signature, i.e. a facial expression or distinct prosodic cue, which may limit the applicability of the protocol across individuals and cultures, if we assume there is variability in such emotions (Barrett et al., 2019; Carlisi et al., 2020), and be considered a limitation, we argue that the ATPT still has utility in training emotion recognition by developing priors which may inform emotional experience in future social situations where such cues are recognised.

Additionally, the current training protocol only includes face with text trials and does not train on face alone or text alone trials. This may be considered a limitation as in real life social exchange auditory information and facial expressions may be the only sources of information present to portray an emotion. In our data, face only trials appear to be the hardest trial type to perform and thus, in this sense, it would seem logical to train face alone pairings. We however opted to employ face with text trials throughout training to facilitate a greater level of learning by providing both face and text information. By doing this, the participants is actually receiving training in affective prosody recognition as well as the interpretation of emotions from faces. The validity of our choice is evidenced in our finding that the magnitude of change across trial types did not differ; significant improvement was observed equally across face, face with text and text only trials. Thus, we argue that employing face with text trials throughout training improved learning and may lead to greater improvement in emotion recognition in real life situations.

One other noteworthy limitation, as we highlight in our previous work utilising this paradigm (Mulcahy, Davies, et al., 2019), was we were unable to adequately quantify accuracy toward discrete basic emotions given the low number of trials (6 per emotion). Finally, the current study had no control group of non-autistic individuals. Thus, we cannot conclude that ATPT training is solely beneficial for autistic participants. In fact, ATPT training could be beneficial for individuals with a range of other conditions that encounter emotion comprehension and social communication difficulties, including alexithymia (Parker, Taylor, & Bagby, 2001) and Attention Deficit Hyperactive Disorder (ADHD) (Da Fonseca, Seguier,

Santos, Poinso, & Deruelle, 2009), and future work should look to explore this.

In conclusion, the results of the current study validate the use of the APTP paradigm as a novel tool for training affective prosody recognition in autistic populations. Enhanced social emotional understanding remains pertinent for smooth social interaction and improved communication. Thus, APTP training has important clinical implications for mitigating core autistic symptomatology to improve daily functioning and social well-being.

Chapter 7

General discussion

7.1 Overview

Interoception is a central component involved in autonomic and emotional processes (Critchley & Garfinkel, 2017), where cardiac signals, operating at conscious and unconscious levels, may contribute to the altered emotional profiles often observed in autistic individuals. Thus, understanding how interoceptive signals influence emotional experience in autistic adults may help us to understand emotion difficulties, particularly the high rates of comorbid affective symptomatology, notably anxiety, in this population. Such work has important implications for the development and implementation of treatment paradigms aimed at mitigating such comorbid symptomatology.

A large portion of the literature on interoception in autistic individuals has focused on behavioural performance on interoceptive tasks, namely heartbeat tracking and discrimination, yet, with one exception (Failla et al., 2020), relatively little work has explored the functioning of neural systems responsible for interoceptive processes in autistic individuals. Additionally, whilst there is work linking impaired interoceptive ability to emotional difficulties in autism, no work has sought to develop and implement an interoceptive training paradigm to reduce affective symptomatology in this population.

To this end, using a combination of behavioural task, subjective reports and functional MRI scanning, the work in this thesis has investigated the relationship between interoception and emotion in autistic adults. I have also evaluated the impact of novel interoceptive, aimed at reducing anxiety, and exteroceptive, aimed at improving social communication and interaction, training paradigms. This thesis has shown that interoceptive signals do influence emotional experience in autistic adults and this profile can be differentiated from non-autistic adults. Additionally, it also demonstrates that interoceptive and exteroceptive (affective prosody recognition) ability can be improved following targeted training which has important clinical implications for mitigating emotion, and thus social, difficulties in this population. This discussion chapter will consolidate empirical findings and highlight areas for future work.

7.2 Key findings

7.2.1 Autistic adults present with an altered interoceptive profile

Historically, the majority of work on sensory difficulties in autism has focused in the exteroceptive domain (e.g. vision and hearing) however recent work has highlighted the importance of interoceptive processing. Self and caregiver reports have documented a range of difficulties with the awareness and integration if interoceptive signals (Elwin et al., 2012) and subjective reports have suggested that autistic adults report less body awareness compared to neurotypical adults (Fiene & Brownlow, 2015). Emerging empirical work has begun to further disentangle interoceptive processing in autism which paints a complex picture of sensory difficulties which, by large, and combined with the findings from this thesis, suggest an altered interoceptive profile in autism.

Objectively, using the heartbeat tracking task, autistic children have superior ability to count their heartbeat over long time durations (100 seconds) yet no group differences, when compared with neurotypical children, over shorter windows (Schauder et al., 2015) suggesting potential sustained attention to interoceptive cues in autistic individuals. Reduced interoceptive accuracy on heartbeat counting tasks has also been reported in adults, along with increased sensibility (Garfinkel, Tiley, et al., 2016), although no difference compared to neurotypicals was reported in accuracy on the heartbeat discrimination task or in interoceptive insight. In this work, the discrepancy between objective accuracy and subjective sensibility, the trait prediction error (ITPE), predicated anxiety. These findings have also been extended into a developmental sample where autistic children report reduced heartbeat tracking, yet similar discrimination accuracy, compared to neurotypical children (Palser et al., 2018). The relationship between the ITPE and anxiety was also replicated despite the lack of group differences in interoceptive sensibility observed in children. These findings are, in part, consistent with the work showing an altered developmental trajectory in autism where impairments are present in children, when IQ (< 115) is considered (Mash et al., 2017), but not in adults (Nicholson et al., 2019). Further work also reports reduced heartbeat tracking accuracy, yet no difference in heartbeat discrimination accuracy, and reduced interoceptive sensibility, as measured by the MAIA, in autistic adults

compared to neurotypical controls matched on age, sex and IQ (Mul et al., 2018). More recent work links interoceptive ability to core autistic features (Palser et al., 2020), namely sensibility was related to social-affective features whilst accuracy was related to restricted and repetitive behaviours.

Evidence to the contrary has however suggested autistic individuals do not differ from neurotypicals in interoceptive ability. In two studies measuring autistic traits in neurotypical participants, no relationship was found between autistic traits and heartbeat tracking accuracy, a finding that was replicated in two follow up studies comparing autistic to neurotypical adults (Nicholson et al., 2018; Shah et al., 2016). In one neuroimaging study (Failla et al., 2020), behaviourally no difference in heartbeat tracking accuracy, between autistic and neurotypical individuals, was found and insula, and whole brain, response did not differ between the two groups. They do, however, report that insula response interacted with group to predict autistic social traits and suggest this may represent altered integration of interoceptive and exteroceptive (social) information in autistic individuals.

In this thesis, I have presented work that supports both similarities and differences in interoceptive processing in autistic, compared to neurotypical, adults. In chapter 4, employing both the heartbeat tracking and discrimination tasks, I found that, at the group level, autistic adults do not differ in objective behavioural tests of interoception, i.e. interoceptive accuracy, consistent with work showing no difference in heartbeat tracking (Failla et al., 2020; Nicholson et al., 2018; Shah et al., 2016) or discrimination (Garfinkel, Tiley, et al., 2016; Palser et al., 2018) accuracy. We also observed no group differences in interoceptive insight, consistent with previous work (Garfinkel, Tiley, et al., 2016). I did however replicated the finding of elevated sensibility, when measured via the BPQ (Garfinkel, Tiley, et al., 2016), and reduced sensibility, when measured by the MAIA (Mul et al., 2018). Given that the BPQ arguably measures a more hyper-vigilant attention style, consistent with the work associating the BPQ with anxiety (Anderson & Hope, 2009; Gregor & Zvolensky, 2008; Olatunji et al., 2007), yet interestingly, in this thesis, BPQ scores correlated with anxiety in all, autistic and neurotypical, participants, whilst the MAIA assesses a more beneficial mindfulness interoceptive approach. Thus, these results suggest that greater attention to interoceptive cues is problematic for anxiety symptoms across populations and autistic individuals

pay greater attention to interoceptive cues yet do not use them for efficient body regulation, arguably contributing to the maintenance of anxiety disorders.

Relatedly, despite previous work linking the discrepancy between objective accuracy and subjective belief about interoceptive ability to anxiety (Garfinkel, Tiley, et al., 2016; Palser et al., 2018), I found no evidence to suggest that the trait prediction error (ITPE) differed in autistic versus neurotypical individuals and none to suggest the ITPE was related to anxiety in autistic adults in my limited neuroimaging sample. In fact, I observed a significant relationship between the heartbeat tracking ITPE and trait anxiety in neurotypical participants only and, in a regression analysis, found tracking ITPE to be the only significant predictor of trait anxiety, independent of group status. Interestingly, this relationship was in the opposite direction to that observed by (Garfinkel, Tiley, et al., 2016), such that the propensity for all individuals (autistic and neurotypical) to underestimate their interoceptive ability was associated with heightened anxiety. The notion that anxiety may manifest as a result of an altered prediction signal, i.e. a heightened discrepancy between observed and expected bodily states, has long been recognised (Paulus & Stein, 2006, 2010). My results support this notion yet show it across all participants, with a weighting towards neurotypical participants, thus somewhat consistent with previous literature that show this finding across autistic and neurotypical individuals (Garfinkel, Tiley, et al., 2016; Palser et al., 2018). At a more basic level, we observed an interesting finding with heartbeat discrimination accuracy; when we included heartbeat tracking ITPE in the model, group status and heartbeat discrimination accuracy best predicted anxiety suggesting the presence of an autism diagnoses and increased heartbeat discrimination accuracy was associated with greater anxiety. Thus, in sum, my results show that all participants (autistic and neurotypical) are more susceptible to anxiety with an aberrant interoceptive prediction signal, yet, in autistic individuals only, the ability to accurately perceive one's heartbeat, and the presence of a potential misinterpretation of this signal associated with an autism diagnosis, contributes to heightened anxiety symptomatology. The coupling of these vulnerabilities in autistic individuals may, in part, contribute to the high prevalence of anxiety often observed in this population.

Thus far, my findings discussed show subtle evidence of altered interocept-

ive processing in autistic adults yet also show autistic adults do not differ from neurotypical individuals on a number of metrics. However, I will now discuss findings that demonstrate clear group differences in the neural processing of interoceptive information. There is a plethora of work linking interoceptive ability to the recruitment of insula and cingulate cortices (Craig, 2002, 2008; Critchley et al., 2004) yet current work in autism actually suggests that insula response does not differ from neurotypical controls (Failla et al., 2020). In chapter 4, we did actually replicate this finding by showing similar activation patterns, of bilateral insula and cingulate cortices, across autistic and neurotypical participants, when attending to one's own heart. We also demonstrate autistic and neurotypical individuals both recruit insula cortices and amygdala during the processing of emotional faces time-locked to the cardiac cycle (chapter 3). However, group differences start to emerge in the domain of functional connectivity, with more targeted measures examining the influence of phasic cardiac signals (i.e. systole versus diastole).

In the heartbeat discrimination paradigm, stimuli are timed to occur at cardiac systole (roughly 250ms after R-wave), when baroreceptors fire (to stabilise blood pressure) or during diastole (roughly around R-wave), based on the assumption that people tend to perceive stimuli as in time with their heartbeat during cardiac systole, although individual variation may exist (Brener & Ring, 2016). As well as being the period of peak differentiation, systolic signalling has also been linked to the inhibition of sensory processing (Edwards et al., 2002; Gray et al., 2009; McIntyre et al., 2008; Schulz et al., 2009), memory (Garfinkel et al., 2013) and visual search (Galvez-Pol et al., 2020) whilst systolic enhancement of emotion processing (Garfinkel et al., 2014), racial bias (Azevedo et al., 2017), facial recognition (Fiacconi et al., 2016) and motor reactivity (Makowski et al., 2020) have also been observed. In brain, in neurotypical individuals, interoceptive regions, namely insula, amygdala and cingulate cortices, show greater activation during cardiac signalling (Garfinkel et al., 2014). Given that baroreceptor activity clearly represents an important process contributing to interoceptive, behavioural and emotional processes (Mulcahy, Larsson, et al., 2019), it is important that no work has yet investigated how this channel functions in autistic, relative to neurotypical, individuals.

In chapter 3, I employed a cardiac contingent fear processing paradigm and found significant group differences between autistic and neurotypicals across systolic and diastolic cardiac phases. Despite showing activation of insula and amygdala in both groups when processing fear faces, independent of the emotional content of the stimuli and of the cardiac phase, autistic participants showed reduced activation of insula and cingulate cortices, suggesting an altered neural response when processing face stimuli in autistic individuals. Conversely, this finding was not replicated in chapter 4, where I found no group differences in brain activation overall (i.e. regardless of the task condition), suggesting a similar neural response between autistic and neurotypical participants during interoceptive/exteroceptive attention. Additionally, in chapter 4, I found no group difference in brain activation when participants attended to interoceptive versus exteroceptive stimuli, with both groups showing activation of insula and cingulate cortices. However, returning to chapter 3, I found autistic participants had reduced activation of right insula and cingulate cortices during cardiac systole and cardiac diastole, regardless of the emotional content of the stimuli. Additionally, functional connectivity of right insula was significantly reduced in autistic participants during cardiac systole thus demonstrating altered processing of interoceptive signals in this population.

The finding of reduced functional connectivity of right insula in autistic adults during cardiac systole is critical and one of the key take-home messages of this thesis. Autism has been proposed as a 'disorder' of connectivity (Belmonte et al., 2004) and indeed numerous studies have reported reduced activation and functional connectivity of insula cortices in autism (Ebisch et al., 2011; Francis et al., 2019; Odriozola et al., 2016). One study argues that functional connectivity of anterior insula and anterior cingulate cortex may represent the neural marker most predictive of an autism diagnosis (Barttfeld et al., 2012). This is the first study to show reduced functional connectivity of right insula cortex during systolic processing in autism and this finding has important clinical implications. Namely, I propose that this result provides insight into a dysregulated interoceptive mechanism that has important implications for anxiety symptomatology in autistic adults. In chapter 3, I show that fear processing is enhanced in both autistic and neurotypical individuals at cardiac systole with increasing levels of anxiety, yet

autistic individuals have significantly increased levels of anxiety compared to neurotypicals. Our neuroimaging findings implicate insula cortices in the processing of threatening information and show group differences in systolic signalling involving insula cortex activation and functional connectivity. Thus, the insula cortex is involved in integrating interoceptive signals to inform emotional experience and, despite no group differences at the behavioural level, shows reduced activation and connectivity in autism which may increase vulnerability to anxiety.

In chapter 4, I also showed altered connectivity of bilateral insula that can differentiate autistic from neurotypical individuals, but only in relation to interoceptive accuracy on the heartbeat tracking task and interoceptive insight on the heartbeat discrimination task. Both findings show reduced functional connectivity in autistic adults. The finding of reduced connectivity correlated with interoceptive accuracy likely represents a neural marker underlying atypical conscious processing of interoceptive signals (Critchley et al., 2004), although not present at the behavioural level in this study, but reported elsewhere (Garfinkel, Tiley, et al., 2016; Mul et al., 2018; Palser et al., 2018). I speculatively propose that the distinction between interoceptive insight between groups, i.e. increased functional connectivity of left insula correlated with heartbeat discrimination insight in neurotypical participants only, and thus reduced functional connectivity in autistic adults, may represent a neural marker underscoring the observation in chapter 2 that related reduced interoceptive insight with reduced affective prosody recognition in autistic adults (Mulcahy, Davies, Quadt, Critchley, Garfinkel, 2019). However, future empirical work is needed to clarify this, for example by employing the affective prosody paradigm and interoceptive tasks during fMRI in autistic and neurotypical participants.

Thus, in sum, I have presented novel work to show that in some interoceptive domains autistic individuals do not markedly differ from neurotypical individuals. However, with more targeted analyses examining multiple dimensions of interoception, and with the examination of functional connectivity, significant differences begin to emerge. I have shown that a) autistic adults do not necessarily differ at the group level in their ability to consciously perceive interoceptive signals or in their ability to appraise their interoceptive ability yet b) autistic adults report a hyper sensitivity to interoceptive sensations, coupled with a blunted utilisation

of such sensations, and c) autistics adults show reduced activation and functional connectivity of primary interoceptive regions, namely insula cortices, during cardiac systole and, finally, d) an altered functional connectivity profile of insula cortices that is directly coupled to distinct facets of interoception. This work has important clinical implication for the contribution of interoceptive signals to emotional and social processes in autism.

7.2.2 Cardiac interoceptive signals influence emotional experience in autistic adults

The notion that interoceptive signals influence emotional experience has long been recognised (Lange et al., 1967) and, indeed, people typically ascribe certain bodily feelings when describing their emotional experience (Nummenmaa, Glerean, Hari, & Hietanen, 2014). In the study of interoception in emotion, cardiac signals have dominated the field, due to their quantifiable phasic nature, where arterial baroreceptors signal the timing and strength of heartbeats, via vagus and glossopharyngeal nerves to brainstem, to stabilize blood pressure. In states of heightened arousal (e.g. emotional stress), the baroreflex is suppressed to allow the simultaneous rise of heartrate and blood pressure (Critchley & Garfinkel, 2017). In this regard, as I have already discussed, work can impede on this mechanism by timing stimuli to occur during baroreceptor activity to examine the impact of interoceptive signals on behaviour and emotion. As interoceptive signals span a continuum however, ranging from the aforementioned higher level measurements of the afferent signals, through objective, subjective and metacognitive indices (Quadt et al., 2018), subtle differences on emotional experience are present at each level of the hierarchy.

Individuals who score high in interoceptive accuracy may report increased intensity of emotional feelings (Barrett et al., 2004; Pollatos et al., 2007; Wiens et al., 2000), have better affective regulation (Füstös et al., 2013) and are better able to verbalize emotions (Bornemann & Singer, 2017). Similarly, individuals who report greater objective accuracy and subjective sensitivity (interoceptive sensibility) to internal bodily sensations often report greater anxiety symptomatology (Anderson & Hope, 2009; Dunn et al., 2010; Gregor & Zvolensky, 2008; Olatunji et al., 2007; Pollatos et al., 2007; Stevens et al., 2011), although results

are not always consistent (De Pascalis et al., 1984; Ehlers et al., 1988). In the domain of interoceptive insight, i.e. interoceptive metacognition; how confidence predicts accuracy (Garfinkel et al., 2015), there is scarcely little work available, although emerging work is starting to implicate insight in emotional processing (Canales-Johnson et al., 2015; Ewing et al., 2017; Khalsa et al., 2008) and indeed the notion that the correspondence between actual and expected bodily states influences emotional experience has long been recognised (Paulus & Stein, 2006, 2010).

In autism, despite some work showing group differences in interoceptive ability (Elwin et al., 2012; Fiene & Brownlow, 2015; Garfinkel, Tiley, et al., 2016; Mul et al., 2018; Palser et al., 2018), little work has investigated the impact of interoceptive signals on emotional experience, with some noteworthy exceptions (Garfinkel, Tiley, et al., 2016; Palser et al., 2018). There is evidence of how interoceptive signals influence emotion in the alexithymia work (described as a difficulty identifying and describing feelings) where some argue that the differences observed in objective accuracy are explained by alexithymia and not autism (Gaigg, Cornell, & Bird, 2018; Nicholson et al., 2018; Shah et al., 2016) whilst others report group differences in accuracy, independent of alexithymia (Mul et al., 2018). The work in this thesis thus provides a step forward in understanding how interoceptive signals, across dimensions, influence emotional experience in autistic adults.

In chapter 6, I employed a novel affective prosody recognition paradigm and found autistic, relative to neurotypical, adults were significantly impaired at recognizing emotions from voices. Interestingly, in autistic adults, this ability was directly linked to interoceptive insight such that greater insight was associated with improved affective prosody recognition. This relationship was independent from interoceptive accuracy or sensibility, which show no relationship with affective prosody recognition. Such a finding is consistent with the work that suggests understanding your own emotional state may be crucial for the understanding of others (Singer et al., 2009), as well as when placed in a predictive coding framework (Ondobaka, Kilner, & Friston, 2017). Neuroimaging work also supports this notion as activation in insula cortex is observed both when experiencing and when viewing others emotions (Wicker et al., 2003). There is also evidence of somatosensory cortex recruitment during emotion perception (Nummenmaa, Hirvonen,

Parkkola, & Hietanen, 2008) which, when damaged, results in impaired emotion recognition of others (Pourtois et al., 2004). Such work is also consistent with my tentative hypothesis that the relationship between interoceptive insight and functional connectivity of left insula, as seen in chapter 4, may represent an altered neural system contributing to deficits in affective prosody recognition. In chapter 4, I also observed a relationship between objective interoceptive (discrimination) accuracy and trait anxiety that was unique to the autistic group. Whilst there is evidence linking the perception of heart signals to anxiety (e.g. Anderson & Hope, 2009; Dunn et al., 2010; Pollatos et al., 2007) this is the first work to show such an effect in autistic adults, an interesting extension of other work that links the mismatch between accuracy and self-reported sensitivity to anxiety (Garfinkel, Tiley, et al., 2016; Palser et al., 2018).

As well as showing a relationship between objectively perceived interoceptive signals and emotion in chapters 2 and 4, I also showed that unconscious cardiac signals can influence emotional experience. Specifically, in autistic and neurotypical participants, we observed a significant inhibitory effect of neutral faces that were presented during cardiac systole whilst no such effect was observed for fear faces suggesting a subtle break-through of inhibitory processing for fear faces, consistent with previous work (Garfinkel et al., 2014), but extended into autistic adults. We also found a relationship with affective symptomatology; individuals who scored high in trait anxiety, after controlling for depression, provided greater intensity ratings at cardiac systole. We also observed recruitment of regions known to be involved in emotion and interoceptive processes, including amygdala and insula cortices, across all participants. Thus, in this novel work, I have replicated previous work linking phasic cardiac signals to emotional experience in neurotypicals (e.g. Azevedo et al., 2017; Garfinkel et al., 2014; Gray et al., 2012) and extended this to show that such signals also influence emotional experience in autistic adults. As previously mentioned, I observed no group differences in the processing of emotional information but showed subtle under-connectivity of right insula during cardiac systole which may contribute to the higher levels of emotion difficulties, namely anxiety, observed in autism.

In the domain of interoceptive sensibility, throughout this thesis, I have found subtle relationships to suggest subjective belief regarding interoceptive sensitiv-

ity, using the BPQ, is related to distinct aspects of emotional experience. Across chapters 2 and 4, we observed a correlation between the BPQ and trait anxiety, consistent with the notion that greater perception of interoceptive signals may heighten susceptibility to anxiety (Anderson & Hope, 2009; Dunn et al., 2010; Gregor & Zvolensky, 2008; Olatunji et al., 2007; Pollatos et al., 2007; Stevens et al., 2011). In chapter 4, BPQ scores also correlated with depression in autistic participants, above neurotypical participants, again consistent with the work linking internal physiology with affective symptomatology, but extending to autistic adults. Likewise, in chapter 3, activation in cuneus, occipital gyrus and cerebellum during fear processing at systole correlated with depressive symptoms across all participants providing the first evidence of a relationship between comorbid depression and fear processing as a function of systolic signaling in autism. Whilst we showed no relationship between activation, with attention toward the heart, and anxiety or depression in chapter 4, we did show functional connectivity of right insula with cerebellum correlated with trait anxiety in all participants. Thus, it would appear that the cerebellum, be it through activation or functional connectivity, is involved in emotional experience, influencing both anxiety and depressive symptoms. This observation was not however unique to autism but was consistent across all participants and is an interesting extension of the work implicating cerebellar involvement in autonomic and emotional control (Barrett, 2017; Schutter & Van Honk, 2005).

We cannot discuss the contribution of interoception to emotional experience in autistic adults without addressing the contribution of alexithymia. Alexithymia, described as a difficulty in identifying and describing one's emotions (Apfel & Sifneos, 1979), is highly comorbid in autism, at roughly between 40% and 65% (Bird & Cook, 2013; Griffin, Lombardo, & Auyeung, 2016; Hill et al., 2004), and work argues that interoceptive impairments are a product of alexithymia not autism (Bird & Cook, 2013; Gaigg et al., 2018; Shah et al., 2016), although evidence is mixed with some showing group differences in interoceptive ability, independent of alexithymia (Mul et al., 2018), and others who show no relationship at all (Nicholson et al., 2018). In this thesis, in chapter 2 and 4, I have shown that alexithymia is related to autism, both in the correlation between autistic and alexithymia traits and in the analysis of functional connectivity showing greater functional

connectivity of right insula with increasing alexithymia scores in autistic participants only. Additionally, in chapter 4, alexithymic scores were greater in autistic compared to neurotypical individuals. However, alexithymia was not associated with interoceptive dimensions, apart from the positive correlation between BPQ scores and alexithymia traits seen in all participants (i.e. not specific to autism) and also did not impact affective prosody recognition ability, as noted in chapter 2. Interestingly, interoceptive training significantly reduced alexithymic traits, a change that was accompanied by altered functional connectivity of left insula, yet this reduction was not associated with change in interoceptive ability. I suggest the reduction in alexithymia may in fact result from the now increased sensitivity to emotions which can thus operate independent from measured interoceptive ability. The extent to which emotion sensitivity has been increased however remains an avenue for future work. Thus, overall I show alexithymia is related to autism yet did not impact interoceptive ability in our sample. Extending this notion further, it is possible that the high concordance between alexithymia and autism typically observed may suggest that the two are not distinct, i.e. alexithymia may be, based on our current understanding, a term used to describe emotion difficulties that actually represent core features of autism. Indeed, the current tools used to identify and characterize alexithymia remain inadequate (Kooiman, Spinhoven, & Trijsburg, 2002) and we do not yet know if, for example, sub-syndromes of alexithymia exist and how this presents in autistic adults or if alexithymia is a cause or consequence of autistic behaviour (Poquérusse, Pastore, Dellantonio, & Esposito, 2018).

In sum, I have shown that cardiac interoceptive signals, across conscious and unconscious levels, selectively influence emotional experience in autistic adults, perhaps causally influencing the development and maintenance of co-morbid affective symptomatology. I have shown that a) the ability to identify emotions from voices is directly related to interoceptive insight, i.e. the ability to reflect on objective performance, b) the objective perception of heartbeats and the subjective belief regarding interoceptive sensitivity may contribute to anxiety, c) unconscious cardiac afferent signals influence the perception of fear and neutral faces and, finally, d) I have identified a set of neural signatures responsible for the processing of interoceptive and emotional processes that has implications for common

co-morbid affective symptomatology in autism.

7.2.3 Interoceptive and exteroceptive training paradigms increase emotion sensitivity in autistic adults

In this thesis, I have presented evidence from two novel training paradigms. The first, an interoceptive training paradigm that targeted heartbeat perception and, the second, an exteroceptive training paradigm that targeted affective prosody recognition in autistic adults. Both have validity in aiming to reduce symptomatology thought to be associated with autism, namely interoceptive differences (Garfinkel, Tiley, et al., 2016; Mul et al., 2018; Palser et al., 2018), which have implications for emotional experience and affective disorders (Garfinkel, Tiley, et al., 2016), and deficits in social communication and interaction that may be exacerbated by deficits in the recognition of affective prosody.

Based on the work by (Garfinkel, Tiley, et al., 2016), which has recently been replicated in children (Palser et al., 2018), showing that individuals with a mismatch between interoceptive accuracy and interoceptive sensibility (i.e. performance on heartbeat detection paradigms and subjective belief regarding interoceptive sensitivity), termed the interoceptive trait prediction error (ITPE), have greater levels of anxiety, the aim of chapter 4, study 2, was to employ a novel interoceptive training paradigm to better align interoceptive dimensions and thus reduce anxiety. This is the first work to apply targeted interoceptive training in such a manner to reduced anxiety in autistic adults, however previous work has used interoceptive components as a form of therapy, for example using mindfulness with a body scan component (Farb et al., 2013; Serpa et al., 2014; Spek et al., 2013) or by targeting breath control (Holtz et al., 2019), whilst others have focused on improving distinct aspects of interoception, namely accuracy, through the use of feedback (Ainley et al., 2013, 2012; Canales-Johnson et al., 2015; Schaefer et al., 2014) and exercise (Kirk et al., 2011; Montgomery et al., 1984).

Interestingly, I found no evidence to suggest an altered ITPE profile in autistic adults at baseline (i.e. no group differences) and, in my sample, before training, no unique relationship between the ITPE, on the tracking or discrimination tasks, and anxiety in autistic individuals was found. Indeed, effects emerged in the neurotypical group only, where the heartbeat tracking ITPE was associated with

trait anxiety, and in both groups combined (autistic and neurotypical), where the heartbeat tracking ITPE was the only significant predictor of trait anxiety, independent of group status. Similarly, despite a significant reduction in discrimination ITPE, we observed no change in anxiety or depressive symptomatology following interoceptive training. Of note however is the limited sample used in this analyses, and the Bayesian statistics that suggest insufficient evidence to draw firm conclusions from this data. Indeed, the full clinical trial was run on a larger sample, of which only a subset performed the neuroimaging tasks. Nonetheless, based on this data, the interoceptive training paradigm employed was not effective at reducing anxiety, however, as reported in chapter 4, in the full sample of autistic adults (n=46) a significant reduction in trait anxiety pre versus post interoceptive training was observed. One interesting point to note is our finding from chapter 4 linking objective perception of heartbeat to trait anxiety which showed increased heartbeat perception was associated with increased anxiety in autistic adults. Based on this finding, it seems counter-intuitive to then train autistic individuals to better perceive their heartbeat, indeed accuracy was increased across both tracking and discrimination tasks, however, we found no evidence linking change in interoceptive performance to change in anxiety symptomatology. Thus interoceptive training, at this level, did not increase vulnerability to anxiety and, as is clear in the whole sample (reported elsewhere), the opposite was true and interoceptive training reduced anxiety. I propose that for some, although speculative, interoceptive training may have 'normalised' heartbeat perception through repeated exposure with no aversive consequences as well as improve concordance between objective performance and subjective belief. We were however likely under-powered to display the result observed in the full sample with the limited sample utilised in this thesis.

