brighton and sussex medical school

Relationships between emotional regulation, interoception and alcohol use

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Abstract

Interoception is the general sensitivity towards internal bodily signals. Interoceptive responses are crucial for homeostatic control and guide motivational behaviour, through generation of affective feelings, mainly integrated by the insular cortex. Alexithymia, a personality construct characterized by difficulties in identifying such feelings, is implicated in the development and maintenance of alcohol use disorders (AUD). An emergent theory suggests that alexithymia, rather than being a problem with verbal labelling, is the outcome of an interoceptive failure. Interestingly, disruption of interoceptive processes in alcohol-dependent individuals correlates positively with alexithymia and subjective alcohol craving ratings. In this work, I explored the relationship between interoception, alexithymia and alcohol use.

An online survey was designed to characterise the causal relationship between self-assessment of alexithymia, sensitivity to bodily sensations and alcohol consumption, in a normative sample (N=600). A second study involving magnetic resonance spectroscopy measured the neurochemical and structural neural correlates of alcohol use within the insular cortex. A third study objectively measured interoception (interoceptive accuracy) in social drinkers and tested the impact of intranasal oxytocin on accuracy scores. A fourth experiment, involving multiband functional magnetic resonance imaging, tested further the impact of intranasal oxytocin on behavioural and neural correlates of social emotional processing (operationalized as empathy for pain) in alcohol users. Finally, the last experiment quantified dynamic brain-body interactions (via measurement of blood pressure) in alexithymia.

I found that alexithymia and difficulties in identifying feelings mediated the relationship between bodily sensations and alcohol consumption, suggesting that difficulties in identifying feelings might be the outcome of an interoceptive failure, apparently predisposing to AUD. This phenomenon can be explained, in part, by differences in insular glutamatergic neurochemistry and structural integrity associated with alcohol use. At the behavioural level, social alcohol use was not directly associated with impaired interoceptive accuracy. However, intranasal oxytocin improved interoceptive accuracy on the discrimination task compared to placebo in heavy drinkers, but not in low-to-moderate drinkers. This finding suggests reduced flexibility of attentional resource-allocation between internal and external environmental signals, in heavy drinkers. Importantly, this impairment can be corrected by acute intranasal oxytocin. Finally, I found that arousal was associated with better performances. No significant relationship between alexithymia and alcohol intake was observed.

In conclusion, this thesis demonstrates the role that interoception plays in alexithymia and AUD, by considering and integrating behavioural, physiological and neural dimensions. My observations motivate the need to take into account interoceptive processes and (other) regulatory impairments in the treatment of AUD. Therapeutic modulation of interoception can potentially reduce alexithymic features and consequently decrease the likelihood of AUD.

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Finally, I would like to end these acknowledgements with a note to myself:

Sophie, if you decide to stay in academia and to become a supervisor. Please, remember the state you were in when you wrote these words. Please, do not underestimate the work of students or of anyone else. Never.

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

Dated 14.05.2018

Acronyms and definitions

AIC	Akaike Information Criterion
ACC	Anterior Cingulate Cortex
AI	Anterior Insula
AL	Alexithymic
ANS	Autonomic Nervous System
AUD	Alcohol Use Disorder
AUDIT	Alcohol Use Disorders Identification Test
AUQ	Alcohol Use Questionnaire
BDI	Beck Depression Index
BPQ	Body Perception Questionnaire
BPQ_A	BPQ Awareness subscale
CEN	Central-Executive Network
CSF	Cerebrospinal Fluid
DDF	Difficulties in Describing Feelings
DIF	Difficulties in Identifying Feelings
DMN	Default Mode Network
ECG	Electrocardiography
EFE	Emotional Facial Expression
EOF	externally oriented thoughts
EPIC	Embodied Predictive Interoception Coding
ERN	Error-Related Negativity
ERP	Event-Related Potentials
FDR	False Discovery Rate
fMRI	functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric acid
Gln	Glutamine
Glu	Glutamate
Glx	Glutamate plus glutamine
GM	Grey Matter
HEP	Heartbeat-Evoked Potential
HRV	Heart Rate Variability
IAPE	Implicit Affect Primes Effort
IBI	Interbeat Interval
INT	Intermediate
IU	International Unit
MCC	Middle Cingulate Cortex
MRS	Magnetic Resonance Spectroscopy
NAA	N-acetylaspartate
NAAG	N-acetyl-aspartylglutamate
NAL	Non Alexithymic
NMDA receptor	N-methyl-D-aspartate receptor
NTS	Nucleus of the Solitary Tract
OCD	Obsessive-Compulsive Disorder
OCDS	Obsessive Compulsive Drinking Scale
OT	Oxytocin