Whilst I showed no effect of interoceptive training on anxiety in my limited sample, Interoceptive training did subtly impact emotional processing in other ways. In chapter 5, employing a cardiac-contingent fear-faces paradigm pre versus post interoceptive training, we found evidence to suggest interoceptive training increased intensity ratings of fear and neutral faces, independent of cardiac phase. This finding is in line with the work implicating increased interoceptive accuracy, as was seen in this sample following training, with increased emotional sensitiv-

ity (Barrett et al., 2004; Pollatos et al., 2007; Wiens et al., 2000). However, at the behavioural level, I found no evidence linking improvement in interoceptive accuracy, or change in trait prediction error, to performance on the 'fearfaces' paradigm, pre versus post training. Despite no reduction in anxiety, I did observe an interesting relationship with change in anxiety scores, reflecting the importance of considering individual differences. Individuals who were the most susceptible to interoceptive training, indexed through reduced anxiety, showed increased intensity ratings towards face stimuli, whereas individuals for whom interoceptive training increased anxiety, showed a pattern of reduced intensity ratings towards face stimuli. I propose that this finding reflects the impact of interoceptive signals whereby some individuals, following targeted training, now better utilise interoceptive signals in the processing of emotional information (i.e. reduced anxiety and increased emotion sensitivity) whilst others may have a maladaptive response, created by a noisy interoceptive channel, which thus increases anxiety and reduces emotion sensitivity. Such a hypothesis is however speculative and, given the noted observation showing no relationship between change in interoceptive performance and change in anxiety, following interoceptive training, should be interpreted with caution.

In brain, through targeted analyses, my findings also support the notion that interoceptive training influences emotional experience. Across chapters 4 and 5, we found no evidence to suggest altered brain activation when attending to your heart or when processing fearful faces, following interoceptive training. However, functional connectivity of left insula with temporal regions, during attention toward the heart, and functional connectivity of right amygdala with precentral gyrus and left insula with vermis, during fear face processing, was significantly increased following interoceptive training. Similarly, changes in behavioural tests of interoception, namely accuracy, were directly coupled to changes in functional connectivity of right and left insula with cingulate and frontal cortex during heartbeat attention. Similarly, in chapter 5, increases in interoceptive accuracy, as a result of interoceptive training, were directly coupled to activation changes, of parahippocampal gyrus and hippocampus, and connectivity changes of amygdala and insula cortices during fear processing post training, compared to pre training. Additionally, increase in anxiety following interoceptive training also correlated

with increases in functional connectivity of amygdala and insula cortices, with regions involved in emotion and autonomic control, post versus pre interoceptive training, when processing fear faces relative to neutral faces and when processing all faces during cardiac systole, suggesting a hyper-connectivity anxiety system. The latter finding highlights the need to tailor interoceptive training paradigms to suit individual needs to prevent maladaptive outcomes, i.e. increased anxiety with accompanying hyperactive neural changes which may facilitate anxiety symptomatology. However the finding of no relationship between change in interoceptive accuracy and anxiety in my limited sample, suggests interoceptive training did not impact anxiety and thus anxiety may have increased independent of training, i.e. due to other factors not measured here. Nonetheless, we still show direct coupling between change in anxiety and interoceptive training change in functional connectivity of regions known to be recruited in emotional processes, namely insula, amygdala and cingulate cortices (Craig, 2002; Critchley et al., 2004; Garfinkel et al., 2014). I speculatively propose that such neural changes will increase emotion sensitivity in autistic adults in other domains, an avenue that deserves more investigation (see section 7.4 for future recommendations).

Whilst I only observed subtle behavioural changes in emotional sensitivity following interoceptive training despite more profound neural changes, in chapter 6 I demonstrated strong behavioural changes following training in affective prosody recognition. Affective prosody, referring to the emotional intonation of speech, is fundamental for smooth social interaction (Wang & Tsao, 2015) and thus represents a targetable component where autistic adults may face difficulties, as seen in chapter 2 and in other work (Golan et al., 2006; Lindner & Rosén, 2006; Peppé et al., 2007; Rosenblau et al., 2017). Following a novel affective prosody recognition training paradigm, employed in chapter 6, autistic adults significantly improved in their ability to recognise and identify emotional voices. Importantly, improvement was seen across trials and across emotion (valence and complexity) categories. Interestingly, change in prosody accuracy did not alter interoceptive dimensions, despite our work showing attenuated prosodic accuracy with reduced interoceptive insight. We did, however, observe a subtle drop in trait anxiety suggesting prosody training may reduce anxiety, likely in the domain of social functioning. I do however speculatively propose that the STAI may not be specific enough to

measure social anxiety, which I propose would be the most affected by prosody training, and thus employing such a tool would reveal more prominent effects. The extent to which this will impact real-life social interaction also remains to be seen, however, the results of chapter 6 lend support to the notion that affective prosody recognition training improves recognition ability, thus increasing sensitivity to emotional stimuli from the intonation of speech, which will subsequently improve social functioning in autistic adults.

In sum, I have shown how interoceptive and exteroceptive (affective prosody recognition) processing can enhance emotion sensitivity in autistic adults. Targeted interoceptive training can a) improve interoceptive ability and b) subtly increase sensitivity towards emotional stimuli with accompanying changes in emotional neural networks, whilst targeted prosody training can c) improve the detection and recognition of emotional voices, suggesting that both interoceptive and prosodic training paradigms d) have important implications for social and emotional functioning in autistic adults.

7.3 Limitations of this thesis

This thesis comprised 5 experimental chapters and all have some noteworthy limitations that I will now discuss. Namely, I will consider the criticism relating to the methodology used to assess cardiac interoceptive ability and I will discuss the issues with using self-report measures in experimental research, and note limitations of the specific questionnaires employed throughout this thesis. Finally I will discuss certain limitations related to the study sample and the study design that may limit the interpretation of my findings.

7.3.1 The problem with the heartbeat tracking and discrimination tasks

The heartbeat discrimination (Katkin et al., 1983; Whitehead et al., 1977) and heartbeat tracking (Schandry, 1981) tasks are two of the most commonly employed heartbeat detection paradigms in the literature and were thus employed here. The use of these two tasks is common because they are non-invasive, compared to a gastric balloon for example, they are relatively straight forward to employ, they

are easy to explain and can thus be understood by diverse populations, they require little equipment and they rely on heartbeat detection; an interoceptive signal that is discrete, continuous, easily manipulated and most people can, to some extent, perceive their heartbeat. Despite this, there are some noteworthy limitations of both tasks that require discussion.

Firstly, the heartbeat tracking task is arguably influenced by participant prior knowledge and belief about their heartbeat (Murphy, Millgate, et al., 2018; Ring & Brener, 1996; Ring et al., 2015). In support of this, the manipulation of pacemakers to increase/decrease heart rate does not alter the number of heartbeats participants report (Windmann, Schonecke, Fröhlig, & Maldener, 1999). Thus, arguably, the heartbeat tracking task does not reply on purely interoceptive processes for accurate performance. Indeed, in chapter 4, we showed no group differences in time estimation between autistic and neurotypical participants yet heartbeat tracking accuracy significantly positively correlated with time estimation ability in all participants and in each group individually suggesting a potential contribution of time estimation toward interoceptive tracking accuracy. Therefore, at the behavioural level, in my sample, I cannot exclude the possibility that interoceptive accuracy was influenced by time estimation ability, although if this were the case, this was not a feature distinct to autism and affected all participants.

Further criticisms of the heartbeat tracking task have been the recent topic of much debate in the literature. Namely the claim that tracking accuracy largely represents under-reporting of heartbeats, the notion that reported and actual heartbeats should correlate, which is not always observed, the finding that accuracy may be negatively correlated with heartrate and, finally, the tendency for reduced accuracy over longer time windows (Zamariola et al., 2018), although such a relationship is not always observed (Ainley et al., 2020). In response to such claims, arguably, under-reporting of heartbeats is more reasonable than over-reporting (i.e. illusionary) heartbeats and if instructions are clear, i.e. they do not pose the task as a cognitive phenomenon, such as guessing, such effects should still reflect interoceptive processes (Ainley et al., 2020). Additionally, the relationship with heart rate should, arguably, be expected as one would expect a relationship between the organ system being studied and performance on the

task, i.e. increasing task validity (Ainley et al., 2020). Such a relationship may be altered by task design, e.g. completing the task in supine position, which reduces heart rate and may alter interoceptive accuracy (Zamariola et al., 2018). Thus, consistent measurement procedures, as was the case in my thesis where all participants completed heartbeat tracking sitting upright, are needed. Such points however remain a topic of much debate and thus deserve consideration (Ainley et al., 2020; Desmedt et al., 2020; Corneille et al., 2020; Zimprich et al., 2020). As a final note, in this thesis, the formula used to calculate interoceptive accuracy was independent of the amount of heartbeats in the trial by normalising the absolute error in perceived heartbeats as a function of the overall number of heartbeats (Garfinkel, Tiley, et al., 2016; Garfinkel et al., 2015; Hart, McGowan, Minati, & Critchley, 2013) which thus operated independently of the participants heart rate.

Regarding the heartbeat discrimination task, one of the major criticisms is the assumption that all people perceive their heartbeat at the same point in time. That is, auditory tones defined as synchronous are presented 250ms after R-wave whilst asynchronous tones are presented at or around R-wave, when individual variation may exist (Brener & Ring, 2016), however recent work posits that, on average, most people tend to perceive their heartbeat 250ms from R-wave (Betka et al., 2020) which validates the interoception paradigm employed in this thesis. Both tasks can also be influenced by a number of individual variables, including BMI (Montgomery et al., 1984), heart rate (Knapp-Kline & Kline, 2005), depression, alexithymia and anxiety (Murphy, Millgate, et al., 2018) and age (Murphy, Geary, Millgate, Catmur, & Bird, 2018). I was thus careful to investigate the impact of these variables, including age, heart rate, depression, alexithymia and anxiety, on interoceptive processes, both behaviorally and at the neural level. In between-group analyses, participants where matched on age, gender and education to control for the impact of these potentially confounding variables.

In chapter 4, we have also highlighted that both interoceptive tasks likely tap different underlying processes; namely sustained attention required for heartbeat tracking and interoceptive/exteroceptive integration required for heartbeat discrimination (Hickman et al., 2020). Nonetheless, with the discussed limitations acknowledged, research still argues for their usability due to the consistent association linking performance on both tasks to emotional experience (Critchley

& Garfinkel, 2017) and to distinct neural signatures implicated in interoceptive processes, namely insula cortices (Critchley et al., 2004), through both functional and heartbeat evoked potential approaches (Pollatos & Schandry, 2004).

7.3.2 Self-report measures

Throughout this thesis I have employed a number of self-report measures which each have individual strength and limitations, as well as more broader challenges. Questionnaires in general are subject to social desirable effects, i.e. participants reporting what they think they should rather than their actual feelings, and always suffer from the limitation related to individual variation in reporting. That is, participants interpreting questions to mean different things, which arguably may be considered a strength, or lacking awareness to accurately report on their own characteristics and behaviour. This is particularly important in autistic individuals who may suffer from an altered sense of self (Quattrocki & Friston, 2014). The extent to which people report symptoms is also an important consideration; the threshold at which people perceive and are affected by a symptom may substantial differ from their tendency to report the symptom.

Regarding the specific questionnaires utilized in this thesis; the AQ, that was used as a measure of autistic traits, has been found to be a poor predictor of clinically assessed autism (Ashwood et al., 2016), although this is less an issue in this thesis as the AQ was not used as a proxy for autism; all autistic participants had a confirmed autism spectrum condition diagnosis. The TAS-20, whilst remaining one of the most commonly used tools to asses alexithymia, remains problematic as it requires participants to report on the very condition that they may have difficulty with. That is, the validity of this measure is dependent on the participant having sufficient insight into their difficulties which may be compromised by the condition itself. Indeed, such an issue could be ascribed to all questionnaires measuring affect, including the STAI and PHQ-9. The TAS-20 also arguably measures distress, not alexithymia (Preece et al., 2020), although it still remains the most widely used tool in the literature.

One final consideration relates to the BPQ, which we have already mentioned in chapter 4. This questionnaire arguably confounds sensitivity with attention and thus makes it hard to disentangle the mechanism being studied. Additionally, the

BPQ measures subjective sensitivity/attention to a broad range of interoceptive signals which may make it hard to compare to, for example, objective tasks that tap purely cardiac processes. That does not however retract from its usability as a broad overview of altered subjective sensitivity/attention to bodily signals is useful, as I discuss in section 7.4 below, yet it would also be interesting if domain specific questionnaires (i.e. cardiac sensitivity) may better reflect objective performance. Nonetheless, measuring a participants belief, rather than objective performance, remains an important avenue of research and provide insight into distinct mechanisms. This is practically illustrated in the trait predication error showing a relationship between anxiety and the discrepancy between subjective belief and objective interoceptive performance (Garfinkel, Tiley, et al., 2016; Palser et al., 2018). The optimal approach then is to consider multiple dimensions, as was the case in this thesis, to establish how subjective and objective indices of interoception, as well as affective disorders, operate in autistic individuals.

7.3.3 Study sample

As previously noted, the autistic participants discussed in this thesis were recruited as part of a large study and thus, all participants were subject to repeated testing. In an ideal environment a different group of participants would have been recruited to complete each task described in the separate chapters to improve the generalisability and reliability of the results. However, such a large scale endeavor would be costly and difficult to recruit for and was thus beyond the scope of this thesis. Similarly, the number of individuals who dropped out/did not complete the second scanning session was large (n=14) and thus the participants included in chapter 4, study 2, and chapter 5 are considerable smaller and represent a subset of the sample used in chapters 4, study 1, and chapter 3. Therefore, the sample may have been under-powered to detect a significant effect.

Regarding the neurotypical participants recruited in this thesis, there was some distinction between the samples; the sample in chapter 2 were only included in this analyses whilst the remain chapters incorporated the same sample of neurotypical individuals. This again presents a similar limitation relating to generalizability and reliability of results. Additionally, in chapter 2, the neurotypical participants did not complete the interoception tasks which made it hard to draw conclusions

regarding the autistic specificity of our finding relating interoceptive insight to prosodic accuracy. Similarly, no neurotypical control group completed interoceptive or prosodic training which meant I had no behavioural or neuroimaging data to compare pre versus post scores, between autistic and neurotypical groups. Indeed, it would have been interesting to see, for example, if, following prosody, training autistic individuals performed at a level similar to that of neurotypicals, given our finding in chapter 2 showing reduced performance in autistic participants. Such investigations remain an avenue for future work. There is also evidence that individuals who consent to scanning score significantly lower in measures of trait anxiety (Charpentier et al., 2020) which could limit the generalisability of our fMRI findings. However, such an issue does not appear to be relevant to this thesis, at least for the autistic sample, as autistic participants who consented to scanning did not statistically differ in levels of trait anxiety compared to those in the interoception group who did not undergo scanning as well as when compared to the remaining whole sample.

As a final note, as is the case with most autism research, the findings are limited in that the autistic participants recruited here are more cognitively able than others. Indeed, the prevalence of learning disabilities in autistic individuals is high, at roughly 30-40% (Friedman, 2015). Whilst every attempt was made to be inclusive regarding specific difficulties the autistic participants included in this thesis faced, for example some individuals who were non-verbal took part, in order to be able to complete the tasks employed throughout this thesis, both experimental and self-report, a certain level of cognitive function was required. Thus, participants who applied to take part in the study but had a learning disability did not meet the inclusion criteria. Given the spectral nature of autism, we cannot reliably conclude that interoception is different in autism without work that investigates interoceptive ability in autistic individuals across the spectrum, including those with low cognitive function. Whilst this remains a limitation of this thesis, it also speaks to a larger issue in the autism/interoception literature. The current tasks are not suitable, both through subjective self-report and objective task performance, to investigate interoception reliably in such populations which makes this a pressing issue for future work.

7.3.4 Study design

As well as limitations with the sample used throughout this thesis, there also remains some limitations regarding the study designs. Perhaps the largest limitation that impacts chapters 4, study 2, and chapter 5, relates to the contribution of time as an uncontrolled variable in fMRI scanning. Scanning the same person at two points in time using the same task can produce markedly different fMRI response due to a number of variables that cannot be easily identified and are near impossible to control for. It is thus difficult for me to conclude that the results identified, showing pre versus post differences in brain activation, are not a mere product of time. Indeed, the findings identified are in line with our hypothesis, for example altered connectivity of insula cortices following interoceptive training, which would suggest such findings could be ascribed to training effects, however I cannot be sure. The optimum way to design this study would be to include a control group who completed a different training procedure, for example prosody training, but also completed the same tasks during fMRI pre versus post training. Such a design would allow me to check for a group x time interaction which would mitigate exogenous variables and allow me to conclude with more certainty whether changes in brain activation or connectivity were a direct results of training. Such a design was not however possible, due to funding restrictions, but it remains an important avenue for future work. Indeed our findings provide tentative evidence that interoceptive training does alter neural connectivity and it would be clinically and academically useful to verify this finding with such a design.

Regarding the interoceptive tasks employed during fMRI, as already noted in chapter 4, due to a programming error I was unable to investigate cardiac contingent heart processing (i.e. systole versus diastole) at the neural level. Given our finding in chapter 3 showing altered activation and connectivity during cardiac systole, this was an important avenue of research that was unfortunately not possible in this thesis. This issue also excluded the use of accuracy measures calculated during fMRI scanning which meant offline interoceptive measures were used which was not ideal. In this analysis I used both heartbeat tracking and discrimination accuracy measures to relate behavioural performance on these tasks to neural activation. However, during fMRI participants were completing the

heartbeat discrimination task which, as previously discussed, requires internal (interoceptive) and external (exteroceptive) integration to complete. Thus, it is likely that distinct neural signatures are activated across the two tasks which means included heartbeat tracking scores as a covariate, as was done in this thesis, may not produce activation/connectivity maps comparable to those that may arise if participants were completing the heartbeat tracking task during fMRI.

Finally, in chapters 4 and 5 I employed a novel interoceptive training paradigm that, despite showing utility in improving interoceptive ability in autistic adults, suffers from some limitations that can be improved in future applications of interoceptive training. Firstly, the time between training session was not stringently controlled which meant significant variation existed in the time to complete training across all participants. Such flexibility was required to achieve the sample size recruited here however future work should look to enforce more stringent training durations. Secondly, as part of training, all participants completed exercise to increase their heart rate, however no specific guidelines regarding exercise type or duration was provided, instead we opted for an exercise type that was preferred by the participants. Whilst individual variation in exercise required to increase heart rate is undoubtedly present, a more robust and replicable training procedure would require stringent exercise criteria.

7.4 Future directions

The work presented in this thesis contributes to our understanding of interoceptive processes in autistic adults and how they may causally impact on emotional experience in this population. In each data chapter of this thesis I have proposed specific avenues for future work, following on from each study, yet two broad themes arise that warrant discussion here. Firstly, a greater understanding of interoceptive processes across the senses (i.e. not just in the cardiac domain) and how these function and impact on emotion and behaviour is warranted. Similarly, this thesis has shown very subtle and specific links between interoception and emotion in autistic adults; there however remains a vast field of emotion difficulties present in autism that may be influenced by interoceptive processes which may causally contribute to the manifestation and maintenance of core autistic symptomatology and associated co-morbid affective symptomatology. Thus, secondly, a

deeper exploration of the relationship between emotion and interception in autism is warranted. I will now discuss my suggestions for avenues of future work.

7.4.1 Cross-modality interoception in autism

The work in this thesis has focused on interoception in the cardiac domain however interoception commonly refers to all signals arising from the internal (visceral) body, for example respiratory and gastric signals. The neural systems responsible for transferring interoceptive signals arguably share neural architecture across modalities, namely in insula cortices (Craig, 2002). However, there appears to be moderate correlations between tests of gastric and cardiac interoception (Herbert et al., 2012; Whitehead & Drescher, 1980) yet cardiac and respiratory interoception show little alignment (Garfinkel, Manassei, et al., 2016; Pollatos, Herbert, Mai, & Kammer, 2016). Thus, it is possible that interoceptive differences in other domains may underscore autistic and co-morbid affective symptomatology yet no work has of yet investigated this. Indeed, in this thesis, I have shown elevated levels of subjective sensitivity to bodily sensations as measured by the BPQ which encompasses questions related to, for example, gastric and respiratory sensations. Other work also suggests reduced thirst and satiety awareness in autistic individuals (Fiene & Brownlow, 2015) and such alterations in homeostatic processes, such as hunger and thirst perception, may have implications in the etiology of eating disorders observed in autism (Keen, 2008). Future work should therefore look to examine the extent to which interoception, if at all, is altered in other domains, including gastric and respiratory, to better understand how such alterations may contribute to core autistic and co-morbid affective symptomatology.

7.4.2 Interoception, emotion and autism

In this thesis, I have developed work investigating interoception, and how interoception contributes to emotional experience, in autism. I have shown that the autistic interoceptive profile can be differentiated from neurotypical individuals and that interoceptive signals differentially contribute to emotional experience in autism, an effect that may be amplified following interoceptive training. Despite this advancement, the relationship between interoception and emotion in autism remains in its infancy and there remains a vast array of research avenues that

can better characterize emotion in autism and how such emotion processing may be impacted by interoceptive signals in autism. For example, future work could employ a paradigm using response latencies as an indirect measure of emotional clarity (Lischetzke, Cuccodoro, Gauger, Todeschini, & Eid, 2005), referring to the extent to which an individual experiences their emotions lucidly (i.e. their ability to subjectively rate how they momentarily feel with faster reaction time (RT) indicating greater emotional clarity). Measuring RT this way provides an inobtrusive measure of accessibility to emotional feeling state which may be coupled to alexithymic or autistic traits. One other possible avenue involves investigating the concordance between autonomic response and subjective emotional ratings (e.g. Silani et al., 2008) which may be altered by interoceptive ability. Such suggestions are exploratory but serve to underscore the breadth of work that is still required to fully understand how interoceptive signals influence emotion in autism.

Similarly, this thesis represents the first example of an interoceptive training paradigm employed in autistic adults aimed at directly affecting emotional experience (anxiety) in this population. Whilst we show no effect on anxiety in this sample, we did show a subtle enhancement of emotional sensitivity in chapter 5 which warrants the replication of such an investigation and the inclusion of a wider range of emotional tasks pre versus post interoceptive training, such as those described above. Indeed, based on the literature linking interoceptive accuracy to emotional experience (Barrett et al., 2004; Pollatos et al., 2007; Wiens et al., 2000) and my finding of increased interoceptive accuracy following training and the subsequent accompanying neural changes, both during fear face processing and heartbeat perception, I hypothesise that emotion sensitivity will be enhanced in other domains. As a final note, as already mentioned in section 7.3.3 above, a pertinent avenue for future work is the extent to which emotional and interoceptive experience is altered in autistic individuals across the spectrum. Given the diversity between autistic symptom presentation, especially in the sensory domain where different autistic individuals can present with both/either hyper and hypo-sensitivity, the extent to which interoceptive signals are altered, and the extent to which they impact emotional experience, is likely different for individuals with different symptom profiles. Such individual-difference investigations will significantly advance our understanding of how interoceptive signals influence

and contribute to emotional experience in autistic individuals.

7.5 Conclusion

It is now widely accepted that the bidirectional signals between the brain and body causally influence emotional experience. In autism, elements of interoceptive signaling are arguably altered which may contribute to the altered emotional experience and the development of comorbid affective symptomatology, namely anxiety, often reported. In this thesis, my results extend previous work to show that the interoceptive profile of autistic adults can be differentiated from neurotypical adults, namely through reduced activation and connectivity of interoceptive regions, primarily insula cortices, in autistic individuals. I also show that interoceptive signals influence emotional experience in autistic adults, through the recognition of emotional voices and the intensity rating of emotional faces. Finally, I show that targeted interoceptive training can improve behavioural interoceptive ability and alter the neural systems responsible for autonomic, interoceptive and emotional processes which has important implications for increasing emotion sensitivity in autistic adults. My work highlights the need for future work to better understand how emotional experience is influenced by interoceptive signals and calls for the implementation of stringent interoceptive training paradigms that may improve interoceptive ability and thus positively improve emotional experience in autistic adults. The need for more inclusive research, with autistic individuals across the spectrum, is paramount to improve our understanding of interoceptive function in autistic adults. Ultimately, a greater understanding of how interoceptive signals influence emotional experience in autism is needed to better inform training paradigms that have the potential to reduce comorbid affective symptomatology and alleviate some of the difficulties, including social-emotional experience, that define the condition.

Bibliography

Adhikari, A., Lerner, T. N., Finkelstein, J., Pak, S., Jennings, J. H., Davidson, T. J., ... others (2015). Basomedial amygdala mediates top-down control of anxiety and fear. *Nature*, *527*(7577), 179–185.

Adolfi, F., Couto, B., Richter, F., Decety, J., Lopez, J., Sigman, M., ... Ibanez, A. (2017). Convergence of interoception, emotion, and social cognition: A twofold fmri meta-analysis and lesion approach. *Cortex*, *88*, 124–142.

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *Journal of cognitive neuroscience*, *13*(2), 232–240.

Adolphs, R., Tranel, D., & Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. *Brain and cognition*, *52*(1), 61–69.

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. R. (1995). Fear and the human amygdala. *Journal of neuroscience*, *15*(9), 5879–5891.

Agam, Y., Joseph, R. M., Barton, J. J., & Manoach, D. S. (2010). Reduced cognitive control of response inhibition by the anterior cingulate cortex in autism spectrum disorders. *Neuroimage*, *52*(1), 336–347.

Ainley, V., Maister, L., Brokfeld, J., Farmer, H., & Tsakiris, M. (2013). More of myself: Manipulating interoceptive awareness by heightened attention to bodily and narrative aspects of the self. *Consciousness and cognition*, *22*(4), 1231–1238.

Ainley, V., Tajadura-Jiménez, A., Fotopoulou, A., & Tsakiris, M. (2012). Looking into myself: Changes in interoceptive sensitivity during mirror self-observation. *Psychophysiology*, *49*(11), 1672–1676.

Ainley, V., Tsakiris, M., Pollatos, O., Schulz, A., & Herbert, B. M. (2020). Comment on "zamariola et al.(2018), interoceptive accuracy scores are problematic: Evidence from simple bivariate correlations"—the empirical data base, the conceptual reasoning and the analysis behind this statement are misconceived and do not support the authors' conclusions. *Biological Psychology*, *152*, 107870.

Akbari, C. C., & Davis, A. H. (2019). Treating expressive affective prosody in autism spectrum disorder. *Communication Disorders Quarterly*, *40*(2), 117–124.

Anderson, E. R., & Hope, D. A. (2009). The relationship among social phobia, objective and perceived physiological reactivity, and anxiety sensitivity in an adolescent population. *Journal of anxiety disorders*, *23*(1), 18–26.

Anteraper, S., Guell, X., D'Mello, A., Joshi, N., Whitfield-Gabrieli, S., & Joshi, G. (2019). Disrupted cerebrocerebellar intrinsic functional connectivity in young adults with high-functioning autism spectrum disorder: a data-driven, whole-brain, high-temporal resolution functional magnetic resonance imaging study. *Brain connectivity*, *9*(1), 48–59.

Antony, M. M., Brown, T. A., Craske, M. G., Barlow, D. H., Mitchell, W. B., & Meadows, E. A. (1995). Accuracy of heartbeat perception in panic disorder, social phobia, and nonanxious subjects. *Journal of Anxiety Disorders*, *9*(5), 355–371.

Apfel, R. J., & Sifneos, P. E. (1979). Alexithymia: Concept and measurement. *Psychotherapy and psychosomatics*, *32*(1-4), 180–190.

Ashwood, K., Gillan, N., Horder, J., Hayward, H., Woodhouse, E., McEwen, F., ... others (2016). Predicting the diagnosis of autism in adults using the autism-spectrum quotient (aq) questionnaire. *Psychological medicine*, *46*(12), 2595–2604.

Azevedo, R. T., Badoud, D., & Tsakiris, M. (2018). Afferent cardiac signals modulate attentional engagement to low spatial frequency fearful faces. *Cortex*, *104*, 232–240.

Azevedo, R. T., Garfinkel, S. N., Critchley, H. D., & Tsakiris, M. (2017). Cardiac afferent activity modulates the expression of racial stereotypes. *Nature Communications*, *8*(1), 1–9.

Babaev, O., Chatain, C. P., & Krueger-Burg, D. (2018). Inhibition in the amygdala anxiety circuitry. *Experimental & molecular medicine*, *50*(4), 1–16.

Bagby, R. M., Parker, J. D., & Taylor, G. J. (1994). The twenty-item toronto alexithymia scale—i. item selection and cross-validation of the factor structure. *Journal of psychosomatic research*, *38*(1), 23–32.

Baker, J., & Kimpinski, K. (2020). Evidence of impaired cerebellar connectivity at rest and during autonomic maneuvers in patients with autonomic failure. *The Cerebellum*, *19*(1), 30–39.

Banker, L., & Tadi, P. (2019). Neuroanatomy, precentral gyrus. In *Statpearls [internet]*. StatPearls Publishing.

Baron-Cohen, S. (1997). *Mindblindness: An essay on autism and theory of mind*. MIT press.

Baron-Cohen, S. (2009). Autism and the empathizing–systemizing (es) theory. In *Developmental social cognitive neuroscience* (pp. 139–152). Psychology Press.

Baron-Cohen, S., Richler, J., Bisarya, D., Gurunathan, N., & Wheelwright, S. (2003). The systemizing quotient: an investigation of adults with asperger syndrome or high–functioning autism, and normal sex differences. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*(1430), 361–374.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The autism-spectrum quotient (aq): Evidence from asperger syndrome/high-functioning autism, malesand females, scientists and mathematicians. *Journal of autism and developmental disorders*, *31*(1), 5–17.

Barrett. (2017). The theory of constructed emotion: an active inference account of interoception and categorization. *Social cognitive and affective neuroscience*, *12*(1), 1–23.

Barrett, L. F., Adolphs, R., Marsella, S., Martinez, A. M., & Pollak, S. D. (2019). Emotional expressions reconsidered: Challenges to inferring emotion from human facial movements. *Psychological science in the public interest*, *20*(1), 1–68.

Barrett, L. F., Quigley, K. S., Bliss-Moreau, E., & Aronson, K. R. (2004). Interoceptive sensitivity and self-reports of emotional experience. *Journal of*

personality and social psychology, *87*(5), 684.

Barrett, L. F., & Simmons, W. K. (2015). Interoceptive predictions in the brain. *Nature reviews neuroscience*, *16*(7), 419–429.

Barsky, A. J., Cleary, P. D., Sarnie, M. K., & Ruskin, J. N. (1994). Panic disorder, palpitations, and the awareness of cardiac activity. *Journal of Nervous and Mental Disease*.

Barttfeld, P., Wicker, B., Cukier, S., Navarta, S., Lew, S., Leiguarda, R., & Sigman, M. (2012). State-dependent changes of connectivity patterns and functional brain network topology in autism spectrum disorder. *Neuropsychologia*, *50*(14), 3653–3662.

Beacher, F. D., Radulescu, E., Minati, L., Baron-Cohen, S., Lombardo, M. V., Lai, M.-C., ... others (2012). Sex differences and autism: brain function during verbal fluency and mental rotation. *PloS one*, *7*(6).

Bechara, A., Tranel, D., Damasio, H., & Damasio, A. R. (1996). Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. *Cerebral cortex*, *6*(2), 215–225.

Becker, J. L., Rogers, E. C., & Burrows, B. (2017). Animal-assisted social skills training for children with autism spectrum disorders. *anthrozoös*, *30*(2), 307–326.

Bellon-Harn, M. L., Harn, W. E., & Watson, G. D. (2007). Targeting prosody in an eight-year-old child with high-functioning autism during an interactive approach to therapy. *Child Language Teaching and Therapy*, *23*(2), 157–179.

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., & Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *Journal of Neuroscience*, *24*(42), 9228–9231.

Betka, S., Łukowska, M., Silva, M., King, J., Garfinkel, S., & Critchley, H. (2020). Feeling the beat: Temporal and spatial perception of heartbeat sensations. *bioRxiv*. Retrieved from `https://www.biorxiv.org/content/early/2020/07/28/2020.07.27.222810` doi:10.1101/2020.07.27.222810

Bird, G., & Cook, R. (2013). Mixed emotions: the contribution of alexithymia to the emotional symptoms of autism. *Translational psychiatry*, *3*(7), e285–e285.

Bird, G., Silani, G., Brindley, R., White, S., Frith, U., & Singer, T. (2010). Empathic brain responses in insula are modulated by levels of alexithymia but not autism. *Brain*, *133*(5), 1515–1525.

Black, M. H., Chen, N. T., Iyer, K. K., Lipp, O. V., Bölte, S., Falkmer, M., ... Girdler, S. (2017). Mechanisms of facial emotion recognition in autism spectrum disorders: Insights from eye tracking and electroencephalography. *Neuroscience & Biobehavioral Reviews*, *80*, 488–515.

Bogaerts, K., Walentynowicz, M., Houte, M. V. D., Constantinou, E., & den Bergh, O. V. (2018). The interoceptive awareness questionnaire (iaq) differentiates between and within patient groups with stress-related bodily complaints versus healthy controls..

Bornemann, B., & Singer, T. (2017). Taking time to feel our body: Steady increases in heartbeat perception accuracy and decreases in alexithymia over 9 months of contemplative mental training. *Psychophysiology*, *54*(3), 469–482.

Brener, J., & Jones, J. M. (1974). Interoceptive discrimination in intact humans:

Detection of cardiac activity. *Physiology & Behavior*, *13*(6), 763–767.

Brener, J., & Kluvitse, C. (1988). Heartbeat detection: judgments of the simultaneity of external stimuli and heartbeats. *Psychophysiology*, *25*(5), 554–561.

Brener, J., & Ring, C. (2016). Towards a psychophysics of interoceptive processes: the measurement of heartbeat detection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1708), 20160015.

Brennand, R., Schepman, A., & Rodway, P. (2011). Vocal emotion perception in pseudo-sentences by secondary-school children with autism spectrum disorder. *Research in Autism Spectrum Disorders*, *5*(4), 1567–1573.

Brooks, S. J., & Stein, D. J. (2015). A systematic review of the neural bases of psychotherapy for anxiety and related disorders. *Dialogues in clinical neuroscience*, *17*(3), 261.

Buchanan, T. W., Lutz, K., Mirzazade, S., Specht, K., Shah, N. J., Zilles, K., & Jäncke, L. (2000). Recognition of emotional prosody and verbal components of spoken language: an fmri study. *Cognitive Brain Research*, *9*(3), 227–238.

Butler, T., Pan, H., Tuescher, O., Engelien, A., Goldstein, M., Epstein, J., ... others (2007). Human fear-related motor neurocircuitry. *Neuroscience*, *150*(1), 1–7.

Cai, R. Y., Richdale, A. L., Dissanayake, C., & Uljarević, M. (2019). Resting heart rate variability, emotion regulation, psychological wellbeing and autism symptomatology in adults with and without autism. *International Journal of Psychophysiology*, *137*, 54–62.

Calder, A. J., Keane, J., Manes, F., Antoun, N., & Young, A. W. (2000). Impaired recognition and experience of disgust following brain injury. *Nature neuroscience*, *3*(11), 1077–1078.

Calhoun, V. D., Wager, T. D., Krishnan, A., Rosch, K. S., Seymour, K. E., Nebel, M. B., ... Kiehl, K. (2017). The impact of t1 versus epi spatial normalization templates for fmri data analyses. *Human brain mapping*, *38*(11), 5331–5342.

Canales-Johnson, A., Silva, C., Huepe, D., Rivera-Rei, Á., Noreika, V., Garcia, M. d. C., ... others (2015). Auditory feedback differentially modulates behavioral and neural markers of objective and subjective performance when tapping to your heartbeat. *Cerebral Cortex*, *25*(11), 4490–4503.

Cannon, W. B. (1914). The interrelations of emotions as suggested by recent physiological researches. *The American Journal of Psychology*, *25*(2), 256–282.

Cannon, W. B. (1927). The james-lange theory of emotions: A critical examination and an alternative theory. *The American journal of psychology*, *39*(1/4), 106–124.

Caria, A., Sitaram, R., Veit, R., Begliomini, C., & Birbaumer, N. (2010). Volitional control of anterior insula activity modulates the response to aversive stimuli. a real-time functional magnetic resonance imaging study. *Biological psychiatry*, *68*(5), 425–432.

Carlisi, C., Reed, K., Helmink, F. G., Lachlan, R., Cosker, D. P., Viding, E., & Mareschal, I. (2020). Using evolutionary algorithms to uncover individual differences in how humans represent facial emotion.

Charpentier, C. J., Faulkner, P., Ly, V., Tollenaar, M. S., Kluen, L. M., Fransen, A., ... others (2020). How representative are neuroimaging samples? large-

scale evidence for trait anxiety differences between mri and behaviour-only research participants.

Cheng, W., Rolls, E. T., Zhang, J., Sheng, W., Ma, L., Wan, L., ... Feng, J. (2017). Functional connectivity decreases in autism in emotion, self, and face circuits identified by knowledge-based enrichment analysis. *NeuroImage*, *148*, 169–178.

Chevallier, C., Noveck, I., Happé, F., & Wilson, D. (2011). What's in a voice? prosody as a test case for the theory of mind account of autism. *Neuropsychologia*, *49*(3), 507–517.

Cibralic, S., Kohlhoff, J., Wallace, N., McMahon, C., & Eapen, V. (2019). A systematic review of emotion regulation in children with autism spectrum disorder. *Research in Autism Spectrum Disorders*, *68*, 101422.

Clark, D. M. (1986). A cognitive approach to panic. *Behaviour research and therapy*, *24*(4), 461–470.

Clemens, W., & MacDonald, D. (1976). Relationship between heart beat discrimination and heart-rate control. In *Psychophysiology* (Vol. 13, pp. 176–176).

Cook, R., Brewer, R., Shah, P., & Bird, G. (2013). Alexithymia, not autism, predicts poor recognition of emotional facial expressions. *Psychological science*, *24*(5), 723–732.

Cooper, E. A., Garlick, J., Featherstone, E., Voon, V., Singer, T., Critchley, H. D., & Harrison, N. A. (2014). You turn me cold: evidence for temperature contagion. *PloS one*, *9*(12).

Corneille, O., Desmedt, O., Zamariola, G., Luminet, O., & Maurage, P. (2020). A heartfelt response to zimprich et al.(2020), and ainley et al.(2020)'s commentaries: Acknowledging issues with the hct would benefit interoception research. *Biological Psychology*, *152*, 107869.

Couto, B., Adolfi, F., Sedeño, L., Salles, A., Canales-Johnson, A., Alvarez-Abut, P., ... others (2015). Disentangling interoception: insights from focal strokes affecting the perception of external and internal milieus. *Frontiers in psychology*, *6*, 503.

Craig, A. (2014). Topographically organized projection to posterior insular cortex from the posterior portion of the ventral medial nucleus in the long-tailed macaque monkey. *Journal of Comparative Neurology*, *522*(1), 36–63.

Craig, A. D. (2002). How do you feel? interoception: the sense of the physiological condition of the body. *Nature reviews neuroscience*, *3*(8), 655–666.

Craig, A. D. (2003). Interoception: the sense of the physiological condition of the body. *Current opinion in neurobiology*, *13*(4), 500–505.

Craig, A. D. (2008). Interoception and emotion: a neuroanatomical perspective. *Handbook of emotions*, *3*(602), 272–88.

Craig, A. D., & Craig, A. (2009). How do you feel–now? the anterior insula and human awareness. *Nature reviews neuroscience*, *10*(1).

Critchley, H., Ewing, D. L., Gould van Praag, C., Habash-Bailey, H., Eccles, J., Meeten, F., & Garfinkel, S. N. (2019). Transdiagnostic expression of interoceptive abnormalities in psychiatric conditions. *Available at SSRN 3487844*.

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *Journal of comparative neurology*, *493*(1), 154–166.

Critchley, H. D., Daly, E. M., Bullmore, E. T., Williams, S. C., Van Amelsvoort, T., Robertson, D. M., ... others (2000). The functional neuroanatomy of

social behaviour: changes in cerebral blood flow when people with autistic disorder process facial expressions. *Brain*, *123*(11), 2203–2212.

Critchley, H. D., & Garfinkel, S. N. (2017). Interoception and emotion. *Current opinion in psychology*, *17*, 7–14.

Critchley, H. D., & Harrison, N. A. (2013). Visceral influences on brain and behavior. *Neuron*, *77*(4), 624–638.

Critchley, H. D., Melmed, R. N., Featherstone, E., Mathias, C. J., & Dolan, R. J. (2002). Volitional control of autonomic arousal: a functional magnetic resonance study. *Neuroimage*, *16*, 909–919.

Critchley, H. D., Rotshtein, P., Nagai, Y., O'Doherty, J., Mathias, C. J., & Dolan, R. J. (2005). Activity in the human brain predicting differential heart rate responses to emotional facial expressions. *Neuroimage*, *24*(3), 751–762.

Critchley, H. D., Wiens, S., Rotshtein, P., Öhman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nature neuroscience*, *7*(2), 189–195.

Da Fonseca, D., Seguier, V., Santos, A., Poinso, F., & Deruelle, C. (2009). Emotion understanding in children with adhd. *Child psychiatry and human development*, *40*(1), 111.

Dalton, K. M., Nacewicz, B. M., Johnstone, T., Schaefer, H. S., Gernsbacher, M. A., Goldsmith, H. H., ... Davidson, R. J. (2005). Gaze fixation and the neural circuitry of face processing in autism. *Nature neuroscience*, *8*(4), 519–526.

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1346), 1413–1420.

Damasio, A. R., Tranel, D., & Damasio, H. (1990). Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *Behavioural brain research*, *41*(2), 81–94.

Dampney, R., Horiuchi, J., Killinger, S., Sheriff, M., Tan, P., & McDowall, L. (2005). Long-term regulation of arterial blood pressure by hypothalamic nuclei: some critical questions. *Clinical and Experimental Pharmacology and Physiology*, *32*(5-6), 419–425.

Dapretto, M., Davies, M. S., Pfeifer, J. H., Scott, A. A., Sigman, M., Bookheimer, S. Y., & Iacoboni, M. (2006). Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. *Nature neuroscience*, *9*(1), 28–30.

Daubenmier, J. J. (2005). The relationship of yoga, body awareness, and body responsiveness to self-objectification and disordered eating. *Psychology of Women Quarterly*, *29*(2), 207–219.

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual review of neuroscience*, *15*(1), 353–375.

De Pascalis, V., Alberti, M. L., & Pandolfo, R. (1984). Anxiety, perception, and control of heart rate. *Perceptual and motor skills*, *59*(1), 203–211.

Desmedt, O., Corneille, O., Luminet, O., Murphy, J., Bird, G., & Maurage, P. (2020). Contribution of time estimation and knowledge to heartbeat counting task performance under original and adapted instructions. *Biological Psychology*, 107904.

Didehbani, N., Allen, T., Kandalaft, M., Krawczyk, D., & Chapman, S. (2016). Virtual reality social cognition training for children with high functioning

autism. *Computers in human behavior*, *62*, 703–711.

Domschke, K., Stevens, S., Pfleiderer, B., & Gerlach, A. L. (2010). Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. *Clinical psychology review*, *30*(1), 1–11.

Duerden, E. G., Taylor, M. J., Soorya, L. V., Wang, T., Fan, J., & Anagnostou, E. (2013). Neural correlates of inhibition of socially relevant stimuli in adults with autism spectrum disorder. *brain research*, *1533*, 80–90.

Dunn, B. D., Dalgleish, T., Ogilvie, A. D., & Lawrence, A. D. (2007). Heartbeat perception in depression. *Behaviour research and therapy*, *45*(8), 1921–1930.

Dunn, B. D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., & Dalgleish, T. (2010). Can you feel the beat? interoceptive awareness is an interactive function of anxiety-and depression-specific symptom dimensions. *Behaviour research and therapy*, *48*(11), 1133–1138.

Duval, E. R., Javanbakht, A., & Liberzon, I. (2015). Neural circuits in anxiety and stress disorders: a focused review. *Therapeutics and clinical risk management*, *11*, 115.

Ebisch, S. J., Gallese, V., Willems, R. M., Mantini, D., Groen, W. B., Romani, G. L., ... Bekkering, H. (2011). Altered intrinsic functional connectivity of anterior and posterior insula regions in high-functioning participants with autism spectrum disorder. *Human brain mapping*, *32*(7), 1013–1028.

Eckman, P. S., & Shean, G. D. (1997). Habituation of cognitive and physiological arousal and social anxiety. *Behaviour Research and Therapy*, *35*(12), 1113–1121.

Edwards, L., Mcintyre, D., Carroll, D., Ring, C., & Martin, U. (2002). The human nociceptive flexion reflex threshold is higher during systole than diastole. *Psychophysiology*, *39*(5), 678–681.

Ehlers, A., Margraf, J., Roth, W. T., Taylor, C. B., & Birbaumer, N. (1988). Anxiety induced by false heart rate feedback in patients with panic disorder. *Behaviour research and therapy*, *26*(1), 1–11.

Eickhoff, S. B., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., & Zilles, K. (2005). A new spm toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage*, *25*(4), 1325–1335.

Ekman, P. (1992). An argument for basic emotions. *Cognition & emotion*, *6*(3-4), 169–200.

Ekman, P., & Friesen, W. V. (1974). Detecting deception from the body or face. *Journal of personality and Social Psychology*, *29*(3), 288.

Elwin, M., Ek, L., Schröder, A., & Kjellin, L. (2012). Autobiographical accounts of sensing in asperger syndrome and high-functioning autism. *Archives of Psychiatric Nursing*, *26*(5), 420–429.

Epstein, L. H., Cinciripini, P. M., McCoy, J. F., & Marshall, W. R. (1977). Heart rate as a discriminative stimulus. *Psychophysiology*, *14*(2), 143–149.

Epstein, L. H., & Stein, D. B. (1974). Feedback-influenced heart rate discrimination. *Journal of Abnormal Psychology*, *83*(5), 585.

Ewing, D. L., Manassei, M., van Praag, C. G., Philippides, A. O., Critchley, H. D., & Garfinkel, S. N. (2017). Sleep and the heart: Interoceptive differences linked to poor experiential sleep quality in anxiety and depression. *Biological psychology*, *127*, 163–172.

Factor, R. S., Ryan, S. M., Farley, J. P., Ollendick, T. H., & Scarpa, A. (2017). Does the presence of anxiety and adhd symptoms add to social impairment in children with autism spectrum disorder? *Journal of Autism and Developmental Disorders*, *47*(4), 1122–1134.

Failla, M. D., Bryant, L. K., Heflin, B. H., Mash, L. E., Schauder, K., Davis, S., ... Cascio, C. J. (2020). Neural correlates of cardiac interoceptive focus across development: Implications for social symptoms in autism spectrum disorder. *Autism Research*.

Faillenot, I., Heckemann, R. A., Frot, M., & Hammers, A. (2017). Macroanatomy and 3d probabilistic atlas of the human insula. *NeuroImage*, *150*, 88–98.

Farb, N. A., Segal, Z. V., & Anderson, A. K. (2013). Mindfulness meditation training alters cortical representations of interoceptive attention. *Social cognitive and affective neuroscience*, *8*(1), 15–26.

Fiacconi, C. M., Peter, E. L., Owais, S., & Köhler, S. (2016). Knowing by heart: Visceral feedback shapes recognition memory judgments. *Journal of Experimental Psychology: General*, *145*(5), 559.

Fiene, L., & Brownlow, C. (2015). Investigating interoception and body awareness in adults with and without autism spectrum disorder. *Autism Research*, *8*(6), 709–716.

Foulkes, L., Bird, G., Gökçen, E., McCrory, E., & Viding, E. (2015). Common and distinct impacts of autistic traits and alexithymia on social reward. *PloS one*, *10*(4), e0121018.

Francis, S. M., Camchong, J., Brickman, L., Goelkel-Garcia, L., Mueller, B. A., Tseng, A., ... Jacob, S. (2019). Hypoconnectivity of insular resting-state networks in adolescents with autism spectrum disorder. *Psychiatry Research: Neuroimaging*, *283*, 104–112.

Friedman, B. H., & Thayer, J. F. (1998). Anxiety and autonomic flexibility: a cardiovascular approach. *Biological psychology*, *47*(3), 243–263.

Friedman, H. S. (2015). *Encyclopedia of mental health*. Academic Press.

Füstös, J., Gramann, K., Herbert, B. M., & Pollatos, O. (2013). On the embodiment of emotion regulation: interoceptive awareness facilitates reappraisal. *Social cognitive and affective neuroscience*, *8*(8), 911–917.

Gahery, Y., & Vigier, D. (1974). Inhibitory effects in the cuneate nucleus produced by vago-aortic afferent fibers. *Brain research*, *75*(2), 241–246.

Gaigg, S. B., Cornell, A. S., & Bird, G. (2018). The psychophysiological mechanisms of alexithymia in autism spectrum disorder. *Autism*, *22*(2), 227–231.

Gallese, V., & Goldman, A. (1998). Mirror neurons and the simulation theory of mind-reading. *Trends in cognitive sciences*, *2*(12), 493–501.

Galvez-Pol, A., McConnell, R., & Kilner, J. M. (2020). Active sampling in visual search is coupled to the cardiac cycle. *Cognition*, *196*, 104149.

Garfinkel, S. N., Barrett, A. B., Minati, L., Dolan, R. J., Seth, A. K., & Critchley, H. D. (2013). What the heart forgets: Cardiac timing influences memory for words and is modulated by metacognition and interoceptive sensitivity. *Psychophysiology*, *50*(6), 505–512.

Garfinkel, S. N., & Critchley, H. D. (2013). Interoception, emotion and brain: new insights link internal physiology to social behaviour. commentary on: "anterior insular cortex mediates bodily sensibility and social anxiety" by terasawa et al.(2012). *Social cognitive and affective neuroscience*, *8*(3), 231–234.

Garfinkel, S. N., & Critchley, H. D. (2016). Threat and the body: how the heart supports fear processing. *Trends in cognitive sciences*, *20*(1), 34–46.

Garfinkel, S. N., Manassei, M. F., Hamilton-Fletcher, G., In den Bosch, Y., Critchley, H. D., & Engels, M. (2016). Interoceptive dimensions across cardiac and respiratory axes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1708), 20160014.

Garfinkel, S. N., Minati, L., Gray, M. A., Seth, A. K., Dolan, R. J., & Critchley, H. D. (2014). Fear from the heart: sensitivity to fear stimuli depends on individual heartbeats. *Journal of Neuroscience*, *34*(19), 6573–6582.

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., & Critchley, H. D. (2015). Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. *Biological psychology*, *104*, 65–74.

Garfinkel, S. N., Tiley, C., O'Keeffe, S., Harrison, N. A., Seth, A. K., & Critchley, H. D. (2016). Discrepancies between dimensions of interoception in autism: Implications for emotion and anxiety. *Biological psychology*, *114*, 117–126.

Gianaros, P. J., Sheu, L. K., Matthews, K. A., Jennings, J. R., Manuck, S. B., & Hariri, A. R. (2008). Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *Journal of Neuroscience*, *28*(4), 990–999.

Gilbert, S. J., Bird, G., Brindley, R., Frith, C. D., & Burgess, P. W. (2008). Atypical recruitment of medial prefrontal cortex in autism spectrum disorders: An fmri study of two executive function tasks. *Neuropsychologia*, *46*(9), 2281–2291.

Gilbert, S. J., Meuwese, J. D., Towgood, K. J., Frith, C. D., & Burgess, P. W. (2009). Abnormal functional specialization within medial prefrontal cortex in high-functioning autism: a multi-voxel similarity analysis. *Brain*, *132*(4), 869–878.

Globerson, E., Amir, N., Kishon-Rabin, L., & Golan, O. (2015). Prosody recognition in adults with high-functioning autism spectrum disorders: From psychoacoustics to cognition. *Autism Research*, *8*(2), 153–163.

Goeleven, E., De Raedt, R., Leyman, L., & Verschuere, B. (2008). The karolinska directed emotional faces: a validation study. *Cognition and emotion*, *22*(6), 1094–1118.

Golan, O., Baron-Cohen, S., & Hill, J. (2006). The cambridge mindreading (cam) face-voice battery: Testing complex emotion recognition in adults with and without asperger syndrome. *Journal of autism and developmental disorders*, *36*(2), 169–183.

Grabauskaitė, A., Baranauskas, M., & Griškova-Bulanova, I. (2017). Interoception and gender: What aspects should we pay attention to? *Consciousness and cognition*, *48*, 129–137.

Gray, M. A., Beacher, F. D., Minati, L., Nagai, Y., Kemp, A. H., Harrison, N. A., & Critchley, H. D. (2012). Emotional appraisal is influenced by cardiac afferent information. *Emotion*, *12*(1), 180.

Gray, M. A., Rylander, K., Harrison, N. A., Wallin, B. G., & Critchley, H. D. (2009). Following one's heart: cardiac rhythms gate central initiation of sympathetic reflexes. *Journal of Neuroscience*, *29*(6), 1817–1825.

Green, D. M., Swets, J. A., et al. (1966). *Signal detection theory and psychophysics* (Vol. 1). Wiley New York.

Gregor, K. L., & Zvolensky, M. J. (2008). Anxiety sensitivity and perceived control

over anxiety-related events: Evaluating the singular and interactive effects in the prediction of anxious and fearful responding to bodily sensations. *Behaviour Research and Therapy*, *46*(9), 1017–1025.

Griffin, C., Lombardo, M. V., & Auyeung, B. (2016). Alexithymia in children with and without autism spectrum disorders. *Autism Research*, *9*(7), 773–780.

Grossman, R. B., Bemis, R. H., Skwerer, D. P., & Tager-Flusberg, H. (2010). Lexical and affective prosody in children with high-functioning autism. *Journal of Speech, Language, and Hearing Research*.

Gu, X., Eilam-Stock, T., Zhou, T., Anagnostou, E., Kolevzon, A., Soorya, L., ... Fan, J. (2015). Autonomic and brain responses associated with empathy deficits in autism spectrum disorder. *Human brain mapping*, *36*(9), 3323–3338.

Gu, X., Hof, P. R., Friston, K. J., & Fan, J. (2013). Anterior insular cortex and emotional awareness. *Journal of Comparative Neurology*, *521*(15), 3371–3388.

Guo, X., Duan, X., Suckling, J., Chen, H., Liao, W., Cui, Q., & Chen, H. (2019). Partially impaired functional connectivity states between right anterior insula and default mode network in autism spectrum disorder. *Human brain mapping*, *40*(4), 1264–1275.

Gupta, M. A. (2013). Review of somatic symptoms in post-traumatic stress disorder. *International review of psychiatry*, *25*(1), 86–99.

Hadjikhani, N., Joseph, R. M., Manoach, D. S., Naik, P., Snyder, J., Dominick, K., ... De Gelder, B. (2009). Body expressions of emotion do not trigger fear contagion in autism spectrum disorder. *Social cognitive and affective neuroscience*, *4*(1), 70–78.

Harms, M. B., Martin, A., & Wallace, G. L. (2010). Facial emotion recognition in autism spectrum disorders: a review of behavioral and neuroimaging studies. *Neuropsychology review*, *20*(3), 290–322.

Harrison, N. A., Gray, M. A., Gianaros, P. J., & Critchley, H. D. (2010). The embodiment of emotional feelings in the brain. *Journal of Neuroscience*, *30*(38), 12878–12884.

Harrison, N. A., Wilson, C. E., & Critchley, H. D. (2007). Processing of observed pupil size modulates perception of sadness and predicts empathy. *Emotion*, *7*(4), 724.

Hart, N., McGowan, J., Minati, L., & Critchley, H. D. (2013). Emotional regulation and bodily sensation: interoceptive awareness is intact in borderline personality disorder. *Journal of personality disorders*, *27*(4), 506–518.

Hatfield, T. R., Brown, R. F., Giummarra, M. J., & Lenggenhager, B. (2019). Autism spectrum disorder and interoception: Abnormalities in global integration? *Autism*, *23*(1), 212–222.

Herbert, B. M., Muth, E. R., Pollatos, O., & Herbert, C. (2012). Interoception across modalities: on the relationship between cardiac awareness and the sensitivity for gastric functions. *PloS one*, *7*(5), e36646.

Herrington, J. D., Maddox, B. B., Kerns, C. M., Rump, K., Worley, J. A., Bush, J. C., ... Miller, J. S. (2017). Amygdala volume differences in autism spectrum disorder are related to anxiety. *Journal of Autism and Developmental Disorders*, *47*(12), 3682–3691.

Hickman, L., Seyedsalehi, A., Cook, J. L., Bird, G., & Murphy, J. (2020). The relationship between heartbeat counting and heartbeat discrimination: a

meta-analysis. *Biological Psychology*, 107949.

Hill, E., Berthoz, S., & Frith, U. (2004). Brief report: Cognitive processing of own emotions in individuals with autistic spectrum disorder and in their relatives. *Journal of autism and developmental disorders*, *34*(2), 229–235.

Hoehn-Saric, R., & McLeod, D. R. (1988). The peripheral sympathetic nervous system: Its role in normal and pathologic anxiety. *Psychiatric Clinics of North America*, *11*(2), 375–386.

Hoehn-Saric, R., McLeod, D. R., Funderburk, F., & Kowalski, P. (2004). Somatic symptoms and physiologic responses in generalized anxiety disorder and panic disorder: An ambulatory monitor study. *Archives of General Psychiatry*, *61*(9), 913–921.

Hogeveen, J., Krug, M. K., Elliott, M. V., & Solomon, M. (2018). Insula-retrosplenial cortex overconnectivity increases internalizing via reduced insight in autism. *Biological psychiatry*, *84*(4), 287–294.

Hollocks, M. J., Lerh, J. W., Magiati, I., Meiser-Stedman, R., & Brugha, T. S. (2019). Anxiety and depression in adults with autism spectrum disorder: a systematic review and meta-analysis. *Psychological medicine*, *49*(4), 559–572.

Holtz, K., Hamm, A. O., & Pané-Farré, C. A. (2019). Repeated interoceptive exposure in individuals with high and low anxiety sensitivity. *Behavior modification*, *43*(4), 467–489.

Hubbard, D. J., Faso, D. J., Assmann, P. F., & Sasson, N. J. (2017). Production and perception of emotional prosody by adults with autism spectrum disorder. *Autism Research*, *10*(12), 1991–2001.

Hubert, B., Wicker, B., Moore, D. G., Monfardini, E., Duverger, H., Da Fonseca, D., & Deruelle, C. (2007). Brief report: recognition of emotional and non-emotional biological motion in individuals with autistic spectrum disorders. *Journal of autism and developmental disorders*, *37*(7), 1386–1392.

Hutchison, A. K. (2015). *Aprosodia therapy: The impact on affective prosody in a child with high functioning autism.* Arkansas State University.

Ibrahim, K., Eilbott, J. A., Ventola, P., He, G., Pelphrey, K. A., McCarthy, G., & Sukhodolsky, D. G. (2019). Reduced amygdala–prefrontal functional connectivity in children with autism spectrum disorder and co-occurring disruptive behavior. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *4*(12), 1031–1041.