PAG	Periaqueductal Gray matter
PB	Parabrachial nucleus
PCC	Posterior Cingulate Cortex
PMM	Predictive Mean Matching
POW	Pulse Oximetry Waveform
PTT	Pulse Transit Time
ROI	region of interest
RT	Reaction time
rTMS	repetitive Transcranial Magnetic Stimulation
SBM	Surface-Based Morphometry
SBP	Systolic Blood Pressure
SN	Salience Network
STAI	Spielberger State/Trait Anxiety Inventory
TAS-20	Toronto Alexithymia Scale-20 items
TE	Echo Time
TIV	Total Intracranial Volume
TNAA	N-acetylaspartate plus N-acetyl-aspartylglutamate
TR	Repetition Time
UNIT	Unit of alcohol unit (8g) per week
VAS	Visual Analogue Scale
VBM	Volume-Based Morphometry
VMb	Ventromedial nucleus of the thalamus
Vmpo	Posterior part of the Ventromedial nucleus of the thalamus
VOI	Voxel of Interest
WHO	World Health Organization
WM	White Matter

Chapter 1 Introduction

“Agnès enviait Paul de pouvoir vivre sans être perceptuellement conscient de son corps. Il inspire, il expire, son poumon travaille comme un grand soufflet automatisé et c’est ainsi qu’il percoit son corps: en l’oubliant joyeusement.”

[Agnès envied Paul as he was able to live without being perceptually aware of his body. He inhales, he exhales, his lung works like big automated bellows and that's how he perceives his body: by forgetting it happily.]

Milan Kundera*, L’immortalité (1990), p148*

1.1 Emotion

The word emotion comes from the Latin verb *emovere,* which means *to move out, to agitate*. But what is an emotion? It is strikingly difficult to find a consensual definition of emotion. Indeed, numerous approaches and definitions of the concept of emotion have been proposed.

For example, evolutionary theories propose that emotions have a purpose in communicating and in favouring survival. Emotions therefore, are considered as a universal product of evolution (Darwin, 1872/1998). This assumption is in line with the later work of Ekman who developed the concept of "basic emotions" (e.g. anger, happiness, fear, sadness and disgust) and showed that basic emotions, as a group of related affective states, are represented centrally (Ekman and Davidson, 1994). When men were instructed to contract facial muscles into prototypical configurations of basic emotions, an emotion-specific pattern was observed at both physiological and subjective levels (Levenson *et al.*, 1990). Moreover, this pattern was consistent across cultures (Levenson *et al.*, 1992), confirming its adaptive dimension.

While evolutionary theories were focusing on the function of emotions, somatic theories (σῶμα-sôma: body in ancient Greek) were interested in brain-body relationships underpinning emotional behaviours. In this context, it is important to introduce the *autonomic nervous system (ANS)*, a mediator of such relationships. The ANS was first proposed and described by Langley (Langley, 1900). Langley defined the ANS as the system of nerves in charge of the regulation of bodies tissue (i.e. viscera, vasculature, glands). After examination of the autonomic outflow, Langley described the parasympathetic, the sympathetic and the enteric systems (Langley, 1921). Both parasympathetic and sympathetic systems consist of a series of efferent preganglionic and postganglionic neurons, under the control of a central autonomic network. Stimulation of parasympathetic neurons produces activation of exocrine glands, non-vascular smooth muscles, pacemaker and atria of the heart. Stimulation of sympathetic neurons produces effector responses from a wide range of tissues such as heart, blood vessels, pancreas and smooth muscles. Langley recognized that the enteric system (έντερα–

éntera: intestines in ancient Greek) functions independently from the central nervous system. Indeed, compared to the sympathetic and parasympathetic systems, the enteric system has a distinct histology. If the sympathetic and parasympathetic systems do not directly interfere with enteric effectors, both systems can modulate the enteric somatosensory motor program (Jänig, 2006).