Jackson, P. L., Meltzoff, A. N., & Decety, J. (2005). How do we perceive the pain of others? a window into the neural processes involved in empathy. *Neuroimage*, *24*(3), 771–779.

Jakobsen, J. C., Gluud, C., Wetterslev, J., & Winkel, P. (2017). When and how should multiple imputation be used for handling missing data in randomised clinical trials–a practical guide with flowcharts. *BMC medical research methodology*, *17*(1), 162.

James, W. (1884). *What is an emotion? mind, os-ix, 188-205.*

Jeffreys, H. (1998). *The theory of probability.* OUP Oxford.

Junek, W. (2007). Mind reading: The interactive guide to emotions. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *16*(4), 182.

Katkin, E., Reed, S., & Deroo, C. (1983). A methodological analysis of 3 techniques for the assessment of individual-differences in heartbeat detection. In *Psychophysiology* (Vol. 20, pp. 452–452).

Ke, F., & Moon, J. (2018). Virtual collaborative gaming as social skills training for high-functioning autistic children. *British Journal of Educational Technology*, *49*(4), 728–741.

Keen, D. V. (2008). Childhood autism, feeding problems and failure to thrive in early infancy. *European child & adolescent psychiatry*, *17*(4), 209–216.

Keysers, C., Gazzola, V., & Wagenmakers, E.-J. (2020). Using bayes factor hypothesis testing in neuroscience to establish evidence of absence. *Nature Neuroscience*, *23*(7), 788–799.

Khalsa, S. S., Adolphs, R., Cameron, O. G., Critchley, H. D., Davenport, P. W., Feinstein, J. S., ... others (2018). Interoception and mental health: a roadmap. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *3*(6), 501–513.

Khalsa, S. S., Rudrauf, D., Damasio, A. R., Davidson, R. J., Lutz, A., & Tranel, D. (2008). Interoceptive awareness in experienced meditators. *Psychophysiology*, *45*(4), 671–677.

Kirk, U., Downar, J., & Montague, P. R. (2011). Interoception drives increased rational decision-making in meditators playing the ultimatum game. *Frontiers in neuroscience*, *5*, 49.

Kleckner, I. R., Wormwood, J. B., Simmons, W. K., Barrett, L. F., & Quigley, K. S. (2015). Methodological recommendations for a heartbeat detection-based measure of interoceptive sensitivity. *Psychophysiology*, *52*(11), 1432–1440.

Knapp-Kline, K., & Kline, J. P. (2005). Heart rate, heart rate variability, and heartbeat detection with the method of constant stimuli: slow and steady wins the race. *Biological psychology*, *69*(3), 387–396.

Konvalinka, I., Xygalatas, D., Bulbulia, J., Schjødt, U., Jegindø, E.-M., Wallot, S., ... Roepstorff, A. (2011). Synchronized arousal between performers and related spectators in a fire-walking ritual. *Proceedings of the National Academy of Sciences*, *108*(20), 8514–8519.

Kooiman, C., Spinhoven, P., & Trijsburg, R. (2002). The assessment of alexithymia: a critical review of the literature and a psychometric study of the toronto alexithymia scale-20. *Journal of psychosomatic research*, *53*(6), 1083–1090.

Koreki, A., Garfkinel, S. N., Mula, M., Agrawal, N., Cope, S., Eilon, T., ... Yogarajah, M. (2020). Trait and state interoceptive abnormalities are associated with dissociation and seizure frequency in patients with functional seizures. *Epilepsia*, *61*(6), 1156–1165.

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological psychology*, *84*(3), 394–421.

Kroenke, K., Spitzer, R. L., & Williams, J. B. (2001). The phq-9: validity of a brief depression severity measure. *Journal of general internal medicine*, *16*(9), 606–613.

Kujala, T., Lepistö, T., Nieminen-von Wendt, T., Näätänen, P., & Näätänen, R. (2005). Neurophysiological evidence for cortical discrimination impairment of prosody in asperger syndrome. *Neuroscience letters*, *383*(3), 260–265.

Kumar, V. J., van Oort, E., Scheffler, K., Beckmann, C. F., & Grodd, W. (2017). Functional anatomy of the human thalamus at rest. *Neuroimage*, *147*, 678–691.

Kunzendorf, S., Klotzsche, F., Akbal, M., Villringer, A., Ohl, S., & Gaebler,

M. (2019). Active information sampling varies across the cardiac cycle. *Psychophysiology*, *56*(5), e13322.

Kurth, F., Zilles, K., Fox, P. T., Laird, A. R., & Eickhoff, S. B. (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Structure and Function*, *214*(5-6), 519–534.

Lacava, P. G., Golan, O., Baron-Cohen, S., & Smith Myles, B. (2007). Using assistive technology to teach emotion recognition to students with asperger syndrome: A pilot study. *Remedial and Special Education*, *28*(3), 174–181.

Lacava, P. G., Rankin, A., Mahlios, E., Cook, K., & Simpson, R. L. (2010). A single case design evaluation of a software and tutor intervention addressing emotion recognition and social interaction in four boys with asd. *Autism*, *14*(3), 161–178.

Lai, M.-C., Lombardo, M. V., Pasco, G., Ruigrok, A. N., Wheelwright, S. J., Sadek, S. A., ... others (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PloS one*, *6*(6).

Lamm, C., & Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Structure and Function*, *214*(5-6), 579–591.

Lane, R. D., Lee, S., Reidel, R., Weldon, V., Kaszniak, A., & Schwartz, G. E. (1996). Impaired verbal and nonverbal emotion recognition in alexithymia. *Psychosomatic medicine*, *58*(3), 203–210.

Lange, C., James, W., & Dunlap, K. (1967). The emotions (reprinted). *Dunlap Keditor Hafner Publishing Co: New York*.

Lassalle, A., Åsberg Johnels, J., Zürcher, N. R., Hippolyte, L., Billstedt, E., Ward, N., ... Hadjikhani, N. (2017). Hypersensitivity to low intensity fearful faces in autism when fixation is constrained to the eyes. *Human brain mapping*, *38*(12), 5943–5957.

Latinus, M., Cléry, H., Andersson, F., Bonnet-Brilhault, F., Fonlupt, P., & Gomot, M. (2019). Inflexibility in autism spectrum disorder: Need for certainty and atypical emotion processing share the blame. *Brain and cognition*, *136*, 103599.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cellular and molecular neurobiology*, *23*(4-5), 727–738.

Lee, T.-W., Dolan, R. J., & Critchley, H. D. (2008). Controlling emotional expression: behavioral and neural correlates of nonimitative emotional responses. *Cerebral Cortex*, *18*(1), 104–113.

Lenhard, W., & Lenhard, A. (2014). Hypothesis tests for comparing correlations. *Bibergau: Psychometrica. Available online at: https://www. psychometrica. de/correlation. html*.

Lerner, M. D., McPartland, J. C., & Morris, J. P. (2013). Multimodal emotion processing in autism spectrum disorders: an event-related potential study. *Developmental cognitive neuroscience*, *3*, 11–21.

Le Sourn-Bissaoui, S., Aguert, M., Girard, P., Chevreuil, C., & Laval, V. (2013). Emotional speech comprehension in children and adolescents with autism spectrum disorders. *Journal of communication disorders*, *46*(4), 309–320.

Leung, R. C., Pang, E. W., Anagnostou, E., & Taylor, M. J. (2018). Young adults with autism spectrum disorder show early atypical neural activity during emotional face processing. *Frontiers in human neuroscience*, *12*,

57.

Lindner, J. L., & Rosén, L. A. (2006). Decoding of emotion through facial expression, prosody and verbal content in children and adolescents with asperger's syndrome. *Journal of autism and developmental disorders*, *36*(6), 769–777.

Lindquist, K. A., Wager, T. D., Kober, H., Bliss-Moreau, E., & Barrett, L. F. (2012). The brain basis of emotion: a meta-analytic review. *The Behavioral and brain sciences*, *35*(3), 121.

Lischetzke, T., Cuccodoro, G., Gauger, A., Todeschini, L., & Eid, M. (2005). Measuring affective clarity indirectly: individual differences in response latencies of state. *Emotion*, *5*(4), 431.

Liss, M., Mailloux, J., & Erchull, M. J. (2008). The relationships between sensory processing sensitivity, alexithymia, autism, depression, and anxiety. *Personality and individual differences*, *45*(3), 255–259.

Lundqvist, D., Flykt, A., & Öhman, A. (1998). The karolinska directed emotional faces (kdef). *CD ROM from Department of Clinical Neuroscience, Psychology section, Karolinska Institutet*, *91*(630), 2–2.

Lynn, A. C., Padmanabhan, A., Simmonds, D., Foran, W., Hallquist, M. N., Luna, B., & O'Hearn, K. (2018). Functional connectivity differences in autism during face and car recognition: underconnectivity and atypical age-related changes. *Developmental science*, *21*(1), e12508.

Lyyra, P., & Parviainen, T. (2018). Behavioral inhibition underlies the link between interoceptive sensitivity and anxiety-related temperamental traits. *Frontiers in psychology*, *9*, 1026.

Machorrinho, J., Veiga, G., Fernandes, J., Mehling, W., & Marmeleira, J. (2019). Multidimensional assessment of interoceptive awareness: Psychometric properties of the portuguese version. *Perceptual and motor skills*, *126*(1), 87–105.

Makovac, E., Garfinkel, S. N., Bassi, A., Basile, B., Macaluso, E., Cercignani, M., ... others (2015). Effect of parasympathetic stimulation on brain activity during appraisal of fearful expressions. *Neuropsychopharmacology*, *40*(7), 1649–1658.

Makovac, E., Meeten, F., Watson, D. R., Herman, A., Garfinkel, S. N., Critchley, H. D., & Ottaviani, C. (2016). Alterations in amygdala-prefrontal functional connectivity account for excessive worry and autonomic dysregulation in generalized anxiety disorder. *Biological Psychiatry*, *80*(10), 786–795.

Makowski, D., Sperduti, M., Blondé, P., Nicolas, S., & Piolino, P. (2020). The heart of cognitive control: Cardiac phase modulates processing speed and inhibition. *Psychophysiology*, *57*(3), e13490.

Mash, L. E., Schauder, K. B., Cochran, C., Park, S., & Cascio, C. J. (2017). Associations between interoceptive cognition and age in autism spectrum disorder and typical development. *Journal of Cognitive Education and Psychology*, *16*(1), 23–37.

Matsuda, S., & Yamamoto, J. (2013). Intervention for increasing the comprehension of affective prosody in children with autism spectrum disorders. *Research in Autism Spectrum Disorders*, *7*(8), 938–946.

Mazefsky, C. A., Herrington, J., Siegel, M., Scarpa, A., Maddox, B. B., Scahill, L., & White, S. W. (2013). The role of emotion regulation in autism spectrum disorder. *Journal of the American Academy of Child & Adolescent*

Psychiatry, *52*(7), 679–688.

McGillivray, J., & Evert, H. (2018). Exploring the effect of gender and age on stress and emotional distress in adults with autism spectrum disorder. *Focus on Autism and Other Developmental Disabilities*, *33*(1), 55–64.

McIntyre, D., Kavussanu, M., & Ring, C. (2008). Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. *PAIN®*, *137*(3), 550–555.

McLennan, J. D., Lord, C., & Schopler, E. (1993). Sex differences in higher functioning people with autism. *Journal of autism and developmental disorders*, *23*(2), 217–227.

Medford, N., & Critchley, H. D. (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Structure and Function*, *214*(5-6), 535–549.

Meessen, J., Mainz, V., Gauggel, S., Volz-Sidiropoulou, E., Sütterlin, S., & Forkmann, T. (2016). The relationship between interoception and metacognition. *Journal of Psychophysiology*.

Mehling, W. E., Price, C., Daubenmier, J. J., Acree, M., Bartmess, E., & Stewart, A. (2012). The multidimensional assessment of interoceptive awareness (maia). *PloS one*.

Montgomery, W. A., Jones, G. E., & Hollandsworth Jr, J. G. (1984). The effects of physical fitness and exercise on cardiac awareness. *Biological Psychology*, *18*(1), 11–22.

Mottron, L., Dawson, M., Soulieres, I., Hubert, B., & Burack, J. (2006). Enhanced perceptual functioning in autism: an update, and eight principles of autistic perception. *Journal of autism and developmental disorders*, *36*(1), 27–43.

Mul, C.-l., Stagg, S. D., Herbelin, B., & Aspell, J. E. (2018). The feeling of me feeling for you: Interoception, alexithymia and empathy in autism. *Journal of Autism and Developmental Disorders*, *48*(9), 2953–2967.

Mulcahy, J. S., Davies, M., Quadt, L., Critchley, H. D., & Garfinkel, S. N. (2019). Interoceptive awareness mitigates deficits in emotional prosody recognition in autism. *Biological psychology*, *146*, 107711.

Mulcahy, J. S., Larsson, D. E., Garfinkel, S. N., & Critchley, H. D. (2019). Heart rate variability as a biomarker in health and affective disorders: A perspective on neuroimaging studies. *Neuroimage*, *202*, 116072.

Mundy, P. (2003). Annotation: The neural basis of social impairments in autism: The role of the dorsal medial-frontal cortex and anterior cingulate system. *Journal of Child Psychology and psychiatry*, *44*(6), 793–809.

Murphy, J., Brewer, R., Plans, D., Khalsa, S., Catmur, C., & Bird, G. (2018). Validation of the interoceptive accuracy scale (ias) supports distinction between self-reported interoceptive accuracy and awareness. *PsyArXiv Preprints*, *10*.

Murphy, J., Geary, H., Millgate, E., Catmur, C., & Bird, G. (2018). Direct and indirect effects of age on interoceptive accuracy and awareness across the adult lifespan. *Psychonomic Bulletin & Review*, *25*(3), 1193–1202.

Murphy, J., Millgate, E., Geary, H., Ichijo, E., Coll, M.-P., Brewer, R., ... Bird, G. (2018). Knowledge of resting heart rate mediates the relationship between intelligence and the heartbeat counting task. *Biological psychology*, *133*, 1–3.

Nebel, M. B., Eloyan, A., Barber, A. D., & Mostofsky, S. H. (2014). Precentral

gyrus functional connectivity signatures of autism. *Frontiers in systems neuroscience*, *8*, 80.

Neuhaus, E., Webb, S. J., & Bernier, R. A. (2019). Linking social motivation with social skill: The role of emotion dysregulation in autism spectrum disorder. *Development and psychopathology*, *31*(3), 931–943.

Nicholson, T., Williams, D., Carpenter, K., & Kallitsounaki, A. (2019). Interoception is impaired in children, but not adults, with autism spectrum disorder. *Journal of autism and developmental disorders*, *49*(9), 3625–3637.

Nicholson, T. M., Williams, D. M., Grainger, C., Christensen, J. F., Calvo-Merino, B., & Gaigg, S. B. (2018). Interoceptive impairments do not lie at the heart of autism or alexithymia. *Journal of abnormal psychology*, *127*(6), 612.

Noel, J.-P., Lytle, M., Cascio, C., & Wallace, M. T. (2018). Disrupted integration of exteroceptive and interoceptive signaling in autism spectrum disorder. *Autism Research*, *11*(1), 194–205.

Nummenmaa, L., Glerean, E., Hari, R., & Hietanen, J. K. (2014). Bodily maps of emotions. *Proceedings of the National Academy of Sciences*, *111*(2), 646–651.

Nummenmaa, L., Hirvonen, J., Parkkola, R., & Hietanen, J. K. (2008). Is emotional contagion special? an fmri study on neural systems for affective and cognitive empathy. *Neuroimage*, *43*(3), 571–580.

Odriozola, P., Uddin, L. Q., Lynch, C. J., Kochalka, J., Chen, T., & Menon, V. (2016). Insula response and connectivity during social and non-social attention in children with autism. *Social cognitive and affective neuroscience*, *11*(3), 433–444.

Ohl, S., Wohltat, C., Kliegl, R., Pollatos, O., & Engbert, R. (2016). Microsaccades are coupled to heartbeat. *Journal of Neuroscience*, *36*(4), 1237–1241.

Olatunji, B. O., Deacon, B. J., Abramowitz, J. S., & Valentiner, D. P. (2007). Body vigilance in nonclinical and anxiety disorder samples: structure, correlates, and prediction of health concerns. *Behavior Therapy*, *38*(4), 392–401.

Ondobaka, S., Kilner, J., & Friston, K. (2017). The role of interoceptive inference in theory of mind. *Brain and cognition*, *112*, 64–68.

Ottaviani, C., Watson, D. R., Meeten, F., Makovac, E., Garfinkel, S. N., & Critchley, H. D. (2016). Neurobiological substrates of cognitive rigidity and autonomic inflexibility in generalized anxiety disorder. *Biological Psychology*, *119*, 31–41.

O'Reilly, H., Lundqvist, D., Pigat, D., Baron, K., Fridenson, S., Tal, S., ... others (2012). The eu-emotion stimulus set. *Cambridge, UK: Autism research centre, University of Cambridge*.

O'Reilly, H., Pigat, D., Fridenson, S., Berggren, S., Tal, S., Golan, O., ... Lundqvist, D. (2016). The eu-emotion stimulus set: a validation study. *Behavior research methods*, *48*(2), 567–576.

Palser, E., Fotopoulou, A., Pellicano, E., & Kilner, J. (2018). The link between interoceptive processing and anxiety in children diagnosed with autism spectrum disorder: Extending adult findings into a developmental sample. *Biological psychology*, *136*, 13–21.

Palser, E., Fotopoulou, A., Pellicano, E., & Kilner, J. (2020). Dissociation in how core autism features relate to interoceptive dimensions: Evidence from cardiac awareness in children. *Journal of autism and developmental disorders*, *50*(2), 572–582.

Palser, E. R., Palmer, C. E., Galvez-Pol, A., Hannah, R., Fotopoulou, A., & Kilner, J. M. (2018). Alexithymia mediates the relationship between interoceptive sensibility and anxiety. *PloS one*, *13*(9), e0203212.

Parker, J. D., Taylor, G. J., & Bagby, M. (1993). Alexithymia and the recognition of facial expressions of emotion. *Psychotherapy and psychosomatics*, *59*(3-4), 197–202.

Parker, J. D., Taylor, G. J., & Bagby, R. M. (2001). The relationship between emotional intelligence and alexithymia. *Personality and Individual differences*, *30*(1), 107–115.

Patriquin, M. A., Hartwig, E. M., Friedman, B. H., Porges, S. W., & Scarpa, A. (2019). Autonomic response in autism spectrum disorder: Relationship to social and cognitive functioning. *Biological psychology*, *145*, 185–197.

Paulus, M. P., & Stein, M. B. (2006). An insular view of anxiety. *Biological psychiatry*, *60*(4), 383–387.

Paulus, M. P., & Stein, M. B. (2010). Interoception in anxiety and depression. *Brain structure and Function*, *214*(5-6), 451–463.

Peppé, S., Cleland, J., Gibbon, F., O'Hare, A., & Castilla, P. M. (2011). Expressive prosody in children with autism spectrum conditions. *Journal of Neurolinguistics*, *24*(1), 41–53.

Peppé, S., McCann, J., Gibbon, F., O'Hare, A., & Rutherford, M. (2007). Receptive and expressive prosodic ability in children with high-functioning autism. *Journal of Speech, Language, and Hearing Research*.

Petrovska, I. V., & Trajkovski, V. (2019). Effects of a computer-based intervention on emotion understanding in children with autism spectrum conditions. *Journal of autism and developmental disorders*, *49*(10), 4244–4255.

Pollatos, O., Herbert, B. M., Mai, S., & Kammer, T. (2016). Changes in interoceptive processes following brain stimulation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1708), 20160016.

Pollatos, O., Kirsch, W., & Schandry, R. (2005). Brain structures involved in interoceptive awareness and cardioafferent signal processing: a dipole source localization study. *Human brain mapping*, *26*(1), 54–64.

Pollatos, O., & Schandry, R. (2004). Accuracy of heartbeat perception is reflected in the amplitude of the heartbeat-evoked brain potential. *Psychophysiology*, *41*(3), 476–482.

Pollatos, O., Traut-Mattausch, E., Schroeder, H., & Schandry, R. (2007). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. *Journal of anxiety disorders*, *21*(7), 931–943.

Poquérusse, J., Pastore, L., Dellantonio, S., & Esposito, G. (2018). Alexithymia and autism spectrum disorder: a complex relationship. *Frontiers in psychology*, *9*, 1196.

Porges, S. (1993). Body perception questionnaire. *Laboratory of Developmental Assessment, University of Maryland*.

Potts, J. T. (2006). Inhibitory neurotransmission in the nucleus tractus solitarii: implications for baroreflex resetting during exercise. *Experimental physiology*, *91*(1), 59–72.

Pourtois, G., Sander, D., Andres, M., Grandjean, D., Reveret, L., Olivier, E., & Vuilleumier, P. (2004). Dissociable roles of the human somatosensory and superior temporal cortices for processing social face signals. *European*

Journal of Neuroscience, *20*(12), 3507–3515.

Preece, D. A., Becerra, R., Boyes, M. E., Northcott, C., McGillivray, L., & Hasking, P. A. (2020). Do self-report measures of alexithymia measure alexithymia or general psychological distress? a factor analytic examination across five samples. *Personality and Individual Differences*, *155*, 109721.

Prkachin, G. C., Casey, C., & Prkachin, K. M. (2009). Alexithymia and perception of facial expressions of emotion. *Personality and Individual Differences*, *46*(4), 412–417.

Quadt, L., Critchley, H. D., & Garfinkel, S. N. (2018). Interoception and emotion: Shared mechanisms and clinical implications. *The interoceptive mind: From homeostasis to awareness*, 123.

Quattrocki, E., & Friston, K. (2014). Autism, oxytocin and interoception. *Neuroscience & Biobehavioral Reviews*, *47*, 410–430.

Radley, K. C., McHugh, M. B., Taber, T., Battaglia, A. A., & Ford, W. B. (2017). School-based social skills training for children with autism spectrum disorder. *Focus on Autism and Other Developmental Disabilities*, *32*(4), 256–268.

Rae, C. L., Botan, V. E., van Praag, C. D. G., Herman, A. M., Nyyssönen, J. A., Watson, D. R., ... Critchley, H. D. (2018). Response inhibition on the stop signal task improves during cardiac contraction. *Scientific reports*, *8*(1), 1–9.

Raven, P. B., Fadel, P. J., & Ogoh, S. (2006). Arterial baroreflex resetting during exercise: a current perspective. *Experimental physiology*, *91*(1), 37–49.

Reker, M., Ohrmann, P., Rauch, A. V., Kugel, H., Bauer, J., Dannlowski, U., ... Suslow, T. (2010). Individual differences in alexithymia and brain response to masked emotion faces. *Cortex*, *46*(5), 658–667.

Reyes, N. M., Pickard, K., & Reaven, J. (2019). Emotion regulation: A treatment target for autism spectrum disorder. *Bulletin of the Menninger Clinic*, *83*(3), 205–234.

Riganello, F., Larroque, S. K., Bahri, M. A., Heine, L., Martial, C., Carrière, M., ... others (2018). A heartbeat away from consciousness: heart rate variability entropy can discriminate disorders of consciousness and is correlated with resting-state fmri brain connectivity of the central autonomic network. *Frontiers in neurology*, *9*, 769.

Rigby, S. N., Stoesz, B. M., & Jakobson, L. S. (2018). Empathy and face processing in adults with and without autism spectrum disorder. *Autism Research*, *11*(6), 942–955.

Ring, C., & Brener, J. (1996). Influence of beliefs about heart rate and actual heart rate on heartbeat counting. *Psychophysiology*, *33*(5), 541–546.

Ring, C., & Brener, J. (2018). Heartbeat counting is unrelated to heartbeat detection: A comparison of methods to quantify interoception. *Psychophysiology*, *55*(9), e13084.

Ring, C., Brener, J., Knapp, K., & Mailloux, J. (2015). Effects of heartbeat feedback on beliefs about heart rate and heartbeat counting: a cautionary tale about interoceptive awareness. *Biological psychology*, *104*, 193–198.

Rivet, T. T., & Matson, J. L. (2011). Review of gender differences in core symptomatology in autism spectrum disorders. *Research in Autism Spectrum Disorders*, *5*(3), 957–976.

Robertson, A. E., & Simmons, D. R. (2015). The sensory experiences of adults

with autism spectrum disorder: A qualitative analysis. *Perception*, *44*(5), 569–586.

Rosenblau, G., Kliemann, D., Dziobek, I., & Heekeren, H. R. (2017). Emotional prosody processing in autism spectrum disorder. *Social cognitive and affective neuroscience*, *12*(2), 224–239.

Rothstein, J. A. (2013). Prosody treatment program. *LinguiSystem, Austin*.

Schachter, S., & Singer, J. (1962). Cognitive, social, and physiological determinants of emotional state. *Psychological review*, *69*(5), 379.

Schaefer, M., Egloff, B., Gerlach, A. L., & Witthöft, M. (2014). Improving heartbeat perception in patients with medically unexplained symptoms reduces symptom distress. *Biological Psychology*, *101*, 69–76.

Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology*, *18*(4), 483–488.

Schauder, K. B., & Bennetto, L. (2016). Toward an interdisciplinary understanding of sensory dysfunction in autism spectrum disorder: an integration of the neural and symptom literatures. *Frontiers in Neuroscience*, *10*, 268.

Schauder, K. B., Mash, L. E., Bryant, L. K., & Cascio, C. J. (2015). Interoceptive ability and body awareness in autism spectrum disorder. *Journal of experimental child psychology*, *131*, 193–200.

Schneider, K., Regenbogen, C., Pauly, K. D., Gossen, A., Schneider, D. A., Mevissen, L., ... Schneider, F. (2013). Evidence for gender-specific endophenotypes in high-functioning autism spectrum disorder during empathy. *Autism Research*, *6*(6), 506–521.

Schooler, J. W., Smallwood, J., Christoff, K., Handy, T. C., Reichle, E. D., & Sayette, M. A. (2011). Meta-awareness, perceptual decoupling and the wandering mind. *Trends in cognitive sciences*, *15*(7), 319–326.

Schulte-Rüther, M., Markowitsch, H. J., Shah, N. J., Fink, G. R., & Piefke, M. (2008). Gender differences in brain networks supporting empathy. *Neuroimage*, *42*(1), 393–403.

Schultz, R. T. (2005). Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. *International Journal of Developmental Neuroscience*, *23*(2-3), 125–141.

Schulz, A., Lass-Hennemann, J., Nees, F., Blumenthal, T. D., Berger, W., & Schachinger, H. (2009). Cardiac modulation of startle eye blink. *Psychophysiology*, *46*(2), 234–240.

Schulz, S. M. (2016). Neural correlates of heart-focused interoception: a functional magnetic resonance imaging meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1708), 20160018.

Schutter, D. J., & Van Honk, J. (2005). The cerebellum on the rise in human emotion. *The Cerebellum*, *4*(4), 290–294.

Serpa, J. G., Taylor, S. L., & Tillisch, K. (2014). Mindfulness-based stress reduction (mbsr) reduces anxiety, depression, and suicidal ideation in veterans. *Medical care*, *52*(12), S19–S24.

Seth, A. K. (2013). Interoceptive inference, emotion, and the embodied self. *Trends in cognitive sciences*, *17*(11), 565–573.

Seth, A. K., Suzuki, K., & Critchley, H. D. (2012). An interoceptive predictive coding model of conscious presence. *Frontiers in psychology*, *2*, 395.

Shaffer, F., & Venner, J. (2013). Heart rate variability anatomy and physiology. *Biofeedback*, *41*(1), 13–25.

Shah, P., Hall, R., Catmur, C., & Bird, G. (2016). Alexithymia, not autism, is associated with impaired interoception. *cortex*, *81*, 215–220.

Shalom, D. B. (2009). The medial prefrontal cortex and integration in autism. *The Neuroscientist*, *15*(6), 589–598.

Sherman, M. T., Barrett, A. B., & Kanai, R. (2015). Inferences about consciousness using subjective reports of confidence. *Behavioral methods in consciousness research*, 87–106.

Sherrington, C. (1907). On the proprio-ceptive system, especially in its reflex aspect. *Brain*, *29*(4), 467–482.

Sherrington, C. (1952). *The integrative action of the nervous system*. CUP Archive.

Shields, S. A., Mallory, M. E., & Simon, A. (1989). The body awareness questionnaire: reliability and validity. *Journal of personality Assessment*, *53*(4), 802–815.

Shriberg, L. D., Paul, R., McSweeny, J. L., Klin, A., Cohen, D. J., & Volkmar, F. R. (2001). Speech and prosody characteristics of adolescents and adults with high-functioning autism and asperger syndrome. *Journal of Speech, Language, and Hearing Research*.

Silani, G., Bird, G., Brindley, R., Singer, T., Frith, C., & Frith, U. (2008). Levels of emotional awareness and autism: an fmri study. *Social neuroscience*, *3*(2), 97–112.

Simmons, E. S., Paul, R., & Shic, F. (2016). Brief report: A mobile application to treat prosodic deficits in autism spectrum disorder and other communication impairments: A pilot study. *Journal of autism and developmental disorders*, *46*(1), 320–327.

Simms, M. L., Kemper, T. L., Timbie, C. M., Bauman, M. L., & Blatt, G. J. (2009). The anterior cingulate cortex in autism: heterogeneity of qualitative and quantitative cytoarchitectonic features suggests possible subgroups. *Acta neuropathologica*, *118*(5), 673–684.

Singer, T., Critchley, H. D., & Preuschoff, K. (2009). A common role of insula in feelings, empathy and uncertainty. *Trends in cognitive sciences*, *13*(8), 334–340.

Singer, T., Seymour, B., O'doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science*, *303*(5661), 1157–1162.

Smith, M. J. L., Montagne, B., Perrett, D. I., Gill, M., & Gallagher, L. (2010). Detecting subtle facial emotion recognition deficits in high-functioning autism using dynamic stimuli of varying intensities. *Neuropsychologia*, *48*(9), 2777–2781.

Smith, R., Thayer, J. F., Khalsa, S. S., & Lane, R. D. (2017). The hierarchical basis of neurovisceral integration. *Neuroscience & biobehavioral reviews*, *75*, 274–296.

Somerville, L. H., Jones, R. M., & Casey, B. (2010). A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. *Brain and cognition*, *72*(1), 124–133.

Spek, A. A., Van Ham, N. C., & Nyklíček, I. (2013). Mindfulness-based therapy in adults with an autism spectrum disorder: a randomized controlled trial. *Research in developmental disabilities*, *34*(1), 246–253.

Spielberger, C. D. (2010). State-trait anxiety inventory. *The Corsini encyclopedia*

of psychology, 1–1.

Spyer, K. (1994). Annual review prize lecture. central nervous mechanisms contributing to cardiovascular control. *The Journal of Physiology*, *474*(1), 1.

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *American Journal of Psychiatry*, *164*(2), 318–327.

Stern, E. R. (2014). Neural circuitry of interoception: New insights into anxiety and obsessive-compulsive disorders. *Current Treatment Options in Psychiatry*, *1*(3), 235–247.

Sterne, J. A., White, I. R., Carlin, J. B., Spratt, M., Royston, P., Kenward, M. G., ... Carpenter, J. R. (2009). Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Bmj*, *338*.

Stevens, S., Gerlach, A. L., Cludius, B., Silkens, A., Craske, M. G., & Hermann, C. (2011). Heartbeat perception in social anxiety before and during speech anticipation. *Behaviour research and therapy*, *49*(2), 138–143.

Tam, F. I., King, J. A., Geisler, D., Korb, F. M., Sareng, J., Ritschel, F., ... Ehrlich, S. (2017). Altered behavioral and amygdala habituation in high-functioning adults with autism spectrum disorder: an fmri study. *Scientific reports*, *7*(1), 1–9.

Tang, J. S., Chen, N. T., Falkmer, M., Blte, S., & Girdler, S. (2019). Atypical visual processing but comparable levels of emotion recognition in adults with autism during the processing of social scenes. *Journal of autism and developmental disorders*, *49*(10), 4009–4018.

Terasawa, Y., Shibata, M., Moriguchi, Y., & Umeda, S. (2013). Anterior insular cortex mediates bodily sensibility and social anxiety. *Social cognitive and affective neuroscience*, *8*(3), 259–266.

Thakkar, K. N., Polli, F. E., Joseph, R. M., Tuch, D. S., Hadjikhani, N., Barton, J. J., & Manoach, D. S. (2008). Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (asd). *Brain*, *131*(9), 2464–2478.

Top Jr, D. N., Stephenson, K. G., Doxey, C. R., Crowley, M. J., Kirwan, C. B., & South, M. (2016). Atypical amygdala response to fear conditioning in autism spectrum disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *1*(4), 308–315.

Tracy, J. L., & Randles, D. (2011). Four models of basic emotions: a review of ekman and cordaro, izard, levenson, and panksepp and watt. *Emotion Review*, *3*(4), 397–405.

Tye, K. M., Prakash, R., Kim, S.-Y., Fenno, L. E., Grosenick, L., Zarabi, H., ... Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, *471*(7338), 358–362.

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience*, *16*(1), 55–61.

Uljarevic, M., & Hamilton, A. (2013). Recognition of emotions in autism: a formal meta-analysis. *Journal of autism and developmental disorders*, *43*(7), 1517–1526.

Van der Does, A. W., Antony, M. M., Ehlers, A., & Barsky, A. J. (2000). Heartbeat perception in panic disorder: a reanalysis. *Behaviour research and therapy*, *38*(1), 47–62.

van der Velde, J., Gromann, P. M., Swart, M., Wiersma, D., de Haan, L., Brugge-

man, R., ... Aleman, A. (2015). Alexithymia influences brain activation during emotion perception but not regulation. *Social Cognitive and Affective Neuroscience*, *10*(2), 285–293.

Van Dyck, Z., Vögele, C., Blechert, J., Lutz, A. P., Schulz, A., & Herbert, B. M. (2016). The water load test as a measure of gastric interoception: Development of a two-stage protocol and application to a healthy female population. *PloS one*, *11*(9), e0163574.

Wan, C. Y., Bazen, L., Baars, R., Libenson, A., Zipse, L., Zuk, J., ... Schlaug, G. (2011). Auditory-motor mapping training as an intervention to facilitate speech output in non-verbal children with autism: a proof of concept study. *PloS one*, *6*(9), e25505.

Wang, J.-E., & Tsao, F.-M. (2015). Emotional prosody perception and its association with pragmatic language in school-aged children with high-function autism. *Research in developmental disabilities*, *37*, 162–170.

Watanabe, T., Yahata, N., Abe, O., Kuwabara, H., Inoue, H., Takano, Y., ... others (2012). Diminished medial prefrontal activity behind autistic social judgments of incongruent information. *PloS one*, *7*(6).

Watson, C., Paxinos, G., & Kayalioglu, G. (2009). The spinal cord: a christopher and dana reeve foundation text and atlas.

Watson, D. R., Garfinkel, S. N., van Praag, C. G., Willmott, D., Wong, K., Meeten, F., & Critchley, H. D. (2019). Computerized exposure therapy for spider phobia: effects of cardiac timing and interoceptive ability on subjective and behavioral outcomes. *Psychosomatic medicine*, *81*(1), 90–99.

White, S. W., & Roberson-Nay, R. (2009). Anxiety, social deficits, and loneliness in youth with autism spectrum disorders. *Journal of autism and developmental disorders*, *39*(7), 1006–1013.

Whitehead, W. E., & Drescher, V. M. (1980). Perception of gastric contractions and self-control of gastric motility. *Psychophysiology*, *17*(6), 552–558.

Whitehead, W. E., Drescher, V. M., Heiman, P., & Blackwell, B. (1977). Relation of heart rate control to heartbeat perception. *Biofeedback and Self-regulation*, *2*(4), 371–392.

Wicker, B., Keysers, C., Plailly, J., Royet, J.-P., Gallese, V., & Rizzolatti, G. (2003). Both of us disgusted in my insula: the common neural basis of seeing and feeling disgust. *Neuron*, *40*(3), 655–664.

Wieckowski, A. T., & White, S. W. (2020). Attention modification to attenuate facial emotion recognition deficits in children with autism: A pilot study. *Journal of Autism and Developmental Disorders*, *50*(1), 30–41.

Wiens, S. (2005). Interoception in emotional experience. *Current opinion in neurology*, *18*(4), 442–447.

Wiens, S., Mezzacappa, E. S., & Katkin, E. S. (2000). Heartbeat detection and the experience of emotions. *Cognition & Emotion*, *14*(3), 417–427.

Wilkinson, M., McIntyre, D., & Edwards, L. (2013). Electrocutaneous pain thresholds are higher during systole than diastole. *Biological psychology*, *94*(1), 71–73.

Williams, D. L., Goldstein, G., & Minshew, N. J. (2006). Neuropsychologic functioning in children with autism: Further evidence for disordered complex information-processing. *Child Neuropsychology*, *12*(4-5), 279–298.

Wilson, K. P., Steinbrenner, J. R., Kalandadze, T., & Handler, L. (2019). Interventions targeting expressive communication in adults with autism spectrum

disorders: A systematic review. *Journal of Speech, Language, and Hearing Research*, *62*(6), 1959–1978.

Windmann, S., Schonecke, O. W., Fröhlig, G., & Maldener, G. (1999). Dissociating beliefs about heart rates and actual heart rates in patients with cardiac pacemakers. *Psychophysiology*, *36*(3), 339–342.

Xu, J., Wang, H., Zhang, L., Xu, Z., Li, T., Zhou, Z., ... Hu, Q. (2018). Both hypo-connectivity and hyper-connectivity of the insular subregions associated with severity in children with autism spectrum disorders. *Frontiers in neuroscience*, *12*, 234.

Yoris, A., Esteves, S., Couto, B., Melloni, M., Kichic, R., Cetkovich, M., ... others (2015). The roles of interoceptive sensitivity and metacognitive interoception in panic. *Behavioral and Brain Functions*, *11*(1), 14.

Zaki, J., Davis, J. I., & Ochsner, K. N. (2012). Overlapping activity in anterior insula during interoception and emotional experience. *Neuroimage*, *62*(1), 493–499.

Zamariola, G., Maurage, P., Luminet, O., & Corneille, O. (2018). Interoceptive accuracy scores from the heartbeat counting task are problematic: Evidence from simple bivariate correlations. *Biological psychology*, *137*, 12–17.

Zantinge, G., van Rijn, S., Stockmann, L., & Swaab, H. (2019). Concordance between physiological arousal and emotion expression during fear in young children with autism spectrum disorders. *Autism*, *23*(3), 629–638.

Zimprich, D., Nusser, L., & Pollatos, O. (2020). Are interoceptive accuracy scores from the heartbeat counting task problematic? a comment on zamariola et al.(2018). *Biological Psychology*, *152*, 107868.

Appendix A

Supplementary results 1

Supplementary results for chapter 2

Heartbeat tracking accuracy: No main effect of heartbeat tracking accuracy was observed ($F(1, 68) = 1.709$, $p = 0.196$) and tracking accuracy did not significantly interact with trial type ($F(1, 68) = 0.967$, $p = 0.383$). Tracking accuracy also did not interact with valence ($F(1, 68) = 0.038$, $p = 0.846$) or complexity ($F(1, 68) = 0.225$, $p = 0.637$) and thus tracking accuracy had no effect on prosody scores.

Heartbeat tracking mean confidence: A significant main effect of heartbeat tracking confidence ($F(1, 66) = 5.018$, $p = 0.028$) revealed overall prosody accuracy increases as heartbeat tracking confidence increased. Confidence also interacted with trial type ($F(2, 132) = 2.971$, $p = 0.021$) reflecting the propensity for participants to perform better on face with text trials as tracking confidence increased ($r = 0.376$, $p = 0.001$) whilst no relationship between tracking confidence and face trials ($r = 0.123$, $p = 0.302$) or text trials ($r = 0.193$, $p = 0.105$) prevailed as significant. No significant interaction between tracking confidence and emotional valence ($F(1, 66) = 0.389$, $p = 0.535$) or complexity ($F(1, 66) = 0.159$, $p = 0.691$) was found.

Heartbeat tracking ITPE: No main effect of heartbeat tracking ITPE was observed ($F(1, 60) = 0.184$, $p = 0.669$) and tracking ITPE did not significantly interact with trial type ($F(1, 60) = 0.025$, $p = 0.975$). Tracking ITPE also did not interact with valence ($F(1, 60) = 0.013$, $p = 0.909$) or complexity ($F(1, 60) = 0.018$, $p = 0.895$) and thus tracking ITPE had no effect on prosody scores.

Heartbeat discrimination ITPE: No main effect of heartbeat discrimination ITPE was observed ($F(1, 59) = 0.424$, $p = 0.517$) and discrimination ITPE did not significantly interact with trial type ($F(1, 59) = 0.011$, $p = 0.989$). Discrim-

ination ITPE also did not interact with valence ($F(1, 59) = 1.031$, $p = 0.314$) or complexity ($F(1, 59) = 1.484$, $p = 0.228$) and thus discrimination ITPE had no effect on prosody scores.

Age: Entering age as a covariate revealed a significant main effect of age ($F(1, 68) = 56.121$, $p = 0.016$) indicating overall accuracy decreased as age increased ($r = -0.261$, $p = 0.025$). No significant age by trial type interaction was observed ($F(1, 68) = 0.658$, $p = 0.420$) and no age by emotional complexity interaction was observed ($F(1, 68) = 0.606$, $p = 0.439$). We did however observe a significant interaction between emotional valence and age ($F(1, 68) = 4.725$, $p = 0.033$) suggesting accuracy towards negative emotions declined with increasing age ($r = -0.317$, $p = 0.006$) whilst no variation in accuracy across ages was observed towards positive emotions ($r = -0.166$, $p = 0.156$).

Supplementary results for chapter 3

Heartbeat tracking accuracy: No significant main effect of heartbeat tracking accuracy was observed ($F(1, 66) = 0.056$, $p = 0.814$) and no significant interaction between tracking accuracy and emotion ($F(1, 66) = 0.327$, $p = 0.569$) or cardiac cycle ($F(1, 66) = 0.124$, $p = 0.726$) was observed. Finally, no three way interaction between tracking accuracy, cardiac cycle and emotion was observed ($F(1, 66) = 0.194$, $p = 0.0661$) suggesting tracking accuracy did not influence responses. No significant relationships were found when broken down by group (i.e. run in each group separately).

Heartbeat tracking mean confidence: No significant main effect of heartbeat tracking confidence was found ($F(1, 66) = 0.871$, $p = 0.354$) and no interaction was observed between tracking confidence and emotion ($F(1, 66) = 0.010$, $p = 0.922$), tracking confidence and cardiac cycle ($F(1, 66) = 0.057$, $p = 0.811$) nor between tracking confidence, cardiac cycle and emotion ($F(1, 66) = 1.966$, $p = 0.166$). No significant relationships were found when broken down by group (i.e. run in each group separately).

Heartbeat tracking ITPE: No significant main effect of tracking ITPE was found ($F(1, 66) = 0.872$, $p = 0.354$) and tracking ITPE did not interact with emotion ($F(1, 66) = 0.108$, $p = 0.743$) or cardiac cycle ($F(1, 66) = 1.827$ $p = 0.181$). There was also no 3-way interaction between tracking ITPE, emotion and cardiac cycle ($F(1, 66) = 0.039$, $p = 0.845$). No significant relationships were

found when broken down by group (i.e. run in each group separately).

Heartbeat discrimination accuracy: No significant main effect of heartbeat discrimination accuracy was observed ($F(1, 66) = 0.084$, $p = 0.773$) and no significant interaction between heartbeat discrimination accuracy and emotion ($F(1, 66) = 3.327$, $p = 0.073$) or cardiac cycle ($F(1, 66) = 2.230$, $p = 0.140$) was observed. Finally, no three-way interaction between heartbeat discrimination accuracy, cardiac cycle and emotion ($F(1, 66) = 0.388$, $p = 0.535$) was observed suggesting heartbeat discrimination accuracy did not influence cardiac contingent fear processing. In the autistic group only, heartbeat discrimination accuracy significantly interacted with emotion reflected the propensity for individuals with greater accuracy to report increased ratings towards fearful faces ($r = 0.381$, $p = 0.024$). In the control group only, discrimination accuracy significantly interacted with cardiac cycle, although post-hoc correlations revealed no significant relationships between accuracy and systole ($r = 0.101$, $p = 0.575$) or diastole ($r = -0.208$, $p = 0.246$). No other significant group relationships were found.

Heartbeat discrimination insight: No significant main effect of heartbeat discrimination insight was observed ($F(1, 66) = 0.011$, $p = 0.917$) and no interaction between heartbeat discrimination insight and emotion ($F(1, 66) = 0.195$, $p = 0.661$) or cardiac cycle ($F(1, 66) = 0.653$, $p = 0.422$) was observed. Finally no three way interaction between heartbeat discrimination insight, cardiac cycle and emotion ($F(1, 66) = 1.226$, $p = 0.272$) was observed suggesting heartbeat discrimination insight did not influence cardiac contingent fear processing. No significant relationships were found when broken down by group (i.e. run in each group separately).

Heartbeat discrimination mean confidence: No significant main effect of heartbeat discrimination confidence was found ($F(1, 66) = 1.102$, $p = 0.198$) discrimination confidence and cardiac cycle ($F(1, 66) = 2.083$, $p = 0.154$) nor between discrimination confidence, cardiac cycle and emotion ($F(1, 66) = 2.528$, $p = 0.117$). We did however observe a significant interaction between heartbeat discrimination and emotion ($F(1, 66) = 6.961$, $p = 0.010$) reflecting the propensity for participants to rate fear faces as more intense with increasing discrimination confidence ($r = 0.333$, $p = 0.005$) whilst no relationship was observed for neutral faces ($r = -0.137$, $p = 0.263$). In autistic participants only, discrimination confidence

significantly interacted with emotion ($F(1, 33) = 22.182$, $p < 0.001$) reflecting the propensity for individuals high in confidence to rate fearful faces as more intense ($r = 0.378$, $p = 0.025$) yet neutral faces as less intense ($r = -0.369$, $p = 0.029$). In neurotypical participants a main effect of confidence was observed ($F(1, 32) = 4.949$, $p = 0.033$) reflecting the propensity for participants to rate all faces as more intense with increasing confidence ($r = 0.285$, $p = 0.025$). No other significant group effects were found.

Heartbeat discrimination ITPE: No significant main effect of ITPE was observed ($F(1, 65) = 0.014$, $p = 0.907$) but we did observe a significant interaction between ITPE and cardiac cycle ($F(1, 65) = 8.758$, $p = 0.004$), although post-hoc correlations with systole ($r = -0.105$, $p = 0.396$) and diastole ($r = 0.119$, $p = 0.337$) were not significant suggesting ITPE did not influence intensity ratings as a function of cardiac cycle. Finally, no significant interaction between ITPE and emotion was ($F(1, 66) = 0.256$, $p = 0.615$) or between ITPE, emotion and cardiac cycle ($F(1, 66) = 0.776$, $p = 0.382$) was observed. No significant relationships were found when broken down by group (i.e. run in each group separately).

Heart rate variability (HRV; RMSSD): No significant main effect of HRV was observed ($F(1, 66) = 0.387$), $p = 0.536$). We did however observe a significant interaction between HRV and emotion ($F(1, 66) = 4.\ 241$, $p = 0.043$) suggesting a subtle increase in fear ratings with reduced HRV, although the post-hoc correlations did not quite reach significance for face ratings ($r = -0.230$, $p = 0.057$) nor neutral faces ($r – 0.086$, $p = 0.482$). No significant interaction between HRV and cardiac cycle ($F(1, 66) = 2.217$, $p = 0.141$) nor between HRV, cardiac cycle and emotion ($F(1, 66) = 0.010$, $p = 0.919$) was observed. No significant relationships were found when broken down by group (i.e. run in each group separately).

Heart rate (HR): No significant main effect of HR was observed ($F(1, 66) = 0.79$, $p = 0.403$) and no significant interaction between HR and emotion ($F(1, 66) = 0.016$) $p = 0.899$), HR and cardiac cycle ($F(1\ ,66) = 1.115$, $p = 0.295$) nor between HR, cardiac cycle and emotion ($F(1, 66) = 0.733$, $p = 0.395$) suggesting mean HR did not influence intensity ratings. No significant relationships were found when broken down by group (i.e. run in each group separately).

BPQ: No significant main effect of BPQ was found ($F(1, 66) = 0.045$, $p = 0.833$) but we did observe a significant interaction between BPQ and cardiac cycle

(F(1, 66) = 4.429, p = 0.039), although post-hoc correlations with systole (r = -0.003, p = 0.982) or diastole (r = 0.136, p = 0.270) were not significant. No significant interaction between emotion and BPQ (F(1, 66) = 1.130, p = 0.292) nor between BPQ, emotion and cardiac cycle (F(1, 66) = 0.115, p = 0.736) was found. No significant relationships were found when broken down by group (i.e. run in each group separately).

MAIA: For the MAIA sub-scales (noticing, not distracting, not worrying, attention regulation, emotional awareness, self-regulation, body listening and trusting), no significant main effects nor interaction effects were found for any variables, all p's > 0.05. We did observe a significant interaction between cardiac cycle and the attention regulation sub-scale (F(1, 66) = 4.214, p = 0.044), although post-hoc correlations with systole (r = -0.006, p = 0.963) and diastole (r = 0.111, p = 0.367) were not significant, and between cardiac cycle and the emotional awareness subscale (F(1, 66) = 4.212, p = 0.044), although again post-hoc correlations with systole (r = 0.038, p = 0.756) and diastole (r = 0.178, p = 0.146) were not significant. We also observed a significant three-way interaction between cardiac cycle, emotion and the emotional awareness sub-scale (F(1, 66) = 4.216, p = 0.044), however the only significant post-hoc correlation to emerge was between the emotional awareness sub-scale and fear diastole (r = 0.298, p = 0.013) whilst correlations between the emotional awareness subscale and fear systole (r = 0.121, p = 0.322), neutral diastole (r = -0.048, p = 0.698 and neutral systole (r = -0.059, p = 0.632) were not significant. No other significant interactions with any of the other MAIA sub-scales were found, all p's > 0.05.

Autistic traits (AQ): No significant main effect of AQ was found (F(1, 67) = 0.662, p = 0.419) and no significant interaction between AQ and emotion (F(1, 67) = 0.001, p = 0.0970), AQ and cardiac cycle (F(1, 67) = 0.008, p = 0.928) nor between AQ, emotion and cardiac cycle (F(1, 67) = 1.368, p = 0.246) was found suggesting autistic traits did not influence intensity ratings. In autistic participants only, a significant main effect of autistic traits was observed (F(1, 33) = 8.321, p = 0.007) reflecting the propensity for those who score lower in autistic traits to rate faces as more intense (r = -0.449, p = 0.007).

Alexithymia (TAS-20): No significant main effect for the TAS-20 total score or any of the sub-scales (difficulty describing feelings, difficulty identifying feelings or

the externally oriented thinking) was observed, all p's > 0.05. We also observed no significant interactions between any of the scores and any of the cardiac/emotion categories, all p's > 0.05. No significant relationships were found when broken down by group (i.e. run in each group separately).

Age: No significant main effect of age was observed (F(1, 67) = 0.171, p = 0.680) suggesting age did not influence overall intensity ratings. Additionally, no interaction between age and emotion (F(1, 67) = 1.060, p = 0.307), cardiac cycle and age (F(1, 67) = 0.918, p = 0.341) nor between cardiac cycle, emotion and age (F(1, 67) = 0.841, p = 0.362) was observed. No significant relationships were found when broken down by group (i.e. run in each group separately).

Supplementary results for chapter 4

Age: Of all the interoceptive variables measured, in all participants, age significantly correlated only with heartbeat tracking mean confidence (r = 0.379, p = 0.002). In the autistic group only, again age only correlated with heartbeat tracking mean confidence (r = 0.347, p = 0.044). In the neurotypical group, age correlated with tracking mean confidence (r = 0.408, p = 0.019), heartbeat discrimination accuracy (r = 0.361, p = 0.039), heartbeat discrimination mean confidence (r = 0.359, p = 0.040) and finally negatively correlated with heartbeat discrimination ITPE (r = -0.368, p = 0.028). In the analysis of affective symptomatology, the only correlation to prevail as significant was between age and trait anxiety in autistic adults (r = -0.376, p = 0.028). No other significant relationships were found.

Heart rate: No significant difference between the two groups in mean heart rate during the task was observed (mean autism 71.80, SD 11.29, mean neurotypical 74.87, SD 11.39; t (67) = -1.082, p = 0.283). In autistic participants, mean heartrate correlated with heartbeat discrimination accuracy (r = -0.402, p = 0.022), heartbeat discrimination mean confidence (r = -0.367, p – 0.045) and discrimination ITPE (r = 0.457, p = 0.009). In neurotypical participants, mean heart rate correlated with heartbeat tracking accuracy (r = -0.428, p = 0.015) and heartbeat tracking ITPE (r = 0.442, p = 0.013). In all participants, mean heartrate correlated with tracking accuracy (r = -0.337, p = 0.006), discrimination mean confidence (r = -0.255, p = 0.042), tracking ITPE (r = 0.342, p = 0.006) and discrimination ITPE (r = 0.320, p = 0.011). No other significant relationships

were found.

Heart rate variability (RMSSD): No significant difference in HRV during the task was observed (mean autism 53.00, SD 41.79, mean neurotypical 65.67, SD 53.79; t(67) = -1.090, p = 0.280). HRV did not correlate with any interoceptive measures or any affective variables across all participants or in each group individually, all p's > 0.05.

Supplementary results for chapter 5

Heartbeat tracking accuracy: No significant main effect and no significant interactions with heartbeat tracking accuracy were found, all p's > 0.05, suggesting change in tracking accuracy did not influence responses. *Heartbeat tracking mean confidence:* Change in heartbeat tracking mean confidence significantly interacted with time (F(1, 17) = 5.296, p = 0.034) suggesting participants who were more confident on heartbeat tracking at final also provided overall greater intensity ratings (r = 0.487, p = 0.034). No other significant main effect or interactions were found, all p's > 0.05.

Heartbeat tracking ITPE: No significant main effect and no significant interactions with heartbeat tracking ITPE were found, all p's > 0.05, suggesting change in tracking ITPE did not influence responses.

Heartbeat discrimination accuracy: No significant main effect and no significant interactions with heartbeat discrimination accuracy were found, all p's > 0.05, suggesting change in discrimination accuracy did not influence responses.

Heartbeat discrimination insight: No significant main effect and no significant interactions with heartbeat discrimination insight were found, all p's > 0.05, suggesting change in discrimination insight did not influence responses.

Heartbeat discrimination mean confidence: A significant main effect of discrimination mean confidence was observed (F(1, 17) = 10.183, p = 0.005) suggesting participants who were more confident on the heartbeat discrimination task at final provided greater intensity ratings overall, regardless of the session (r = 0.612, p = 0.005). No other significant interactions were found, all p's > 0.05.

Heartbeat discrimination ITPE: No significant main effect and no significant interactions with heartbeat discrimination ITPE were found, all p's > 0.05, suggesting change in discrimination ITPE did not influence responses.

BPQ: No significant main effect and no significant interactions with the BPQ

were found, all p's > 0.05, suggesting change in BPQ scores did not influence responses.

MAIA: No significant main effect and no significant interactions with the MAIA, or any sub-scales, were found, all p's > 0.05, suggesting change MAIA scores did not influence responses.

AQ: No significant main effect and no significant interactions with AQ scores were found, all p's > 0.05, suggesting change in autistic traits did not influence responses.

TAS-20: In the analyses of alexithymia, a significant 4-way interaction between time, cardiac cycle, emotion and change in the difficulty describing feelings subscale of the tas-20 ($F(1, 18) = 5.937$, $p = 0.025$) however post-hoc correlations were not significant, all p's > 0.05. No other significant results for total scores or any subscales were found, all p's > 0.05.

Age: Including age in the model revealed no significant main effect and no interactions with age, all p's > 0.05, suggesting age did not impact intensity ratings across emotion or cardiac categories.

Supplementary results for chapter 6

Heartbeat tracking accuracy: No significant main effect or significant interactions with change in heartbeat tracking accuracy were found, all p's > 0.05, suggesting change in tracking accuracy did not influence prosodic accuracy overall or across trial types.

Heartbeat tracking mean confidence: No significant main effect or significant interactions with change in tracking confidence were found, all p's > 0.05, suggesting change in tracking confidence did not influence prosodic accuracy overall or across trial types.

Heartbeat tracking ITPE: No significant main effect or significant interactions with change in tracking ITPE scores were found, all p's > 0.05, suggesting change in ITPE scores did not influence prosodic accuracy overall or across trial types.

Heartbeat discrimination accuracy: No significant main effect or significant interactions with change in discrimination accuracy were found, all p's > 0.05, suggesting change in discrimination accuracy did not influence prosodic accuracy overall or across trial types.

Heartbeat discrimination insight: No significant main effect or significant in-

teractions with change in discrimination insight were found, all p's > 0.05, suggesting change in discrimination insight did not influence prosodic accuracy overall or across trial types.

Heartbeat discrimination mean confidence: No significant main effect or significant interactions with change in discrimination confidence were found, all p's > 0.05, suggesting change in discrimination confidence did not influence prosodic accuracy overall or across trial types.

Heartbeat discrimination ITPE: No significant main effect or significant interactions with change in discrimination ITPE scores were found, all p's > 0.05, suggesting change in discrimination ITPE scores did not influence prosodic accuracy overall or across trial types.

BPQ: No significant main effect or significant interactions with change in BPQ scores were found, all p's > 0.05, suggesting change in BPQ scores did not influence prosodic accuracy overall or across trial types.

MAIA: A significant main effect of change in MAIA not worrying scores ($F(1, 28) = 5.966$, $p = 0.021$) was observed suggesting participants with greater not-worrying scores at final were less accurate overall ($r = -0.379$, $p = 0.039$). We also observed a significant 3-way interaction between time, trial type and change in not worrying scores ($F(2, 56) = 3.912$, $p = 0.026$), although post-hoc correlations did not meet significance, all p's > 0.05, although the relationship between change in accuracy on text trials and change in not worrying scores was just above threshold significant ($r = -0.356$, $p = 0.054$). No other significant relationships with the MAIA were found, all p's > 0.05.

AQ: No significant main effect or significant interactions with change in AQ scores were found, all p's > 0.05, suggesting change in AQ scores did not influence prosodic accuracy overall or across trial types.

TAS-20: No significant main effect or significant interactions with the TAS-20, or any subscales, were found, all p's > 0.05, suggesting alexithymia did not influence prosodic accuracy overall or across trial types. Age: Including age in the model did not influence responses, all p's > 0.05.

Gender (group comparison): Including group as a between-subjects factor (based on the gender differences observed in chapter x) did not reveal any significant group effects, all p's > 0.05, suggesting training did not influence males or

females, as assigned at birth, differently.