An influential theory proposed by James and Lange puts the body back at the heart of the question of emotion by postulating that an object stimulates one or more sense organs; afferent impulses pass through the cortex and allow the perception of the object. Then, the brain triggers autonomic efferent signals, which alter the activity of relevant internal organs. For James and Lange, afferent signals coming from these organs go back to cortical regions allowing the transition from the "object-simply-apprehended" toward the "object-emotionally-felt" (James, 1884, James, 1890, Lange, 1912). This "peripheralist" idea was novel in that it was questioning the sequence of emotional reaction. James and Lange suggest that what was considered the outcome of the emotion, is its cause: specific emotions are shaped by the perception of a specific pattern of peripheral activations. James wrote, "*We feel sorry because we cry, angry because we strike, afraid because we tremble.*" (quoted from James, 1884, p. 190).

This assumption was highly criticised by Cannon, who was following the James' philosophy lectures at Harvard. Cannon replied, "*We do not "feel sorry because we cry", as James contented, but we cry because, when we are sorry […] there are nervous discharges[...] to various viscera, including the lachrymal glands*" (quoted from Cannon, 1914a, p 280). Cannon's ideas are rather "centralist" and suggest that emotional states are represented in the brain rather than being the outcome of peripheral changes. For example, Cannon highlighted that the suppression of visceral afferent signals does not suppress emotion. He also argued that similar visceral changes happen in very different emotional states, but also in non-emotional states and that viscera were too insensitive and slow to be the source of such a fast and complex reaction as an emotion. Cannon and his student Bard, did extensive work on the representation of emotion in the central nervous system - especially at the

thalamic level - and on the contribution of the sympathetic autonomic system in the phenomenon of emotion (Cannon, 1927, Bard, 1932). Later, and based on preliminary work from Cantril and Hunt, Schachter and Singer merged both theories by suggesting that an emotion is an undifferentiated physiological activation which is labelled by cognitive processes (Cantril and Hunt, 1932, Schachter and Singer, 1962). Thus, a state of sympathetic bodily arousal induced by an adrenaline injection will evoked different emotional states depending on the cognitive context: if participants receive no direct explanation about the injection, they report increased levels of euphoria, anger and amusement compared to the informed participants. These feelings are further biased by the context: if bodily arousal was induced in the context of an angry confederate, then the participant would report the experience of anger.(Schachter and Singer, 1962).

More recently, Damasio revisited the James-Lange theory and proposed the somatic marker hypothesis suggesting that cognitive processes are guided by the representation of bodily responses. Indeed, Damasio and colleagues found that subjects with ventromedial prefrontal lesions failed to react to emotionally charged stimuli, as indexed by changes in skin conductance responses (Damasio *et al.*, 1990, Damasio, 1996). In a gambling task, healthy patients (i.e. without frontal damage) learnt throughout the trials: they gradually played more frequently from good decks (i.e. higher chance to win game money than to lose game money) than from disadvantageous ones (i.e. higher chance to lose game money than to win game money). This phenomenon was not observed in subjects with a frontal lesion, instead, they perseverated in playing from the bad decks (Bechara *et al.*, 1994). Interestingly, these patients did not generate differentiated anticipatory skin conductance responses related to the deck's uncertainty, whereas the healthy control did (Bechara *et al.*, 1996). This suggests that changes in internal bodily states give an affective tone to the outcome of an action (e.g. taking a card from a bad deck) and therefore, inform future potential related actions (e.g. anticipating taking a card from a bad deck, *via* an "*as-if-body loop*" mechanism). This, then, contributes to learning processes (e.g. avoiding taking a card from the bad deck). In that sense, Damasio defines the term

emotion as *"a collection of responses triggered from parts of the brain to the body, and from parts of the brain to other parts of the brain, using both neural and humoral routes"* (quoted from Damasio, 1998, p 84). The central activations induced by the emotion result in a wide range of physiological changes within the body, which are re-expressed in the brain and lead to an *emotional/affective state*, via the interoceptive (afferent) channel. Correspondingly, it is important to define what is interoception.

1.1.1 Interoception

Sherrington was interested in the distribution of the receptor organs in the body. He described two fields "*a surface field constituted by the surface layer of the organism, and a deep field constituted by the tissues of the organism beneath the surface sheet*" (quoted from Sherrington, 1907, p 469). Moreover, he considered the surface field to be divided into two subcomponents. The first subcomponent "*lies freely open to the numberless vicissitudes and agencies of the environment* […]. *It possesses as receptive organs not only of touch, &c., in the skin proper, but also the eye, nose and organ of hearing" (p 469).* Sherrington discussed a second subcomponent of the *surface* field, which was commonly called the "*internal surface of the animal, the alimentary or intestinal surface*". As the second subcomponent is "*turned inward upon the alimentary contents*", Sherrington termed it as "*intero-ceptive*" surface. The first and larger subcomponent of the surface field "*looks outward upon the free environment*", therefore, he qualified it as "*extero-ceptive*" surface*" (p 469)*. Sherrington acknowledged the larger number and the greater diversity of receptors present in the exteroceptive field and even mentioned the specificity of some receptors (temperature, light, sound, noxa/pain) to that external sheet. Finally, Sherrington recognised "*receptors which lie in the deep tissues appear adapted for excitation by changes going forward in the organism itself*" that he termed "*proprio-ceptors (*quoted from Sherrington, 1907, p 472).