Appendix B

Supplementary results 2

Supplementary results for chapter 3

Neuroimaging results

All significant clusters were localised according to the Anatomy toolbox (Eickhoff et al., 2005) in SPM12. (L = left hemisphere, R = right hemisphere; x, y, z = co-ordinates of maximum activated voxel in standard MNI152 space; t stat at this voxel. Peaks are listed at $p<0.05$ FDR cluster corrected (cluster-forming threshold: $p<0.001$).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
neurotypical >Autistic (t-contrast)					
1	Precentral Gyrus	R	44 -2 34	539	6.13
2	Paracentral Lobule	L	0 -28 58	221	4.43
	Posterior-medial frontal	L	-6 -18 58		4.26
	Mid cingulate cortex	L	-10 -32 44		3.40
3	Superior temporal gyrus	R	64 -44 20	256	4.27
	Supramarginal gyrus	R	62 -42 24		4.26
4	Middle temporal gyrus	L	-50 -60 16	212	4.22
5	Rolandic operculum	L	-46 -6 14	219	4.89
	Insula lobe	L	-36 -8 2		3.56
6	Supramarginal gyrus	L	-56 -46 34	304	4.38
	Inferior parietal lobule	L	-46 -44 40		3.31
7	Rolandic operculum	R	46 0 16	311	4.48
	Insula lobe	R	34 -8 16		4.43
8	Precuneus	L	-2 -54 22	119	3.97
9	Supramarginal gyrus	R	64 -24 36	182	3.96
	Postcentral gyrus	R	64 -12 22		3.37
10	Rolandic operculum	L	-38 -32 18	119	4.24

Supplementary table 2.1. Local Maxima of significant clusters for group t-contrast: neurotypical > autistic.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
Fear >neutral (t-contrast)					
1	Lingual gyrus	R	14 -70 -6	2892	17.38
2	Precuneus	L	-12 -58 60	288	4.67
3	Amygdala	L	-26 -4 -22	113	5.10
	Putamen	L	-28 4 2		4.13
	Olfactory cortex	L	-24 6 -18		4.11
	Temporal pole	L	-30 4 -22		3.69
	Insula lobe	L	-36 10 -8		3.67
4	Putamen	R	28 6 4	131	4.61
5	Superior parietal lobule	L	-14 -76 46	370	4.66
6	Amygdala	R	22 -4 -16	196	5.07
	Olfactory cortex	R	26 10 -16		3.72
	Insula Lobe	R	34 12 -14		3.27
Neutral >fear (t-contrast)					
1	Precuneus	R	14 -60 46	1709	5.15
	Superior parietal lobule	R	16 -66 54		4.99
	Angular gyrus	R	48 -62 36		4.39
	Superior occipital gyrus	R	26 -66 42		4.13
	Middle occipital gyrus	R	36 -74 34		4.08
2	Lingual gyrus	L	-10 -74 -4	2290	17.92
3	Precentral gyrus	L	-34 4 48	799	4.63
	IFG (pars triangularis)	L	-50 18 20		4.58
	Precentral gyrus	L	-36 6 40		4.27
	Middle frontal gyrus	L	-40 14 36		4.23
4	Inferior parietal lobule	L	-34 -58 44	430	4.53
5	IFG (pars triangularis)	R	52 32 28	171	4.10

Supplementary table 2.2. Local Maxima of significant clusters for emotion contrast: fear > neutral and neutral > fear.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
Systole >diastole (t-contrast)					
1	Thalamus	R	10 -26 14	340	3.91
	Hippocampus	R	22 -34 8		3.74

Supplementary table 2.3. Local Maxima of significant clusters for cardiac cycle contrast: systole > diastole, no diastole > systole activation was observed.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
neurotypical systole >Autism systole (t-contrast)					
1	Precentral gyrus	R	56 0 40	454	5.34
2	Posterior-medial frontal	L	-6 -18 58	337	3.94
	Paracentral lobule	L	-6 22 56		3.73
	Mid cingulate cortex	L	-6 -28 48		3.23
4	Rolandic operculum	L	-46 -6 14	82	4.59
	Precentral gyrus	L	-48 -4 26		3.76
5	Superior temporal gyrus	R	64 -42 22	134	4.07
	Supramarginal gyrus	R	64 -44 34		3.55
6	Middle temporal gyrus	L	-58 -58 20	221	3.88
7	Rolandic operculum	R	46 0 16	85	4.15
	Insula lobe	R	36 -10 18		3.58
neurotypical diastole >Autism diastole (t-contrast)					
1	Precentral gyrus	R	44 -2 34	592	6.08
2	Posterior-medial frontal	L	-6 -18 58	293	4.10
	Paracentral lobule	R	2 -32 62		4.09
	Mid cingulate cortex	L	-6 -32 44		3.36
3	Middle temporal gyrus	L	-58 -58 20	324	4.51
5	Precuneus	L	0 -54 20	198	4.00
	Posterior cingulate cortex	L	0 -52 24		3.91
	Mid cingulate cortex	L	0 -46 34		3.26
6	Rolandic operculum	L	-46 -6 14	269	4.24
	Precentral gyrus	L	-46 -4 26		4.20
	Insula lobe	L	-34 -12 18		3.51
	Postcentral gyrus	L	-42 -12 38		3.43
7	Supramarginal gyrus	L	-54 -46 34	245	4.22
	Inferior parietal lobule	L	-44 -46 38		3.77
8	Insula lobe	R	36 -8 16	476	4.33
	Rolandic operculum	R	40 -8 18		4.27
	Putamen	R	34 -6 6		3.52
9	Superior temporal gyrus	R	64 -44 20	289	3.87
	Supramarginal gyrus	R	62 -42 26		3.64
10	Angular gyrus	L	-46 -70 38	169	3.56
Autism systole >neurotypical systole (t-contrast)					
1	Cuneus	L	-6 -100 14	76	4.72

Supplementary table 2.4. Local Maxima of significant clusters for group by cardiac cycle interaction: neurotypical systole > autism systole; neurotypical diastole > autism diastole; autism systole > neurotypical systole.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
AQ + neutral systole (t-contrast)					
1	Middle frontal gyrus	L	-28 46 2	217	5.54
2	Superior temporal gyrus	L	-54 -6 -6		4.66
3	Angular gyrus	L	-46 -52 28	125	4.87
4	Middle temporal gyrus	L	-46 4 -28	211	5.07
	Temporal pole	L	-50 10 -20		4.81
	Medial temporal pole	L	-48 12 -26		4.66

Supplementary table 2.5. Local Maxima of significant clusters for correlation between autistic traits and activation when viewing neutral faces at systole.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
Fear sys – fear dias + depression/anxiety all (t-contrast)					
1	Cuneus	R	14 -76 26	181	5.12
	Superior occipital gyrus	R	26 -70 20		4.52
	Middle occipital gyrus	R	34 -82 24		3.48
2	Cerebellum (VI)	L	-28 -62 -22	244	4.47
3	Superior occipital gyrus	L	-12 -78 22	211	4.64
	Cuneus	L	-12 -78 34		3.83
4	Cerebellum (VI)	R	32 -58 -24	125	4.12

Supplementary table 2.6. Local Maxima of significant clusters for correlation between fear processing at systole (minus fear diastole) and depression, controlling for anxiety.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	F/t
Main effect left insula PPI with fear >neutral (F-contrast)					
1	Precuneus	L	-2 -54 70	391	29.04
2	Cerebellum	R	34 -68 -22	159	20.51
Main effect right insula PPI on systole trials (F-contrast)					
1	Inferior parietal lobule	R	44 -50 46	1468	29.67
	Angular gyrus	R	42 -60 50		25.73
	Superior parietal lobule	R	40 -58 62		15.77
2	Middle occipital gyrus	L	-24 -58 40	143	24.46
3	Middle frontal gyrus	R	48 12 50	684	18.38
	IFG (p. opercularis)	R	36 6 34		17.56
	Precentral gyrus	R	38 4 50		15.44
4	Superior medial gyrus	R	12 32 42	135	25.26
	Posterior-medial frontal	R	10 24 48		14.46
5	Middle temporal gyrus	R	56 -32 -6	84	23.83
	Inferior temporal gyrus	R	52 -48 -12		17.09
6	Calcarine gyrus	R	16 -80 10	81	16.22
Main effect of group right insula PPI on systole trials (F-contrast)					
1	Angular gyrus	R	48 -54 28	672	30.97
2	Inferior parietal lobule	L	-48 -54 50	138	22.19
	Angular gyrus	L	-48 -64 26		19.54
	Middle occipital gyrus	L	-36 -76 36		13.29
3	Precuneus	R	4 -48 40	376	20.42
	Precuneus	L	-4 -58 10		20.10
	Mid cingulate cortex	R	4 -50 34		18.11
	Posterior cingulate cortex	R	10 -50 30		15.91
4	Middle frontal gyrus	L	-28 22 48	246	24.54
	Superior frontal gyrus	L	-14 22 48		16.07
Group effect, neurotypical >autism right insula PPI systole trials (T-contrast)					
1	Angular gyrus	R	48 -54 28	866	5.57
	Supramarginal gyrus	R	48 -42 30		3.86
2	Inferior parietal lobule	L	-48 -54 50	214	4.71
	Angular gyrus	L	-48 -64 26		4.42
	Middle occipital gyrus	L	-36 -76 36		3.65
3	Precuneus	R	4 -48 40	546	4.52
	Precuneus	L	-4 -58 10		4.49
	Mid cingulate cortex	R	4 -50 34		4.26
	Posterior cingulate cortex	R	10 -50 30		3.99
	Mid cingulate cortex	L	-2 -40 40		3.61
4	Middle frontal gyrus	L	-28 22 48	408	4.95
	Superior frontal gyrus	L	-14 22 48		4.01

Supplementary table 2.7. Local maximum of significant clusters for PPI analysis. Psychophysiological interaction between BOLD activity and emotion, group and cardiac cycle. Seed regions were selected based on the general lineal model (GLM) findings.

Appendix C

Supplementary results 3

Supplementary results for chapter 4

All significant clusters were localised according to the Anatomy toolbox (Eickhoff et al., 2005) in SPM12. (L = left hemisphere, R = right hemisphere; x, y, z = co-ordinates of maximum activated voxel in standard MNI152 space; t stat at this voxel. Peaks are listed at $p<0.05$ FDR cluster corrected (cluster-forming threshold: $p<0.001$).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	F/t-score
Condition (F-contrast)					
1	Supramarginal Gyrus	L	-54 -32 30	3579	63.99
	Inferior parietal lobule	L	-58 -38 40		56.60
	Angular gyrus	L	-52 -58 33		24.66
	Middle temporal gyrus	L	-52 -70 21		19.08
2	Posterior-medial frontal	L	-10 -6 66	2976	48.42
	Posterior-medial frontal	R	12 -4 58		45.48
	Mid cingulate cortex	R	8 8 38		23.59
	Precentral gyrus	L	-30 -8 54		17.51
	Anterior cingulate cortex	L	-4 22 30		16.42
3	Middle frontal gyrus	L	-26 40 30	1515	42.03
	IFG (pars triangularis)	L	-34 36 12		23.61
	Superior frontal gyrus	L	-20 22 38		12.33
4	Supramarginal gyrus	R	62 -34 39	2454	36.69
	Inferior parietal lobule	R	58 -48 46		24.02
	Angular gyrus	R	50 -62 50		23.33
5	Rolandic operculum	L	-46 0 8	1336	47.73
	Insula lobe	L	-32 2 12		37.38
	Putamen	L	-24 -12 8		23.33
6	Insula lobe	R	36 8 10	464	41.46
	Putamen	R	28 0 10		26.29
	Pallidum	R	24 -4 6		23.60
	Rolandic operculum	R	56 6 8		21.65
7	Precuneus	L	-4 -54 48	1845	35.37
	Cuneus	L	-10 -60 24		16.13
	Precuneus	R	10 -50 36		12.52
8	Middle frontal gyrus	R	24 46 30	1268	25.83
9	Paracentral lobule	L	-4 -30 64	501	29.90
	Paracentral lobule	R	10 -32 70		19.60
	Postcentral gyrus	R	12 -34 82		18.91
10	Cerebellum (VI)	R	34 -46 -32	827	40.43
	Cerebellum (VIII)	R	30 -46 -44		26.87
	Cerebellum (VII)	R	38 -56 -44		12.15
11	Mid cingulate cortex	L	-10 -36 38	345	20.63
	Mid cingulate cortex	R	2 -26 32		19.36
12	Precentral gyrus	R	40 -28 70	453	24.00
	Postcentral gyrus	R	34 -24 48		20.66

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	F/t-score
Heart >Note (t-contrast)					
1	Supramarginal gyrus	L	-54 -32 30	4172	8.00
	Inferior parietal lobule	L	-58 -38 40		7.52
	Angular gyrus	L	-52 -54 28		4.97
	Middle temporal gyrus	L	-52 -66 16		4.37
2	Posterior-medial frontal	L	-10 -6 66	3538	6.96
	Posterior-medial frontal	R	12 -4 58		6.74
	Mid cingulate cortex	R	8 8 38		4.86
	Precentral gyrus	L	-30 -8 54		4.18
	Anterior cingulate cortex	L	-4 22 30		4.05
3	Middle frontal gyrus	L	-26 40 30	1722	6.48
	IFG (pars triangularis)	L	-34 36 12		4.86
	Superior frontal gyrus	L	-20 22 38		3.51
4	Precuneus	L	-4 -54 48	2793	5.95
	Mid cingulate cortex	L	-10 -36 38		4.54
	Mid cingulate cortex	R	2 -26 32		4.40
	Cuneus	L	-10 -60 24		4.02
5	Supramarginal gyrus	R	62 -30 34	1540	6.06
	Inferior parietal lobule	R	58 -48 46		4.90
	Angular gyrus	R	50 -62 50		4.83
6	Insula lobe	L	-32 2 12	531	6.11
	Putamen	L	-24 -12 8		5.53
	Caudate nucleus	L	-16 0 16		3.26
7	Insula lobe	R	36 8 10	2078	6.44
	Putamen	R	28 0 10		5.13
	Pallidum	R	24 -4 6		4.86
	Rolandic operculum	R	56 6 8		4.65
8	Middle frontal gyrus	R	24 46 30	2230	5.08
9	Cerebellum (VI)	R	34 -46 -32	1041	6.36
	Cerebellum (VIII)	R	30 -46 -44		5.18
	Cerebellum (VII)	R	38 -56 -44		3.49
Note >Heart (t-contrast)					
1	Paracentral lobule	L	-4 -30 64	616	5.47
	Paracentral lobule	R	10 -32 70		4.43
	Postcentral gyrus	R	12 -34 82		4.35
2	Precentral gyrus	R	40 -28 70	403	4.90
	Postcentral gyrus	R	34 -24 48		4.55

Supplementary table 3.1. Main effect of condition (heart versus note). Significant clusters according to the main effect (F-contrast) of condition, and significant t-contrasts for heart > note and note > heart.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
All participants, task (F-contrast); right insula					
1	Supramarginal gyrus	L	-56 -42 06	876	28.08
	Angular gyrus	L			
	Middle temporal gyrus	L			
	Middle temporal gyrus	L			
	Superior temporal gyrus	L			
	Planum temporale	L			
	Parietal operculum cortex	L			
2	Precentral gyrus	L	-30 -34 68	656	22.65
	Postcentral gyrus	L			
	Superior frontal gyrus	L			
	Precentral gyrus	R			
	Supp motor cortex	R			
3	Precentral gyrus	R	54 08 18	414	19.01
	Postcentral gyrus	R			
	Inferior frontal gyrus	R			
	Central opercular cortex	R			
4	Middle frontal gyrus	L	-40 14 24	329	17.72
	Inferior frontal gyrus	L			
	Precentral gyrus	L			
5	Angular gyrus	R	52 -44 16	295	17.34
	Supramarginal gyrus	R			
	Middle temporale gyrus	R			
	Lateral occipital cortex	R			
6	Lingual gyrus	R	10 -66 -14	187	16.53
	Cerebellum 6	R			
	Vermis 6	M			
	Vermis 4 5	M			
	Occipital fusiform gyrus	R			
	Cerebellum 4 5	R			
7	Central opercular cortex	L	-46 -06 06	184	15.89
	Insular cortex	L			
	Putamen	L			
	Heschl's gyrus	L			
	Planum polare	L			
8	Postcentral gyrus	L	-52 -26 48	131	14.86
	Supramarginal gyrus	L			
9	Precentral gyrus	L	-38 -02 46	94	14.27
	Middle frontal gyrus	L			
10	Postcentral gyrus	L	-56 -20 28	94	13.77
	Supramarginal gyrus	L			
11	Insula cortex	R	36 -04 12	89	12.79
	Central opercular cortex	R			
12	Superior frontal gyrus	R	18 -02 58	72	12.67
13	Occipital pole	L	-04 -102 16	49	9.84
All participants, heart >note; right insula					
1	Postcentral gyrus	L	-58 -22 28	121	4.70
	Supramarginal gyrus	L			
All participants, note >heart; right insula					
1	Angular gyrus	R	56 -54 18	144	4.43
	Lateral occipital cortex	R			
	Middle temporal gyrus	R			

Supplementary table 3.2. Functional connectivity of right insula across all participants during the interoception task (F-contrast) and when processing heart > note and note > heart trials (t-contrasts).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
All participants, task (F-contrast); left insula					
1	Postcentral gyrus	L	-48 -22 -49	2511	22.24
	Precentral gyrus	L			
	Superior parietal lobule	L			
	Superior frontal gyrus	L			
	Supramarginal gyrus	L			
	Occipital cortex	L			
	Middle frontal gyrus	L			
2	Angular gyrus	L	-38 -50 16	435	20.95
	Supramarginal gyrus	L			
	Middle temporal gyrus	L			
	Superior temporal gyrus	L			
3	Precentral gyrus	R	54 02 30	260	18.46
	Inferior frontal gyrus	R			
4	Central opercular cortex	L	-42 -06 10	193	16.58
	Insular cortex	L			
	Precentral gyrus	L			
	Inferior frontal gyrus	L			
	Heschl's gyrus	L			
5	Precuneus cortex	M	-04 -44 42	171	15.84
	Cingulate gyrus	M			
6	Middle frontal gyrus	L	-42 16 30	127	14.96
	Inferior frontal gyrus	L			
7	Posterior cingulate gyrus	M	06 -48 14	113	13.42
	Precuneus cortex	M			
8	Lateral occipital cortex	L	-38 -72 26	111	13.31
9	Lateral occipital cortex	L	-14 -92 40	110	12.64
	Occipital pole	L			
	Cuneal cortex	L			
10	Postcentral gyrus	R	54 -14 28	108	12.16
	Central opercular cortex	R			
11	Middle temporal gyrus	L	-54 -14 -12	108	11.82
	Inferior temporal gyrus	L			
	Superior temporal gyrus	L			
12	Temporal fusiform cortex	L	-32 -44 -14	105	11.29
	Lingual gyrus	L			
	Temporal fusiform cortex	L			
	Parahippocampal gyrus	L			
13	Occipital fusiform gyrus	L	-34 -80 02	80	9.92
	Lateral occipital cortex	L			
14	Occipital pole	R	12 -88 20	76	9.81
	Cuneal cortex	R			
15	Subcallosal cortex	M	-08 18 -06	75	9.75
	Accumbens	L			
	Caudate	L			
16	Occipital pole	L	-08 -90 00	46	9.71
	Intracalcarine cortex	L			
	Lingual gyrus	L			

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
All participants, heart >note; left insula					
1	Superior parietal lobule	L	-30 -38 38	425	5.16
	Postcentral gyrus	L			
2	Postcentral gyrus	R	58 -14 20	167	4.67
	Central opercular cortex	R			
	Supramarginal gyrus	R			
	Parietal operculum cortex	R			
3	Precentral gyrus	L	-28 -06 58	130	4.43
	Superior frontal gyrus	L			
	Middle frontal gyrus	L			
4	Precentral gyrus	R	56 02 32	113	4.39
All participants, note >heart; left insula					
1	Middle temporal gyrus	L	-52 -14 -10	306	5.60
	Inferior temporal gyrus	L			
	Superior temporal gyrus	L			
2	Lateral occipital cortex	L	-24 -92 26	199	4.51
	Occipital pole	L			
3	Occipital fusiform gyrus	L	-28 -74 -08	147	4.24
	Lateral occipital cortex	L			
	Lingual gyrus	L			
4	Occipital pole	L	-22 -90 36	97	4.23
	Lateral occipital cortex	L			
	Cuneal cortex	L			
5	Occipital pole	L	-08 -90 00	67	4.16
	Intracalcarine cortex	L			
	Lingual gyrus	L			

Supplementary table 3.3. Functional connectivity of left insula across all participants during the interoception task (F-contrast) and when processing heart > note and note > heart trials (t-contrasts).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
ITPE tracking, neurotypical participants, heart >note					
1	Superior temporal gyrus	R	60 -08 -04	508	5.08
BPQ, neurotypical participants, heart >note					
1	Anterior cingulate cortex	R	-04 28 08	630	5.05
	Anterior cingulate cortex	L	-4 42 00		4.46
	Superior medial gyrus	L	-14 38 20		3.87
	Mid orbital gyrus	R	06 40 -06		3.86
2	Middle frontal gyrus	L	-24 14 60	339	4.19

Supplementary table 3.4. Relationship between tracking ITPE and BPQ scores and BOLD activation in neurotypical participants.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Heartbeat tracking accuracy, neurotypical, right insula, heart >note					
1	Middle frontal gyrus	L	-32 44 42	128	4.95
	Frontal pole	L			
2	Supplementary motor cortex	R	00 00 52	75	4.54
	Cingulate gyrus	M			
	Supplementary motor cortex	L			
3	Putamen	L	-22 -04 04	63	5.46
	Pallidum	L			
	Insula cortex	L			
4	Superior frontal gyrus	L	-14 -02 74	56	5.82
	Precentral gyrus	L			
	Supplementary motor cortex	L			
Heartbeat tracking accuracy, neurotypical, left insula, heart >note					
1	Superior frontal gyrus	L	-16 38 50	153	4.98
	Frontal pole	L			
2	Middle frontal gyrus	L	-42 32 36	126	4.40
Heartbeat tracking accuracy, neurotypical >autistic, right insula, heart >note					
1	Putamen	L	-26 -14 -02	94	4.69
	Pallidum	L			
2	Middle frontal gyrus	L	-34 32 36	72	4.42
	Frontal pole	L			
Heartbeat tracking accuracy, neurotypical >autistic, left insula, heart >note					
1	Putamen	L	-24 00 -02	430	5.78
	Pallidum	L			
	Frontal orbital cortex	L			
2	Frontal pole	L	-26 58 02	165	5.39
3	Frontal pole	L	-04 62 24	74	4.17

Supplementary table 3.5. Relationship between functional connectivity of left and right insula with interoceptive accuracy.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Heartbeat discrimination insight, all participants, right insula, heart >note					
1	Middle temporal gyrus	R	48 -20 -06	214	5.38
	Superior temporal gyrus	R			
2	Middle temporal gyrus	L	-60 -14 -16	150	4.70
	Inferior temporal gyrus	L			
Heartbeat discrimination insight, all participants, left insula, heart >note					
1	Cerebellum 4 5	R	22 -34 -20	421	7.33
	Hippocampus	R			
	Parahippocampal gyrus	R			
	Cerebellum 3	R			
	Temporal fusiform cortex	R			
	Amygdala	R			
	Brain stem	M			
	Temporal fusiform cortex	R			
	Vermis 3	M			
	Parahippocampal gyrus	R			
	Vermis 4 5	M			
2	Parietal operculum cortex	R	32 -14 16	306	5.55
	Insula cortex	R			
	Heschl's gyrus	R			
	Planum temporale right	R			
	Central opercular cortex	R			
3	Postcentral gyrus	R	30 -28 62	214	4.36
	Precentral gyrus	R			
4	Middle temporal gyrus	R	62 -14 -16	214	4.81
	Superior temporal gyrus	R			
5	Cerebellum 4 5	L	-22 -40 -26	131	5.54
	Parahippocampal gyrus	L			
	Temporal fusiform gyrus	L			
	Brain stem	M			
6	Thalamus	R	14 -18 10	82	5.05
7	Middle temporal gyrus	L	-70 -26 -08	61	4.21
8	Brain stem	M	10 -22 -04	55	4.53
	Thalamus	R			
Heartbeat discrimination insight, autistic participants, right insula, heart >note					
1	Middle temporal gyrus	R	48 -20 -06	208	5.01
	Superior temporal gyrus	R			
2	Paracingulate gyrus	L	08 36 44	217	4.65
	Superior frontal gyrus	R			
	Paracingulate gyrus	R			
	Frontal pole	R			

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Heartbeat discrimination insight, autistic participants, left insula, heart >note					
1	Insula cortex	R	14 -26 00	780	7.83
	Thalamus	R			
	Parietal operculum cortex	R			
	Central operculum cortex	R			
	Heschl's gyrus	R			
	Planum temporale	R			
	Supramarginal gyrus	R			
	Brain stem	M			
	Planum polare	R			
	Putamen	R			
2	Middle temporal gyrus	R	58 -14 -20	92	5.40
	Inferior temporal gyrus	R			
Heartbeat discrimination insight, neurotypical participants, right insula, heart >note					
1	Frontal pole	R	38 60 10	99	4.80
Heartbeat discrimination insight, neurotypical participants, left insula, heart >note					
1	Cerebellum 4 5	R	22 -34 -22	200	6.51
	Temporal fusiform cortex	R			
	Parahippocampal gyrus	R			
	Lingual gyrus	R			
	Cerebellum 3	R			
2	Precentral gyrus	R	28 -28 64	69	4.86
	Postcentral gyrus	R			
Heartbeat discrimination insight, neurotypical >autistic, left insula, heart >note					
1	Occipital fusiform gyrus	R	28 -84 -14	230	5.20
	Lateral occipital cortex	R			
	Occipital pole	R			
	Cerebellum crus1	R			
2	Precuneus cortex	M	04 -64 54	184	4.75
	Lateral occipital cortex	R			
	Superior parietal lobule	R			

Supplementary table 3.6. Relationship between functional connectivity of left and right insula with heartbeat discrimination insight.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
BPQ, all participants, left insula, heart >note					
1	Cerebellum 6	R	18 -64 -18	89	5.43
	Lingual gyrus	R			
2	Cerebellum 6	L	-18 -54 -26	86	5.08
	Cerebellum 4 5	L			
BPQ, neurotypical participants, left insula, heart >note					
1	Inferior temporal gyrus	R	50 -54 -14	164	6.23
	Lateral occipital cortex	R			
	Temporal fusiform cortex	R			
	Middle temporal gyrus	R			

Supplementary table 3.7. Relationship between BPQ scores and functional connectivity of left and right insula.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Heartbeat tracking ITPE, neurotypical participants, right insula, heart >note					
1	Frontal orbital cortex	R	34 32 -08	73	5.09
	Frontal pole	R			
Heartbeat tracking ITPE, autistic participants, left insula, heart >note					
1	Cerebellum 8	R	34 -52 -40	127	5.36
	Cerebellum crus1	R			
	Cerebellum 7b	R			
	Cerebellum crus2	R			
Heartbeat discrimination ITPE, neurotypical participants, left insula, heart >note					
1	Parahippocampal gyrus	R	06 -06 -20	81	5.32
	Hippocampus	R			

Supplementary table 3.8. Relationship between heartbeat tracking ITPE and heartbeat discrimination ITPE with functional connectivity of left and right insula.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Trait anxiety, all participants, right insula, heart >note					
1	Cerebellum crus2	R	46 -64 -38	121	5.13
	Cerebellum crus1	R			
Trait anxiety, neurotypical participants, left insula, heart >note					
1	Brain stem	M	-08 -28 -46	120	6.61
	Cerebellum 9	R			

Supplementary table 3.9. Relationship between trait anxiety and functional connectivity of left and right insula.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
AQ, neurotypical participants, right insula, heart >note					
1	Occipital pole	R	28 -96 14	113	5.19
	Lateral occipital cortex	R			
AQ, neurotypical participants, left insula, heart >note					
1	Occipital pole	R	22 -96 12	160	4.85
	Lateral occipital cortex	R			
2	Occipital pole	L	-22 -92 10	65	4.19
	Lateral occipital cortex	L			
3	Brain stem	M	04 -30 -58	48	4.94

Supplementary table 3.10. Relationship between autistic traits and functional connectivity of left and right insula.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
TAS total, all participants, left insula, heart >note					
1	Cerebellum	L	-34 -44 -54	140	5.63
TAS total, autistic participants, right insula, heart >note					
1	Lateral occipital cortex	R	46 -72 -10	96	4.83
2	Frontal pole	L	-16 50 -16	68	5.65
	Frontal medial cortex	M			
TAS total, autistic >neurotypical, right insula, heart >note					
1	Parietal operculum cortex	L	-30 -30 28	97	5.27
2	Frontal pole	L	-16 48 -16	92	4.91
	Frontal medial cortex	M			

Supplementary table 3.11. Relationship between alexithymia and functional connectivity of left and right insula.

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
left insula, autistic participants (pre heart >pre note) <(post heart >post note)					
1	Middle temporal gyrus	L	-58 -02 -30	78	6.38
	Temporal pole	L			
	Inferior temporal gyrus	L			

Supplementary table 3.12. Significant functional connectivity of left insula for the contrast ((pre heart > pre note) < (post heart > post note)).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Right insula, autistic participants, heartbeat tracking accuracy					
1	Insular cortex	Left	-32 16 02	94	5.11
	Frontal operculum cortex	Left			
Left insula, autistic participants, heartbeat discrimination accuracy					
1	Anterior cingulate cortex	M	00 06 36	66	4.92
2	Superior frontal gyrus	L	-12 22 62	59	5.49
3	Anterior cingulate gyrus	M	02 24 22	44	4.49
right insula, autistic participants, heartbeat discrimination accuracy					
1	Frontal medial cortex	M	10 34 -12	78	4.60
	Paracingulate gyrus	R			
	Paracingulate gyrus	L			

Supplementary table 3.13. Relationship between change in interoceptive accuracy and functional connectivity of left and right insula for the contrast ((pre heart > pre note) < (post heart > post note)).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
left insula, autistic participants, MAIA emotional awareness					
1	Hippocampus	R	28 -36 00	62	5.61
right insula, autistic participants, MAIA emotional awareness					
1	Cerebellum 4 5	R	10 -56 -10	111	6.29
	Cerebellum 6	R			
	Lingual gyrus	R			
	Vermis 4 5	M			
	Vermis 6	M			
	Temporal fusiform cortex	R			
2	Lateral occipital cortex	R	40 -78 24	99	4.85

Supplementary table 3.14. Relationship between change in MAIA scores and functional connectivity of left and right insula for the contrast ((pre heart > pre note) < (post heart > post note)).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
right insula, autistic participants, discrimination ITPE					
1	Middle frontal gyrus	R	48 32 22	61	6.15
	Inferior frontal gyrus	R			
	Frontal pole	R			

Supplementary table 3.15. Relationship between change in ITPE on the discrimination task and functional connectivity of left and right insula for the contrast ((pre heart > pre note) < (post heart > post note)).

Cluster	Region	Hemisphere	Peak coordinates (mm; x y z)	Voxel count	F/t-score
Left insula, autistic participants, TAS total					
1	Middle frontal gyrus	R	32 14 58	97	5.89
	Superior frontal gyrus	R			
2	Superior Parietal Lobule	R	42 -50 62	82	5.24
	Angular gyrus	R			
	Lateral occipital cortex	R			
Left insula, autistic participants, PHQ-9					
1	Paracingulate gyrus	R	08 46 22	70	5.04
	Superior frontal gyrus	R			

Supplementary table 3.16. Relationship between change in alexithymia and depression and functional connectivity of left and right insula for the contrast ((pre heart > pre note) < (post heart > post note)).

Appendix D

Supplementary results 4

Supplementary results for chapter 5

All significant clusters were localised according to the Anatomy toolbox (Eickhoff et al., 2005) in SPM12. (L = left hemisphere, R = right hemisphere; x, y, z = co-ordinates of maximum activated voxel in standard MNI152 space; t stat at this voxel. Peaks are listed at $p<0.05$ FDR cluster corrected (cluster-forming threshold: $p<0.001$).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
Fear >neutral (t-contrast)					
1	Lingual gyrus	R	12 -70 -4	2282	10.02
	Cuneus	R	14 -82 26		4.18
2	Superior parietal lobule	L	-14 -74 46	147	5.15
	Superior occipital gyrus	L	-12 -86 44		4.62
3	Superior parietal lobule	L	-16 -60 62	602	4.71
	Precuneus	L	-16 -62 66		4.59
4	Middle occipital gyrus	L	-28 -82 14	531	4.25
5	Amygdala	L	-20 0 -18	96	5.17
	Middle temporal gyrus	L	-38 4 -28		3.86
6	Amygdala	R	28 -2 -13	468	4.45
Neutral >fear (t-contrast)					
1	Lingual gyrus	L	-10 -76 -2	829	10.71
2	Precentral gyrus	L	-38 4 53	110	3.98
	Middle frontal gyrus	L	-32 4 50		3.93

Supplementary table 4.1. Main effect of emotion for the t-contrasts of fear > neutral and neutral > fear.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	F/t-score
Session x cardiac cycle interaction (F-contrast)					
1	Anterior cingulate cortex	R	6 28 20	530	22.65
	Anterior cingulate cortex	L	-2 30 18		16.00
	Superior medial gyrus	R	20 46 6		15.88
2	IFG (pars triangularis)	R	40 16 28	124	16.87
	Middle frontal gyrus	R	48 12 50		16.68
	Precentral gyrus	R	48 0 42		16.08
	Middle frontal gyrus	R	44 10 48		16.08
3	Superior frontal gyrus	L	-16 46 34	421	17.32
	Superior medial gyrus	L	-4 54 28		16.74
	Middle frontal gyrus	L	-24 44 32		14.96
4	Mid cingulate cortex	R	2 -14 32	278	18.68
	Mid cingulate cortex	L	-2 -8 34		17.80
	Anterior cingulate cortex	L	-4 -2 30		17.66
5	Posterior-medial frontal	L	-6 16 66	296	17.04
	Posterior-medial frontal	R	8 18 66		15.60
	Superior frontal gyrus	R	18 18 66		12.41
6	Insula lobe	R	38 24 00	237	16.03
	IFG (pars opercularis)	R	50 18 6		15.94
7	IFG (pars opercularis)	L	-46 10 10	121	22.29
8	Superior frontal gyrus	R	16 36 46	160	15.35
	Superior medial gyrus	R	10 36 44		15.35
	Superior medial gyrus	L	0 48 40		13.16
Pre-systole >post-systole (t-contrast)					
1	Precuneus	L	-2 -48 70	264	4.05
	Precuneus	R	6 -54 62		4.00

Supplementary table 4.2. Session x cardiac cycle interaction (F-contrast) and significant activation for the t-contrast of pre-systole > post-systole.

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
Heartbeat discrimination accuracy ((fear systole post >fear diastole post) >(fear systole pre >fear diastole pre))					
1	Parahippocampal gyrus	R	24 -26 -16	114	6.09
2	Hippocampus	R	30 -30 -8	142	4.75

Supplementary table 4.3. Relationship between change in heartbeat discrimination accuracy and brain activation for the contrast ((fear systole post > fear diastole post) > (fear systole pre > fear diastole pre)).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
PPI right amygdala (post-fear>post-neutral) >(pre-fear>pre-neutral)					
1	Precentral gyrus	L	-24 -12 74	113	6.09
	Postcentral gyrus	L			
	Superior frontal gyrus	L			
PPI left insula (post-fear>post-neutral) >(pre-fear>pre-neutral)					
1	Vermis 3	M	00 -34 -12	70	5.53
	Brain-stem	M			
	Vermis 1 2	M			

Supplementary table 4.4. Functional connectivity of right amygdala and left insula for the contrast ((post-fear > post-neutral) > (pre-fear > pre-neutral)).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
PPI right amygdala (post-fear>post-neutral) >(pre-fear>pre-neutral) with heartbeat discrimination accuracy					
1	Parahippocampal gyrus	R	-18 -82 16	70	6.43
2	Occipital fusiform gyrus	R	30 -74 -02	40	5.74
	Lingual gyrus	R			
PPI left insula (post-fear>post-neutral) >(pre-fear>pre-neutral) with heartbeat discrimination accuracy					
1	Inferior frontal gyrus	R	56 26 22	101	6.54
	Middle frontal gyrus	R			
	Frontal pole	R			
PPI left amygdala (post-fear>post-neutral) >(pre-fear>pre-neutral) with heartbeat tracking accuracy					
1	Inferior frontal gyrus	R	52 12 06	113	6.28
	Precentral gyrus	R			
	Central opercular cortex	R			
	Frontal operculum cortex	R			
2	Central opercular cortex	L	-48 -06 00	65	5.92
	Planum polare	L			
	Insula cortex	L			
	Frontal operculum cortex	L			
3	Insula cortex	R	30 18 04	63	5.63
	Frontal operculum cortex	R			
4	Supplementary motor cortex	L	-06 -10 70	61	5.63
	Precentral gyrus	L			
	Superior frontal gyrus	L			
5	Lingual gyrus	L	-22 -42 -06	52	5.55
	Temporal fusiform cortex	L			
	Hippocampus	L			
6	Insula cortex	L	-32 20 02	36	4.82
	Frontal operculum cortex	L			
PPI right amygdala (post-fear>post-neutral) >(pre-fear>pre-neutral) with heartbeat tracking accuracy					
1	Insula cortex	L	-30 24 04	95	10.07
	Frontal operculum cortex	L			
	Frontal orbital cortex	L			
	Inferior frontal gyrus	L			
2	Middle frontal gyrus	L	-44 10 40	68	9.35
	Inferior frontal gyrus	L			
	Precentral gyrus	L			
3	Amygdala	L	-24 -04 -14	47	5.65
	Hippocampus	L			
PPI right insula (post-fear>post-neutral) >(pre-fear>pre-neutral) with heartbeat tracking accuracy					
1	Frontal pole	L	-52 34 06	54	8.26
	Inferior frontal gyrus	L			
	Frontal orbital cortex	L			
2	Temporal fusiform cortex	L	-38 -52 -06	50	6.10
	Lingual gyrus	L			
3	Inferior frontal gyrus	L	-36 30 -8	37	4.48
	Frontal operculum cortex	L			

Supplementary table 4.5. Relationship between change in interoceptive accuracy and functional connectivity of amygdala and insula cortices for the contrast ((post-fear > post-neutral) > (pre-fear > pre-neutral)).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
PPI left amygdala (post-fear>post-neutral) >(pre-fear>pre-neutral) with anxiety					
1	Cerebellum crus 2	L	-24 -82 -38	242	7.52
	Cerebellum crus 1	L			
	Cerebellum 6	L			
2	Cerebellum crus 1	R	34 -60 -28	79	5.60
	Cerebellum crus 2	R			
	Cerebellum 6	R			
3	Middle frontal gyrus	L	-34 18 56	50	5.48
PPI left insula (post-fear>post-neutral) >(pre-fear>pre-neutral) with anxiety					
1	Lateral occipital cortex	L	-48 -62 34	169	6.69
	Angular gyrus	L			
2	Lateral occipital cortex	R	38 -58 36	73	6.28
	Angular gyrus	R			
3	Middle temporal gyrus	R	50 -19 -14	72	6.14
	Superior temporal gyrus	R			
4	Cerebellum crus 2	R	08 -84 -26	35	5.59
	Cerebellum crus 1	R			
	Cerebellum crus 2	L			
5	Thalamus	R	04 -22 12	31	5.43
	Thalamus	L			
6	Frontal pole	L	-36 49 -10	28	5.27
PPI right insula (post-fear>post-neutral) >(pre-fear>pre-neutral) with anxiety					
1	Cerebellum 4 5	L	-12 -44 -16	43	9.29
	Cerebellum 6	L			
	Temporal fusiform cortex	L			
2	Middle temporal gyrus	L	-59 -24 -22	40	6.50
	Inferior temporal gyrus	L			
3	Postcentral gyrus	R	34 -36 64	36	5.96
	Superior parietal lobule	R			

Supplementary table 4.6. Relationship between change in anxiety and functional connectivity of amygdala and insula cortices for the contrast ((post-fear > post-neutral) > (pre-fear > pre-neutral)).

Cluster	Region	Hemisphere	Coordinates (mm; x y z)	Voxel count	t-score
PPI right insula (post systole >pre systole)					
1	Supramarginal gyrus	R	54 -30 42	243	6.91
	Postcentral gyrus	R			
2	Supramarginal gyrus	L	-52 -32 42	169	6.71
	Postcentral gyrus	L			
3	Precuneus	M	-10 -60 64	164	6.26
	Lateral occipital cortex	L			
	Superior parietal lobule	L			
4	Precuneus	M	-10 -72 50	98	6.16
	Lateral occipital cortex	L			
5	Lateral occipital cortex	L	-30 -88 34	79	6.10
	Occipital pole	L			
6	Superior parietal lobule	L	-28 -42 42	67	6.02
7	Insula cortex	R	40 08 04	63	6.01
	Central opercular cortex	R			
8	Lateral occipital cortex	R	50 -76 16	42	5.73
9	Supramarginal gyrus	R	36 -38 38	40	5.70
	Superior parietal lobule	R			
	Supramarginal gyrus	R			
	Postcentral gyrus	R			
10	Precuneus	M	18 -66 36	39	5.67
11	Cingulate gyrus	M	06 46 06	31	5.45
	Paracingulate gyrus	R			

Supplementary table 4.7. Relationship between change in anxiety and functional connectivity of right insula cortex for the contrast (post systole > pre systole).

Appendix E

Ethical approval

NHS
Health Research Authority

Prof Hugo Critchley
Chair in Psychiatry and Head of Neuroscience, Co-director of the Sackler Centre for Consciousness Science
Brighton & Sussex Medical School
University of Sussex
Falmer
BN1 9RR

Email: hra.approval@nhs.net

16 May 2017

Dear Prof Critchley

Letter of HRA Approval

Study title:	**Aligning Dimensions of Interoceptive Experience (ADIE) to prevent development of anxiety disorders in autism**
IRAS project ID:	**217819**
REC reference:	**17/WM/0125**
Sponsor	**NHS Sussex Partnership Foundation Trust**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators",* issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

IRAS project ID	217819

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **217819**. Please quote this on all correspondence.

Yours sincerely

Kevin Ahmed
Assessor

Telephone: 0207 104 8171
Email: hra.approval@nhs.net

Copy to: *Miss Taffy Bakasa, Sponsor Contact, Sussex Partnership NHS Foundation Trust*

brighton and sussex medical school

BSMS Research Governance Ethics Committee

Certificate of Approval	
Reference Number	ERA/BSMS9B9Y/2/3
Title Of Project	Interoception and Emotion in the Brain (AMENDMENT)
Principal Investigator (PI):	James Mulcahy
Student	James Mulcahy
Collaborators	Professor Sarah Garfinkel, Professor Hugo Critchley, Dr Lisa Quadt
Date Of Approval	02-Jun-2020
Approval Expiry Date	31-Dec-2020
RGEC Chair	Caroline Brooks
Name of Authorised Signatory	Prof Val Jenkins
Date	02-Jun-2020

The Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) has assessed your application and granted Ethical and Research Governance Approval to proceed with the above named project.

Approval is granted on the following basis:

Amendment to extend the study end date to 31/12/2020 to facilitate the recruitment of the remaining participants for the study.

Duration of Approval

Approval covers the period stated above. Research must commence within 12 months of the certificate start date; any delay beyond 12 months and this certificate of approval will lapse necessitating renewed review of the project.

Project Amendments

Any substantial changes or minor amendments to the project following issue of the certificate of approval should be submitted to the Research Governance and Ethics Committee for review and authorisation prior to implementation. Please submit your application for an amendment to the Committee (via rgec@bsms.ac.uk) using the Request for an Amendment Form.

Reporting Adverse and Unexpected Events

Any incidents occurring during the project's lifespan presenting ethical and safety implications must be reported immediately to the Chair of the Research Governance and Ethics Committee. In the event of an adverse (undesirable an unintended) and unexpected event occurring during the project, research must be stopped immediately and events reported to the Chair of the Research Governance and Ethics Committee within 24 hours of its occurrence.

Monitoring

The Medical School has a duty to ensure all its research is conducted in accordance with the University of Sussex's Code of Practice for Research and Research Governance and Ethical Review Framework. In order to ensure compliance auditing may be undertaken annually and /or periodic monitoring of a percentage of approved research studies. If your project is selected you will be given 4 weeks' notice to prepare all study documentation for inspection.

Notification of End of Study

Please notify the Research Governance and Ethics Committee once the study has completed. It is also your responsibility to inform the Committee in the event of early termination of the project or if the work is not completed.

Appendix F

Consent forms and information sheets

brighton and sussex medical school

Sussex Partnership NHS
NHS Foundation Trust

CONSENT FORM - Confidential

Title of project: **Testing a new therapy to prevent anxiety in autism spectrum conditions**

Name of chief investigator: **Prof Hugo Critchley**
Named researchers: Dr Sarah Garfinkel, Dr Clara Strauss, Dr Yoko Nagai

		Please initial box
1	I confirm that I have read and understood the information sheet '**Testing a new therapy to prevent anxiety symptoms**' and have had the opportunity to ask questions.	
2	I confirm that I have had sufficient time to consider whether or not I want to be included in the study.	
3	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my usual care being affected.	
4	I understand that any information I give is completely confidential and will be stored in such a way that it cannot be traced back to me. I agree that the data I provide will be anonymised and stored for further analysis (and that this data will be kept if I withdraw unless I specifically request it to be deleted).	
5	I understand that research data collected during the study may be looked at by individuals from the sponsor organisation, from regulatory authorities, and from the NHS Trust where it is relevant to my taking part in the study. I give permission for these individuals to have access to my research data.	
6	I do/do not (**please delete as appropriate**) agree to be contacted in the future to consider participating in further related research if I am suitable for such studies. I understand that I am under no obligation to be contacted or participate in further research. Phone number: _____ Email:_____ Address:_____ _____	
7	I give permission for my clinical care records to be accessed by members of the research team in relation to this study. All such information will remain strictly confidential.	
8	I do/do not (**please delete as appropriate**) wish to receive a copy of the final results of the study. If so, I would like to receive this by post/email (**please delete as appropriate**) and I agree to provide my contact details. These will be kept separately from the research data.	
9	I agree to take part in the above study	

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

brighton and sussex medical school

Sussex Partnership NHS
NHS Foundation Trust

IMAGING CONSENT FORM - Confidential

Title of project: **Testing a new therapy to prevent anxiety in autism spectrum conditions fMRI investigations**

Name of chief investigator: **Prof Hugo Critchley**
Named researchers: Dr Sarah Garfinkel, Dr Clara Strauss, Dr Yoko Nagai

Please initial box

1 I confirm that I have read and understood the information sheet '**Testing a new therapy to prevent anxiety symptoms, part 2 fMRI investigations**' and have had the opportunity to ask questions. ☐

2 I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐

3 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my usual care being affected. ☐

4 I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data I provide will be anonymised and stored for further analysis (and that this data will be kept if I withdraw unless I specifically request it to be deleted). ☐

5 I understand that research data collected during the study may be looked at by individuals from the sponsor organisation, from regulatory authorities, and from the NHS Trust where it is relevant to my taking part in the study. I give permission for these individuals to have access to my research data. ☐

6 I understand that if there are any unexpected findings that need further investigation you will, with my consent, inform my GP who will notify me if further tests are needed. ☐

7 I give permission for my clinical care records to be accessed by members of the research team in relation to this study. All such information will remain strictly confidential. ☐

8 I agree to take part in the above study ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

brighton and sussex medical school

CONSENT FORM - Confidential

Title of project: **Interoception and Emotion in the Brain**

Name of chief investigator: Dr Sarah Garfinkel
Named researchers: James Mulcahy, Dr Lisa Quadt

Please initial box

1 I confirm that I have read and understood the information sheet '**Interoception and Emotion in the Brain'** and have had the opportunity to ask questions. ☐

2 I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐

3 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason. ☐

4 I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data I provide will be anonymised and stored for further analysis (and that this data will be kept if I withdraw unless I specifically request it to be deleted). ☐

5 I understand that research data collected during the study may be looked at by individuals from the sponsor organisation and from regulatory authorities. I give permission for these individuals to have access to my research data. ☐

6 I understand that if there are any unexpected findings that need further investigation you will, with my consent, inform my GP who will notify me if further tests are needed. ☐

7 I agree to take part in the above study. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

Testing a new therapy to prevent anxiety symptoms in autism spectrum conditions

PARTICIPANT INFORMATION SHEET

We would like to invite you to take part in a research study investigating a new therapy to prevent anxiety symptoms in people with autism. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Part 1 explains the purpose of the study and what will happen to you if you take part. Part 2 gives more detailed information about how the study is run. This project is a collaboration between Sussex Partnership NHS Trust and Brighton and Sussex Medical School.

Take time to decide whether or not you wish to take part and please feel free to discuss your participation with friends and family. Please remember that your decision about whether to take part or not will not affect your care in any way.

What is the purpose of the study?
Some of our recent work has shown anxiety can be increased if there is a discrepancy between how well you feel you can interpret signals, such as your heartbeat, from your body and how well you are able to do this. We have found that helping people to be more aware of their ability, and to increase this helps reduce and may prevent anxiety symptoms. We would like to try out and compare a new treatment teaching you these skills against the current treatment.

Why have I been invited to take part?
We would like to invite people who have a diagnosis of autism spectrum condition**.**

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.

What will happen to me if I take part?
If you decide to take part, you will be invited to an initial interview where you will be fully briefed about the study. You will then be invited to a follow up interview where you have the opportunity to ask any questions you have about taking part. If you agree to take part, we will ask you to sign a consent form and then conduct a screening interview to make sure that you are eligible to take part. We will ask you to fill out a set of questionnaires. These will ask about symptoms you might have (e.g. anxiety, depression) and about the way in which you experience emotion and signals from your body. We will also administer a questionnaire which asks you questions about your interests and thought patterns, this will allow us to understand more about you and your autism. With your permission, we will inform your GP regarding your inclusion into the study.

As part of the study you will be randomly assigned to one of two therapy groups, receiving either an existing therapy to improve recognition of emotion from the way

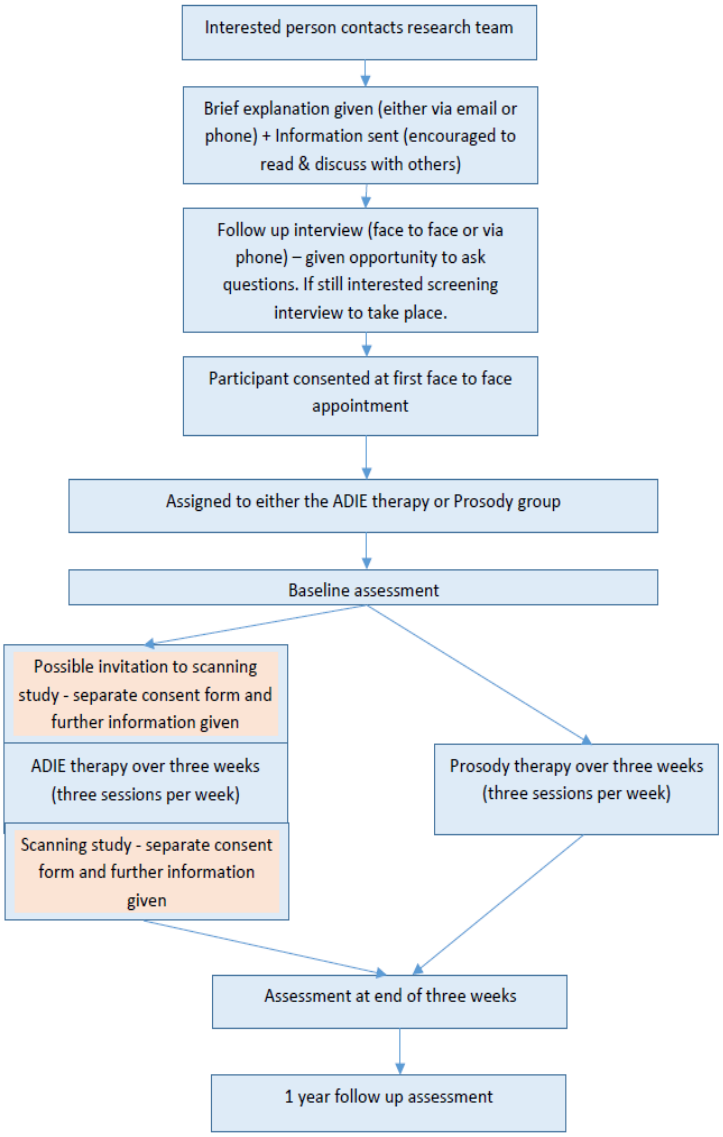
people say things, called prosody, or our new ADIE therapy. You will then receive training according to the group you have been assigned to.

Prosody therapy
You will receive an initial assessment where you will be asked to complete some computer tasks. The computer tasks require application of finger sensors to measure your pulse. You will then receive three therapy sessions per week for three weeks. These will focus on elements of speech such as intonation and rhythm. You will be played phrases which are spoken in ways which convey different emotions (e.g. happy, sad, fearful etc.). The aim of this therapy is to help you better identify the emotion underlying the way in which things are said. You will match phrases to different emotional faces and words, feedback will be provided to help you improve your perception of emotion from the way people say things.

ADIE therapy
You will be asked to complete some computer tasks. The computer tasks require application of finger sensors to measure your pulse. You will then receive three therapy sessions per week for three weeks. These will focus on your ability to read signals from inside your body (what we call interoception) with feedback and guidance. You will be asked to monitor your own heartbeat, but without physically feeling for it (i.e. just by sensing it internally). These tasks of interoception will ask you to count how many heartbeats you feel during a period of time or decide if you think a rhythmic beep is in time or out of time with your own heartbeat. We will give you feedback on how you have done in order to help you become more accurate in your awareness of your own heartbeats.

Both types of therapy will be accompanied by tasks which assess prosody (matching phrases to emotional faces or words), interoception (monitoring your own heartbeat), empathy (the ability to feel for others) and joint hypermobility (how bendy your joints are).

As compensation for your time spent taking part in the study, you will receive £7.50 per hour of your time. Assessment sessions take **approximately** 2 hours, training sessions take around 30 minutes. At the end of the study you will be debriefed and asked about your experience and have the opportunity to ask any questions.


Interested person contacts research team
Brief explanation given (either via email or phone) + Information sent (encouraged to read & discuss with others)
Follow up interview (face to face or via phone) – given opportunity to ask questions. If still interested screening interview to take place.
Participant consented at first face to face appointment
Assigned to either the ADIE therapy or Prosody group
Baseline assessment
Possible invitation to scanning study - separate consent form and further information given
ADIE therapy over three weeks (three sessions per week)
Scanning study - separate consent form and further information given
Prosody therapy over three weeks (three sessions per week)
Assessment at end of three weeks
1 year follow up assessment

Time Table Example (without brain scans)

Week	Day	Session	Content	Time
1	1	Baseline Assessment	• Consent Form • Questionnaires 1-3 • Computer Task 1 • Questionnaire 4 • Computer Task 2 • Questionnaires 5-8 • Computer Task 3 • Questionnaires 9-11	• 10 minutes • 15 minutes • 15 minutes • 10 minutes • 15 minutes • 15 minutes • 15 minutes • 15 minutes Total ~2 hours
	2	Training Session 1	• ECG computer task • Prosody or Interoception Training	• 30 minutes • 15-30 minutes Total ~1 hour
	3	Training Session 2	• Training	• 15-30 minutes
2	4	Training Session 3	• Training	• 15-30 minutes
	5	Training Session 4	• Training	• 15-30 minutes
3	6	Training Session 5	• Training	• 15-30 minutes
	7	Training Session 6	• Prosody or Interoception Training • ECG computer task	• 15-30 minutes • 30 minutes Total ~1 hour
	8	Final Assessment	• Questionnaires 1-3 • Computer Task 1 • Questionnaire 4 • Computer Task 2 • Questionnaires 5-7 • Computer Task 3 • Questionnaires 8-10 • Reading Task	• 15 minutes • 15 minutes • 10 minutes • 15 minutes • 15 minutes • 15 minutes • 15 minutes • 5 minutes Total ~2 hours
After 3 months		3 months follow-up	• 3 Online Questionnaires	• 15 minutes
After 1 year		1 year follow-up	• Questionnaires 1-3 • Computer Task 1 • Questionnaire 4 • Computer Task 2 • Questionnaires 5-7 • Computer Task 3 • Questionnaires 8-10 • Computer Task 4	• 15 minutes • 15 minutes • 10 minutes • 15 minutes • 15 minutes • 15 minutes • 15 minutes • 15 minutes Total ~2 hours

What are the possible risks in taking part?
As far as we know there are no risks to taking part. Information from the study will be protected and anonymous so that people will not have access to the information about who took part or find out results of any one individual.

What are the possible benefits of taking part?
Anxiety symptoms are common in people with autism spectrum conditions and we anticipate that the training you receive will help reduce or prevent any anxiety symptoms you may experience. This research could result in new ways of treating and preventing anxiety in people with autism spectrum conditions.

What will happen if I don't want to carry on with the study?
You may withdraw at any point during the study. If you withdraw from the study we would like, with your consent, to still use the data and results associated with your participation. You are free to not consent to us using data associated with your participation in which case all results will be securely deleted. This will not affect your future care in any way.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers (Dr Sarah Garfinkel: 01273 678584; Dr Clara Strauss: 01273 265896; Dr Yoko Nagai: 01273 876828) or the chief investigator (Prof Hugo Critchley: 01273 678336) in the first instance, who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this either by contacting the Research and Development department at Sussex Partnership NHS Trust (01273 265896) or the Service Experience Team - also known as PALS (01903 843026).

Any complaint about the way you have been dealt with during the study or any issues will be taken very seriously. If taking part in this research project harms you, then you may have grounds for legal action.

Will my taking part in the study be kept confidential?
Yes. We want to emphasise that all results obtained will be strictly confidential and will only be used for research purposes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual participants will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 10 years. After 10 years, this information will be securely destroyed.

What will happen to the results of the research study?
The results will be anonymised (removed of identifying information) and kept in a locked office at Brighton and Sussex Medical School.

The results of the questionnaires, along with all other information collected from you during this research will be kept strictly confidential. The results will be statistically analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication. You are welcome to a copy of any publication resulting from this work which can be obtained by giving us your email address or postal address.

Who has funded this study?
This study is funded by a grant from the MQ Transforming mental health through research charity. Their research aims include finding ways to prevent mental illness, such as anxiety, from developing. Their web page about the study can be found here:

https://www.mqmentalhealth.org/research/profiles/breaking-the-link-between-autism-and-anxiety

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests.

Contact for further Information
Many thanks for reading this. We hope you feel able to take part in our study. If you have any questions, please contact the following people:

[RA or named researcher contact details]

Prof Hugo Critchley (Chief investigator): H.Critchley@bsms.ac.uk
01273 678336

Testing a new therapy to prevent anxiety symptoms in autism spectrum conditions: fMRI investigations

PARTICIPANT INFORMATION SHEET

We would like to invite you to take part in a research study investigating a new treatment to prevent anxiety symptoms in people with autism. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Part 1 explains the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about how the study is run. This project is a collaboration between Sussex Partnership NHS Trust and Brighton and Sussex Medical School.

Take time to decide whether or not you wish to take part and please feel free to discuss your participation with friends and family. Please remember that your decision about whether to take part or not will not affect your care in any way.

What is the purpose of the study?
Some people are likely to respond to our new therapy, which we are calling ADIE (Aligning Dimensions of Interoceptive Experience), better than others. We would like to take brain scans of people participating in our study to see if we can understand why this might be. We will use a non-invasive technique called functional Magnetic Resonance Imaging (fMRI) to study your brain. This technique uses a magnetic field to produce high quality images of the brain without the use of harmful radiation.