In Sherrington's view, exteroception involves the interface between the surface of the body and the external environment, including the main senses; whereas receptors involved in body movement (i.e. skeletal muscles, joints) are part of proprioception. Interoception is restricted to signals coming from

the internal environment (i.e. viscera and glands), this is the exclusive definition of interoception (i.e. not including experiences from the surface of the body as interoceptive).

Recently, the concept of interoception has been expended: Craig postulates that itch, pain, muscular, thermal as well as visceral sensations are senses from the body tissues and, as such, should be listed under the umbrella of interoception (Craig *et al.*, 2000, Craig, 2002, Saper, 2002, Craig, 2003). Craig argues that thermal and pain sensations possess an affective tone, which is modulated by the body's needs (Cabanac *et al.*, 1972). In that sense, he considered that thermoception and nociception are crucial for maintenance of homeostasis and body integrity (Craig, 2002, Craig, 2003). In other words, for Craig, all representations of the physiological condition of the internal milieu should be labelled as interoceptive. To understand Craig's position, it seems important to explore the interoceptive pathways.

1.1.1.1 Interoceptive pathways

Two main interoceptive pathways have been described (Figure 1.1A.b and c); the vagal (Figure 1.1A.b) and the spinal (Figure 1.1A.c) pathways. Interoceptive afferent signals are conveyed from the body to the brain, following the efferent autonomic nerves. Furthermore, this does not mean that visceral neurons can be qualified as sympathetic or parasympathetic *per se*. To date, no apparent reason exists to label different afferent neurons as part of one or the other subdivision of the autonomic system (Jänig, 2006, p. 40-41).

Vagal interoceptive inputs are carried by the vagus nerve (X; following parasympathetic efferent nerves) and have cell bodies in its ganglia (Figure 1.1A.b). Signals are projected to the nucleus of the solitary tract (NTS) and pass through the parabrachial nucleus (PB) as well as through the periaqueductal gray matter (PAG) to be projected on the ventromedial nucleus of the thalamus (VMb). From these structures, the information is sent to the hypothalamus, the amygdala, the anterior cingulate cortex (ACC) and to the mid/posterior dorsal insula (Craig, 2002, Critchley and Harrison, 2013).

Spinal interoceptive inputs have cell bodies in the dorsal root ganglion, then enter the spinal cord in the lamina 1 of the dorsal horn and project to the sympathetic cell columns of the thoracolumbar of the spinal cord (Figure 1.1.A.c). Via the spinothalamic tract, signals reach the posterior part of the ventromedial nucleus of the thalamus (VMpo). In primates, lamina 1 neurons also project to VMb via PB (Craig, 2002, Craig, 2003, Craig, 2009). From VMpo and VMb, inputs project to the mid/posterior dorsal insula. Interestingly, this is distinct from the main interoceptive pathway in sub-primate species where the pathway from the PB and the PAG projects to the dorsal posterior insula (and also to ACC) via medial thalamus (Craig, 2002, Saper, 2002, Craig, 2008, Critchley and Harrison, 2013).

From the mid/posterior dorsal part of the insula, interoceptive inputs are proposed to be re-represented (i.e. projected forward and integrated) in the ipsilateral anterior insula (AI), independently of their origins (i.e. vagal or spinal). However, there is also some evidence suggesting that vagal visceral inputs are represented a second time on the left AI; whereas spinal inputs are represented on the right AI, due to the autonomic hemispheric lateralisation (Figure 1.1B; Craig, 2002). From the right AI, information is conveyed to the orbitofrontal cortex, which has been hypothesised to encode its hedonic value (Craig, 2000, Kringelbach, 2005). The existence of such a neuroanatomical path is in line with the aberrant behaviour observed in patients with frontal lesion and provides a substrate to the somatic markers hypothesis (Damasio *et al.*, 1990, Bechara *et al.*, 1994, Bechara *et al.*, 1996, Damasio, 1996, Craig, 2002).

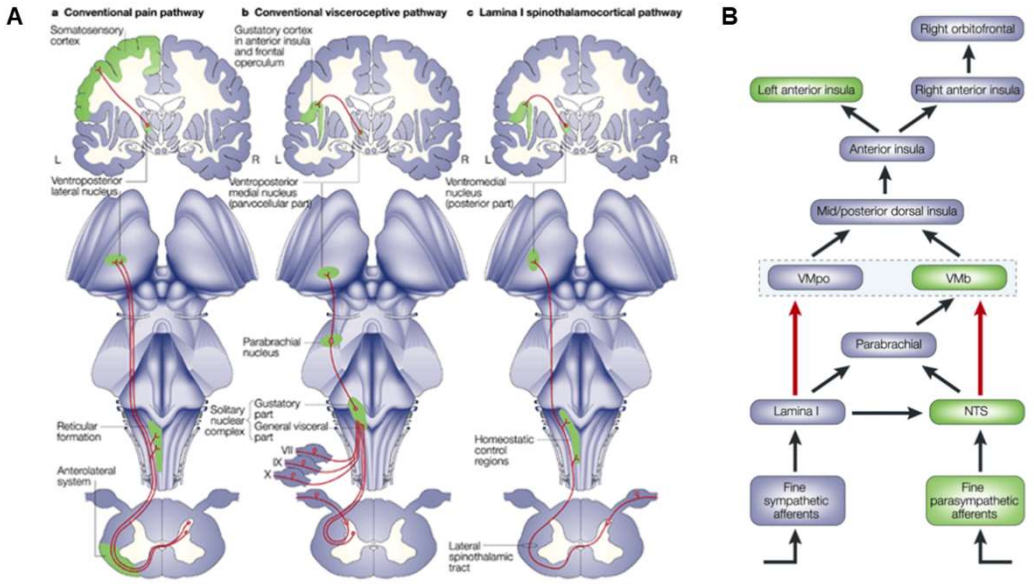


Figure 1.1 Representation of the interoceptive pathways

A. Diagrams of a. conventional pain pathway; b. conventional visceroceptive pathway and c. Lamina I spinothalamocortical pathway; B. Chart representing interoceptive organisation: interoceptive afferences carried along the sympathetic system (spinal) are represented in blue; interoceptive afferences carried along the parasympathetic system (vagal) are represented in green ; reproduced from Craig (2002)

Craig argues that lamina 1 neurons receive inputs from the afferent neurons with small-diameter axons (Aδ, C; Craig, 2002). Sensory fibres Aδ are mylienated fibres characterized by a diameter of 2-5 µm and a conduction velocity of 3-15m/s. C fibres are unmyelinated fibres characterized by a diameter of 0.4-1.2 µm and a conduction velocity of 0.5-2.0m/s (Brading, 1999). These fibres convey activities such as touch, temperature, muscular contraction and nociception, which are represented at the MVpo level and then projected to the posterior insular cortex. For example, in humans, microstimulation of MVpo modulates pain, temperature and visceral sensations (Lenz *et al.*, 1993, Lenz *et al.*, 1994, Davis *et al.*, 1999). Also, in neuroimaging studies, dorsal insular cortex is activated by manipulation of temperature, (chronic or thermal) pain, affective touch or itch (Craig *et al.*, 2000, Kupers *et al.*, 2000, Drzezga *et al.*, 2001, Brooks *et al.*, 2002, Olausson *et al.*, 2010). For Craig, the fact that a wide range of modalities activates the bilateral posterior insulae suggests that it contains a sensory representation of

afferent inputs associated with physiological monitoring of the entire body, which is crucial for homeostasis. In that sense, the insular cortex is a veritable “interoceptive” hub of the brain.