Knowing why some respond better than others will help us improve the therapy we give. We also plan on using these scans to increase awareness among doctors that people with Autism Spectrum Conditions (ASC) can profoundly benefit from psychological therapies.

Why have I been invited to take part?
We would like to invite adults who have a diagnosis of autism spectrum condition.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.

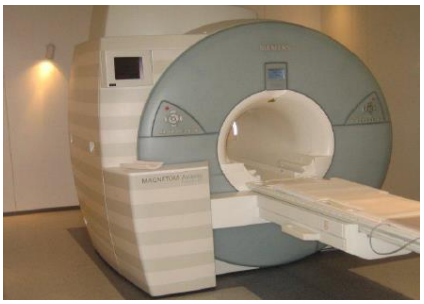
What will happen to me if I take part?

The study involves you coming to the Clinical Imaging Sciences Centre, on the Falmer campus of the University of Sussex. Before you arrive, you will be asked to remove any piercings you may have. When you arrive, you will first be asked to fill in a questionnaire to ensure there are no contraindications to your having a MRI scan. MRI is a widely used and safe technique. However, we usually exclude people with metal implanted in their body for research scans but they may still be able to have medical scans. Fixed dental work is usually safe.

We will then take you to the Trafford Centre (which is just opposite the Clinical Imaging Sciences Centre) into one of the testing rooms where we will show you a

short presentation that will explain the three tasks that you will be undertaking whilst in the scanner. You will be able to ask any questions you may have.

Before going back to the Clinical Imaging Sciences Centre to conduct the scan, we will ask you to lie down and rest for 5 minutes. You will get electrodes attached to your chest and back, and put on a finger monitor. This is so we can get a measure of your pulse, which we need for the scanning session.



The MRI scanner is a short tube with a strong magnet inside. Before going into the scanner, we will ask you to remove any metal items (e.g. watch, earrings, jewellery). If you wear glasses, we will ask you to take them off and we will give you a pair of MRI glasses of a similar strength to wear. We will then ask you to lie down on the scanner bed and we will place a coil over your head which will help you to keep your head still. The bed will then move slowly backwards into the scanner. We will attach a monitor to your finger to record your pulse, and we will give you a button box to hold in your hand. During the task, you can use this button box to indicate your responses. As the scanner is very noisy when it is running we will give you earplugs and headphones to wear. Once you are ready we will start the scanner. We will collect some structural pictures of your brain, in addition to scans that measure brain activity during the tasks. The scanning session will last approximately 60 minutes. Some people may experience discomfort during the scan and you will be given a red button to press if you wish to stop. You can stop the scan at any time and it will not affect your further participation in the study or your care at all.

During the scan, you will complete the three tasks that have previously been explained to you. One involves you viewing a series of faces and being asked to judge their emotional intensity, one requires you to follow a circle on the screen with your eyes and indicate when the circle changes colour, and for the final task you will ~~also~~ be asked to perceive or judge your own heart beat in different conditions.

At the end of the study you will have one final questionnaire to complete and then you will be debriefed and asked about your experience and you will be able to ~~can~~ ask any questions. As compensation for your time spent taking part in the study, you will receive £7.50 per hour of your time.

The neuroimaging part of this study takes place over two sessions. The first and second session are both the same and follow the same structure that has just been described. The second scan will take place roughly three weeks after the first scan, following completion of interoceptive training.

What are the possible risks in taking part?
Providing there are no contraindications, MRI is entirely safe. The brain scans are not used for medical diagnosis. In the very unusual event that a significant anomaly is found on your brain scan, a medical doctor would complete a formal medical report on the scan and may request a clinical scan. Both you and your GP would then be informed of the findings.

What are the possible benefits of taking part?
Taking part is of no direct benefit to you but this research could result in new ways of treating and preventing anxiety in people with autism spectrum conditions.

What will happen if I don't want to carry on with the study?
You may withdraw at any point during the study. If you withdraw from the study we would like, with your consent, to still use the data and results associated with your participation. This will not affect your future care in any way.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers (Dr Sarah Garfinkel: 01273 678584; Dr Clara Strauss: 01273 265896; Dr Yoko Nagai: 01273 876828) or the chief investigator (Prof Hugo Critchley: 01273 678336) in the first instance, who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this either by contacting the Research and Development department at Sussex Partnership NHS Trust (01273 265896) or the Service Experience Team - also known as PALS (01903 843026).

Any complaint about the way you have been dealt with during the study or any issues will be taken very seriously. If taking part in this research project harms you, then you may have grounds for legal action.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual participants will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 10 years. After 10 years, this information will be securely destroyed.

What will happen to the results of the research study?
The results will be anonymised (removed of identifying information) and kept in a locked office at Brighton and Sussex Medical School. The results will be analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication. You are more than welcome to a copy of any publication resulting from this work which can be obtained by giving us your email address or postal address.

Who has funded this study?
This study is funded by a grant from the MQ Transforming mental health through research charity. Their research aims include finding ways to prevent mental illness, such as anxiety, from developing. Their web page about the study can be found here: https://www.mqmentalhealth.org/research/profiles/breaking-the-link-between-autism-and-anxiety

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests.

Contact for further Information
Many thanks for reading this. We hope you feel able to take part in our study. If you have any questions, please contact the following people: Dr Lisa Quadt, Tel: 01273 876771, Email: L.Quadt@bsms.ac.uk. James Mulcahy, Email: J.Mulcahy@bsms.ac.uk.

Prof Hugo Critchley (Chief investigator): H.Critchley@bsms.ac.uk
01273 678336

brighton and sussex medical school

Version 1. 25/09/2018

Interoception and Emotion in the Brain

PARTICIPANT INFORMATION SHEET

We would like to invite you to take part in a research study investigating interoception and emotional processing. Your data will be compared to an ongoing study which is investigating interoceptive ability in autistic individuals. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Part 1 explains the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about how the study is run.

Take time to decide whether or not you wish to take part and please feel free to discuss your participation with friends and family.

What is the purpose of the study?

Currently underway is a project titled Aligning Dimensions of Interoceptive Experience (ADIE) to prevent anxiety in autism which aims to test a new therapy, "ADIE". The "ADIE" therapy is designed to reduce anxiety in autistic people by training them to better interpret arousal changes inside the body, such as a faster heartrate. The "ADIE" project will scan 40 participants pre and post "ADIE" training. The participants recruited from this study will form a control group so data from the first session from the "ADIE" project can be compared with the data collected from this study. The aim of this study is to therefore examine the difference in brain activation between autistic individuals and controls on three tasks examining interoception and emotional processing. We will also compare scores from a number of questionnaires as well as responses on computer based tasks that investigate emotional processing.

Why have I been invited to take part?
You have been invited to take part because you are over the age of 18 and do not have a diagnosis of autism.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and will be sent an eligibility questionnaire to complete. If you are eligible, you will be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.

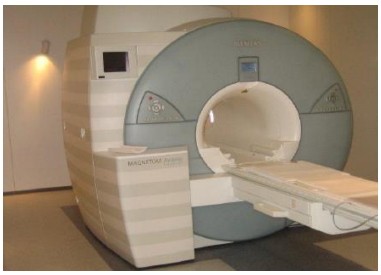
What will happen to me if I take part?

The study will involve you coming to the University of Sussex for 2, 2 hour sessions. If you agree to take part we will conduct a screening interview (via phone or email) to make sure that you are eligible to take part. Provided you are eligible we will invite you to the Trafford Centre at the University of Sussex for your first session. During this session you will complete a number of questionnaires and will be asked to undertake three tasks that require you to wear a pulse oximeter and/or an ECG which measure the activity of your heart.

For the second session you will come to the Clinical Imaging Sciences Centre, on the Falmer campus of the University of Sussex. Before you arrive, you will be asked to remove any piercings you may have. When you arrive, you will first be asked to fill in a questionnaire to ensure there are no contraindications to your having a MRI scan. MRI is a widely used and safe technique. However, we usually exclude people with metal implanted in their body for research scans but they may still be able to have medical scans. Fixed dental work is usually safe.

We will then take you to the Trafford Centre (which is just opposite the Clinical Imaging Sciences Centre) into one of the testing rooms where we will show you a short presentation that will explain the three tasks that you will be undertaking whilst in the scanner. You will be able to ask any questions you may have.

Before going back to the Clinical Imaging Sciences Centre to conduct the scan, we will ask you to lie down and rest for 5 minutes. You will get electrodes attached to your chest and back, and put on a finger monitor. This is so we can get a measure of your pulse, which we need for the scanning session.



The MRI scanner is a short tube with a strong magnet inside. Before going into the scanner, we will ask you to remove any metal items (e.g. watch, earrings, jewellery). If you wear glasses, we will ask you to take them off and we will give you a pair of MRI glasses of a similar strength to wear. We will then ask you to lie down on the scanner bed and we will place a coil over your head which will help you to keep your head still. The bed will then move slowly backwards into the scanner. We will attach a monitor to your finger to record your pulse, and we will give you a button box to hold in your hand. During the task, you can use this button box to indicate your responses. As the scanner is very noisy when it is running we will give you earplugs and headphones to wear. Once you are ready we will start the scanner. We will collect some structural pictures of your brain, in addition to scans that measure brain activity during the tasks. The scanning session will last approximately 60 minutes. Some people may experience discomfort during the scan and you will be given a button to press if you wish to stop. You can stop the scan at any time and it will not affect your further participation in the study.

During the scan, you will complete the three tasks that have previously been explained to you. One involves you viewing a series of faces and being asked to judge their emotional intensity, one requires you to follow a circle on the screen with your eyes and indicate when the circle changes colour, and for the final task you will be asked to perceive or judge your own heart beat in different conditions.

- At the end of the study you will have one final questionnaire to complete and then you will be debriefed and asked about your experience and you will be able to ask any questions. **As compensation for your time spent taking part in the study, you will receive £30.00.**

What are the possible risks in taking part?

Some of the questionnaires you will be given include questions on sensitive issues and there is a possibility that they may induce distress. It is important to note that you do not have to complete any part of this study if you feel it may cause you distress. You are free to withdraw from the study at any point, without giving a reason. Should you feel the questionnaires have impacted your mental health in anyway then we will, with your consent, contact your GP who will be able to direct you towards the appropriate support.

Providing there are no contraindications, MRI is entirely safe. The brain scans are not used for medical diagnosis. In the very unusual event that a significant anomaly is found on your brain scan, a medical doctor would complete a formal medical report on the scan and may request a clinical scan. Both you and your GP would then be informed of the findings.

What are the possible benefits of taking part?
Taking part is of no direct benefit to you but this research could highlight differences in the way people process emotion and perceive their own bodily sensations.

What will happen if I don't want to carry on with the study?
You may withdraw at any point during the study. If you withdraw from the study we would like, with your consent, to still use the data and results associated with your participation.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers (Dr Sarah Garfinkel: 01273 678584) who will do their best to answer your questions.

Any complaint about the way you have been dealt with during the study or any issues will be taken very seriously. If taking part in this research project harms you, then you may have grounds for legal action.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual participants will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 5 years. After 5 years, this information will be securely destroyed.

What will happen to the results of the research study?
The results will be anonymised (removed of identifying information) and kept in a locked office at Brighton and Sussex Medical School. The results will be analysed and findings subsequently published in peer reviewed journals. You will not be identified in any publication. You are more than welcome to a copy of any publication resulting from this work which can be obtained by giving us your email address or postal address.

Who has reviewed the study?
This research has been reviewed and approved by the Brighton and Sussex Medical School research ethics committee.

Contact for further Information
Many thanks for reading this. We hope you feel able to take part in our study. If you have any questions, please contact James Mulcahy, Email: J.Mulcahy@bsms.ac.uk.

Appendix G

Self-report measures

Interoceptive Testing

Participant ID:.....

CSO:.....

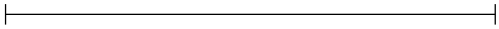
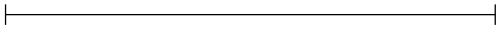
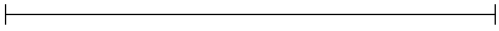
Mental Tracking Heartbeat

Please mark each of the lines below with a single downward stroke, to indicate how confident you are with the answer you give in the heartbeat mental tracking task.

Total guess — *No heartbeat awareness*

Complete confidence — *Full perception of heartbeat*

Example. 

1. 
2. 
3. 
4. 
5. 
6. 

Interoceptive Testing

Participant ID:.....

CSO:.....

Mental Tracking Heart Beat Beliefs

1. Do you know what a heart rate is?

- ☐ Yes
- ☐ No

2. Do you know what your heart rate is?

- ☐ Yes, it is _____
- ☐ No

Interoceptive Testing

Participant ID:……..

CSO:……..

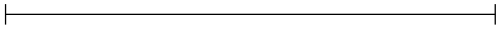
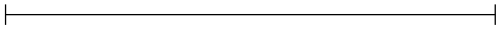
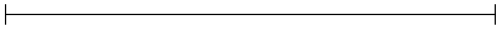
Mental Tracking Time

Please mark each of the lines below with a single downward stroke, to indicate how confident you are with the answer you give in the mental time tracking task.

Total guess — **Complete confidence**

No time awareness — *Full perception of time*

Example. 

1. 
2. 
3. 
4. 
5. 
6. 

Interoceptive Testing

Participant ID:……..

CSO:……..

Heartbeat Perception

Please mark each of the lines below with a single downward stroke, to indicate how confident you are with the answer you give in the heartbeat perception task.

Total guess — **Complete confidence**

No heartbeat awareness — *Full perception of heartbeat*

1. ├──┤
2. ├──┤
3. ├──┤
4. ├──┤
5. ├──┤
6. ├──┤
7. ├──┤
8. ├──┤
9. ├──┤
10. ├──┤
11. ├──┤
12. ├──┤
13. ├──┤
14. ├──┤
15. ├──┤
16. ├──┤
17. ├──┤
18. ├──┤
19. ├──┤
20. ├──┤

BODY PERCEPTION QUESTIONNAIRE

Stephen W. Porges, Ph.D.

The BODY PERCEPTION QUESTIONNARIE has five sub-tests: 1) Awareness, 2) Stress Response, 3) Autonomic Nervous System Reactivity, 4) Stress Style, and 5) Health History Inventory. Each of the 122 items in the BODY PERCEPTION QUESTIONNARE are to be answered on the 5-point scoring scale described in the beginning of each sub-test. Read the instructions for each sub-test and designate your answers for each of the 122 items on the provided answer sheet. Since the BODY PERCEPTION QUESTIONNAIRE will be scored by a computer, use a #2 pencil and make heavy black marks that fill the circle completely. Do not use ink or ballpoint pens. Erase cleanly any answer you wish to change and make no stray marks on the answer sheet.

I: AWARENESS

Image how aware you are of your body processes. Select the answer that most accurately describes you. Rate your awareness on each of the characteristics described below using the following 5-point scale:

a) Never b) Occasionally c) Sometimes d) Usually e) Always

During most situations I am aware of:

1. Swallowing frequently
2. A ringing in my ears
3. An urge to cough to clear my throat
4. My body swaying when I am standing
5. My mouth being dry
6. How fast I am breathing
7. Watering or tearing of my eyes
8. My skin itching
9. Noises associated with my digestion
10. Eye fatigue or pain
11. Muscle tension in my back and neck
12. A swelling of my body or parts of my body
13. An urge to urinate
14. Tremor in my hands
15. An urge to defecate
16. Muscle tension in my arms and legs
17. A bloated feeling because of water retention
18. Muscle tension in my face
19. Goose bumps
20. Facial twitches
21. Being exhausted
22. Stomach and gut pains
23. Rolling or fluttering my eyes
24. Stomach distension or bloatedness
25. Palms sweating
26. Sweat on my forehead
27. Clumsiness or bumping into people

28. Tremor in my lips
29. Sweat in my armpits
30. Sensations of prickling, tingling, or numbness in my body
31. The temperature of my face (especially my ears)
32. Grinding my teeth
33. General jitteriness
34. Muscle pain
35. Joint pain
36. Fullness of my bladder
37. My eye movements
38. Back pain
39. My nose itching
40. The hair on the back of my neck "standing up"
41. Needing to rest
42. Difficulty in focusing
43. An urge to swallow
44. How hard my heart is beating
45. Feeling constipated

II: STRESS RESPONSE

Imagine yourself in a very stressful situation or during periods of severe stress. Using the following 5-point scale, rate your awareness of perceived changes due to stress in each of the global response systems described below

a) Never b) Occasionally c) Sometimes d) Usually e) Always

During stressful situations I am aware of:

46. Vascular responses such as my face becoming flushed or pallid, or feeling faint.
47. Body posture shifts such as being hunched over, head down, and knees locked.
48. Muscle tone or tremor such as arms and legs feeling weak, hands shaking, and lips quivering.
49. Breathing more rapidly and shallowly, and having difficulty in catching my breath.
50. Digestive responses including gastric distress, gas, cramps, and diarrhea.
51. Difficulty in paying attention with my mind wondering or daydreaming.
52. Difficulties in sensory abilities such as problems hearing, seeing, smelling, or feeling touch.
53. Emotional problems such as more frequent feelings of depression, frustration, rage, or anger.
54. Difficulty organizing my thoughts.
55. Difficulty speaking clearly and understandably.

III: AUTONOMIC NERVOUS SYSTEM REACTIVITY

The autonomic nervous system is the part of your nervous system that controls your cardiovascular, respiratory, digestive, and temperature regulation systems. It is also involved in the experience and expression of emotions. The autonomic nervous system functions differently among people. This scale has been developed to measure how your autonomic nervous system reacts.

Multidimensional Assessment of Interoceptive Awareness (MAIA)

Contact: Wolf E. Mehling, MD
Osher Center for Integrative Medicine
University of California, San Francisco
1545 Divisadero St., 4th floor
San Francisco, CA 94115
Phone: 01 (415) 353 9506
mehlingw@ocim.ucsf.edu
http://www.osher.ucsf.edu/maia/

Multidimensional Assessment of Interoceptive Awareness

Permission and Copyright

Although the MAIA survey is copyrighted, it is available without charge and no written permission is required for its use. This assumes agreement with the following as a consequence of using a MAIA survey:

- Please refer to the survey using its complete name – Multidimensional Assessment of Interoceptive Awareness - and provide the appropriate citation.
- Modifications may be made without our written permission. However, please clearly identify any modifications in any publications as having been made by the users. If you modify the survey, please let us know for our records.
- We recommend including entire subscales when selecting items from the MAIA to retain the psychometric features of these subscales (rather than selecting items from subscales).
- If you translate the MAIA into another language, please send us a copy for our records.
- If other investigators are interested in obtaining the survey, please refer them to the source document (PLoS-ONE 2012, and www.osher.ucsf.edu/maia/) to assure they obtain the most recent version and scoring instructions.

Scoring Instructions

Take the average of the items on each scale.
Note: Reverse-score items 5, 6, and 7 on Not-Distracting, and items 8 and 9 on Not-Worrying.

1. **Noticing:** Awareness of uncomfortable, comfortable, and neutral body sensations
   Q1_____ + Q2_____ + Q3_____ + Q4_____ / 4 = _____

2. **Not-Distracting:** Tendency not to ignore or distract oneself from sensations of pain or discomfort
   Q5(**reverse**)_____ + Q6(**reverse**)_____ + Q7(**reverse**)_____ / 3 = _____

3. **Not-Worrying:** Tendency not to worry or experience emotional distress with sensations of pain or discomfort
   Q8(**reverse**)_____ + Q9(**reverse**)_____ + Q10_____ / 3 = _____

4. **Attention Regulation:** Ability to sustain and control attention to body sensations
   Q11_____ + Q12_____ + Q13_____ + Q14_____ + Q15_____ + Q16_____ + Q17_____ / 7 = _____

5. **Emotional Awareness:** Awareness of the connection between body sensations and emotional states
   Q18_____ + Q19_____ + Q20_____ + Q21_____ + Q22_____ / 5 = _____

6. **Self-Regulation:** Ability to regulate distress by attention to body sensations
   Q23_____ + Q24_____ + Q25_____ + Q26_____ / 4= _____

7. **Body Listening:** Active listening to the body for insight
   Q27_____ + Q28_____ + Q29_____ / 3= _____

8. **Trusting:** Experience of one's body as safe and trustworthy
   Q30_____ + Q31_____ + Q32_____ / 3= _____

Below you will find a list of statements. Please indicate how often each statement applies to you generally in daily life.

.	Circle one number on each line					
	Never					Always
1. When I am tense I notice where the tension is located in my body.	0	1	2	3	4	5
2. I notice when I am uncomfortable in my body.	0	1	2	3	4	5
3. I notice where in my body I am comfortable.	0	1	2	3	4	5
4. I notice changes in my breathing, such as whether it slows down or speeds up.	0	1	2	3	4	5
5. I do not notice (I ignore) physical tension or discomfort until they become more severe.	0	1	2	3	4	5
6. I distract myself from sensations of discomfort.	0	1	2	3	4	5
7. When I feel pain or discomfort, I try to power through it.	0	1	2	3	4	5
8. When I feel physical pain, I become upset.	0	1	2	3	4	5
9. I start to worry that something is wrong if I feel any discomfort.	0	1	2	3	4	5
10. I can notice an unpleasant body sensation without worrying about it.	0	1	2	3	4	5
11. I can pay attention to my breath without being distracted by things happening around me.	0	1	2	3	4	5
12. I can maintain awareness of my inner bodily sensations even when there is a lot going on around me.	0	1	2	3	4	5
13. When I am in conversation with someone, I can pay attention to my posture.	0	1	2	3	4	5
14. I can return awareness to my body if I am distracted.	0	1	2	3	4	5
15. I can refocus my attention from thinking to sensing my body.	0	1	2	3	4	5
16. I can maintain awareness of my whole body even when a part of me is in pain or discomfort.	0	1	2	3	4	5

Please indicate how often each statement applies to you generally in daily life.

	Circle one number on each line					
	Never					Always
17. I am able to consciously focus on my body as a whole.	0	1	2	3	4	5
18. I notice how my body changes when I am angry.	0	1	2	3	4	5
19. When something is wrong in my life I can feel it in my body.	0	1	2	3	4	5
20. I notice that my body feels different after a peaceful experience.	0	1	2	3	4	5
21. I notice that my breathing becomes free and easy when I feel comfortable.	0	1	2	3	4	5
22. I notice how my body changes when I feel happy / joyful.	0	1	2	3	4	5
23. When I feel overwhelmed I can find a calm place inside.	0	1	2	3	4	5
24. When I bring awareness to my body I feel a sense of calm.	0	1	2	3	4	5
25. I can use my breath to reduce tension.	0	1	2	3	4	5
26. When I am caught up in thoughts, I can calm my mind by focusing on my body/breathing.	0	1	2	3	4	5
27. I listen for information from my body about my emotional state.	0	1	2	3	4	5
28. When I am upset, I take time to explore how my body feels.	0	1	2	3	4	5
29. I listen to my body to inform me about what to do.	0	1	2	3	4	5
30. I am at home in my body.	0	1	2	3	4	5
31. I feel my body is a safe place.	0	1	2	3	4	5
32. I trust my body sensations.	0	1	2	3	4	5

The Autism Quotient

Indicate how much you agree or disagree with each of the following statements. Just choose the appropriate option.

		definitely agree	slightly agree	slightly disagree	definitely disagree
1.	I prefer to do things with others rather than on my own.				
2.	I prefer to do things the same way over and over again.				
3.	If I try to imagine something, I find it very easy to create a picture in my mind.				
4.	I frequently get so strongly absorbed in one thing that I lose sight of other things.				
5.	I often notice small sounds when others do not.				
6.	I usually notice car number plates or similar strings of information.				
7.	Other people frequently tell me that what I've said is impolite, even though I think it is polite.				
8.	When I'm reading a story, I can easily imagine what the characters might look like.				
9.	I am fascinated by dates.				
10.	In a social group, I can easily keep track of several different people's conversations.				
11.	I find social situations easy.				
12.	I tend to notice details that others do not.				

		definitely agree	slightly agree	slightly disagree	definitely disagree

13.	I would rather go to a library than a party.				
14.	I find making up stories easy.				
15.	I find myself drawn more strongly to people than to things.				
16.	I tend to have very strong interests which I get upset about if I can't pursue.				
17.	I enjoy social chit-chat.				
18.	When I talk, it isn't always easy for others to get a word in edgeways.				
19.	I am fascinated by numbers.				
20.	When I'm reading a story, I find it difficult to work out the characters' intentions.				
21.	I don't particularly enjoy reading fiction.				
22.	I find it hard to make new friends.				
23.	I notice patterns in things all the time.				
24.	I would rather go to the theatre than a museum.				
25.	It does not upset me if my daily routine is disturbed.				
26.	I frequently find that I don't know how to keep a conversation going.				
27.	I find it easy to "read between the lines" when someone is talking to me.				
28.	I usually concentrate more on the whole picture, rather than the small details.				
		definitely agree	**slightly agree**	**slightly disagree**	**definitely disagree**

30.	I don't usually notice small changes in a situation, or a person's appearance.				
31.	I know how to tell if someone listening to me is getting bored.				
32.	I find it easy to do more than one thing at once.				
33.	When I talk on the phone, I'm not sure when it's my turn to speak.				
34.	I enjoy doing things spontaneously.				
35.	I am often the last to understand the point of a joke.				
36.	I find it easy to work out what someone is thinking or feeling just by looking at their face.				
37.	If there is an interruption, I can switch back to what I was doing very quickly.				
38.	I am good at social chit-chat.				
39.	People often tell me that I keep going on and on about the same thing.				
40.	When I was young, I used to enjoy playing games involving pretending with other children.				
41.	I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).				
42.	I find it difficult to imagine what it would be like to be someone else.				
43.	I like to plan any activities I participate in carefully.				
		definitely agree	**slightly agree**	**slightly disagree**	**definitely disagree**

45.	I find it difficult to work out people's intentions.				
46.	New situations make me anxious.				
47.	I enjoy meeting new people.				
48.	I am a good diplomat.				
49.	I am not very good at remembering people's date of birth.				
50.	I find it very easy to play games with children that involve pretending.				

TORONTO ALEXITHYMIA SCALE

PARTICIPANT ID _____

Indicate how much you agree or disagree with each of the following statements. Just tick the appropriate box. **Use the middle box** ('I neither agree or disagree') **only if you are really unable to assess your behaviour.**	I strongly disagree	I quite disagree	I neither agree nor disagree	I quite agree	I strongly agree
1- I am often confused about what emotion I am feeling					
2- It is difficult for me to find the right words for my feelings					
3- I have physical sensations that even doctors don't understand					
4- I am able to describe my feelings easily					
5- I prefer to analyze problems rather than just describe them					
6- When I am upset, I don't know if I am sad, frightened, or angry					
7- I am often puzzled by sensations in my body					
8- I prefer to just let things happen rather than to understand why they turned out that way					
9- I have feelings that I can't quite identify					
10- Being in touch with emotions is essential					
11- I find it hard to describe how I feel about people					
12- People tell me to describe my feelings more					
13- I don't know what's going on inside me					
14- I often don't know why I am angry					
15- I prefer talking to people about their daily activities rather then their feelings					
16- I prefer to watch « light » entertainment shows rather than psychological dramas					
17- It is difficult for me to reveal my innermost feelings, even to close friends					
18- I can feel close to someone, even in moments of silence					
19- I find examination of my feelings useful in solving personal problems					
20- Looking for hidden meanings in movies or plays distracts from their enjoyment					

STAI - State Anxiety Form

Directions: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment.* There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

STAI – Trait Anxiety Form

Directions: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that doesn't really matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks,* how often have you been bothered by any of the following problems? *(use "✓" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite — being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
		add columns	+	+

(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card). TOTAL:

10. If you checked off *any problems,* how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?

- Not difficult at all _____
- Somewhat difficult _____
- Very difficult _____
- Extremely difficult _____

A2663B 10-04-2005

PHQ-9 Patient Depression Questionnaire

For initial diagnosis:

1. Patient completes PHQ-9 Quick Depression Assessment.
2. If there are at least 4 ✓s in the shaded section (including Questions #1 and #2), consider a depressive disorder. Add score to determine severity.

Consider Major Depressive Disorder

- if there are at least 5 ✓s in the shaded section (one of which corresponds to Question #1 or #2)

Consider Other Depressive Disorder

- if there are 2-4 ✓s in the shaded section (one of which corresponds to Question #1 or #2)

Note: Since the questionnaire relies on patient self-report, all responses should be verified by the clinician, and a definitive diagnosis is made on clinical grounds taking into account how well the patient understood the questionnaire, as well as other relevant information from the patient.
Diagnoses of Major Depressive Disorder or Other Depressive Disorder also require impairment of social, occupational, or other important areas of functioning (Question #10) and ruling out normal bereavement, a history of a Manic Episode (Bipolar Disorder), and a physical disorder, medication, or other drug as the biological cause of the depressive symptoms.

To monitor severity over time for newly diagnosed patients or patients in current treatment for depression:

1. Patients may complete questionnaires at baseline and at regular intervals (eg, every 2 weeks) at home and bring them in at their next appointment for scoring or they may complete the questionnaire during each scheduled appointment.
2. Add up ✓s by column. For every ✓: Several days = 1 More than half the days = 2 Nearly every day = 3
3. Add together column scores to get a TOTAL score.
4. Refer to the accompanying **PHQ-9 Scoring Box** to interpret the TOTAL score.
5. Results may be included in patient files to assist you in setting up a treatment goal, determining degree of response, as well as guiding treatment intervention.

Scoring: add up all checked boxes on PHQ-9

For every ✓ Not at all = 0; Several days = 1;
More than half the days = 2; Nearly every day = 3

Interpretation of Total Score

Total Score	Depression Severity
1-4	Minimal depression
5-9	Mild depression
10-14	Moderate depression
15-19	Moderately severe depression
20-27	Severe depression

A2662B 10-04-2005