1.1.1.2 The Insula

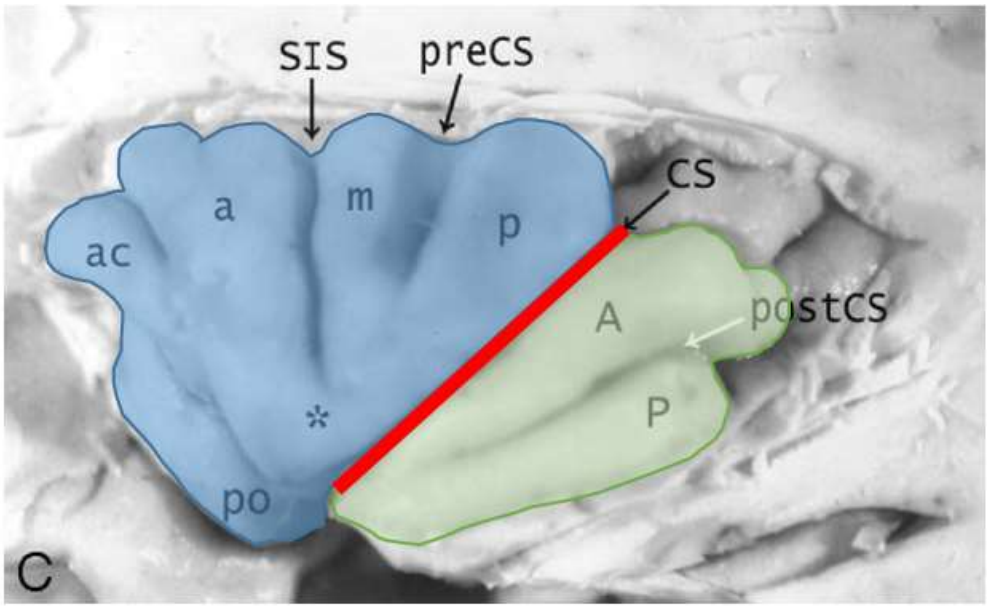


Figure 1.2 Anatomy of the left insular lobe after resection of the frontal and parietal opercula, vessels, and pia-arachnoid.

In blue, the anterior part; in green the posterior part of the insula, both separated by the central sulcus (CS), in red. ac = accessory gyrus; a = anterior short insular gyrus; SIS = short insular sulcus; m = middle short insular gyrus; preCS = precentral sulcus; p = posterior short insular gyrus; po = pole; A = anterior long insular gyrus ; postCS = postcentral insular sulcus; P = posterior long insular gyrus. Figure reproduced and modified from Naidich et al., (2004).

The insula (*island* in Latin) is a hidden part of the cortex, localized underneath the frontal and temporal opercula. This region is divided by a central sulcus in two parts; the anterior part which is composed of three short gyri and the posterior part which is composed of the two long gyri (Guenot *et al.*, 2004; see figure 1.2). However, this anatomical configuration is somewhat variable between individuals (Naidich *et al.*, 2004).

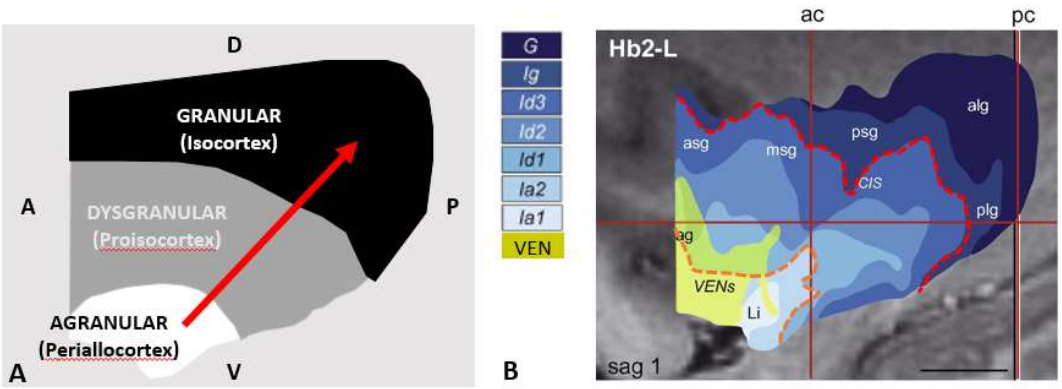


Figure 1.3 Cytoarchitectonic organisation of the insula.

A. Schematic representation of different cortices; evolving from periallocortex (Agranular) to Isocortex (Dysgranular). A = anterior; P = posterior; V= ventral; D = dorsal. B. Sagittal MRI registrations of cytoarchitectonic map on a T1 structural image; von Encomo neurons are represented in green (VEN). The cytoarchitectonic organisation is represented by the different shades of blue from light (Agranular; Ia1, Ia2), intermediate (Dysgranular; Id1, Id2, Id3) to dark ones (Granular; Ig, G); ac = anterior commissure; pc = posterior commissure. Panel B reproduced from Morel et al., (2013).

Microscopically, insular cytoarchitectonic organisation reflects different steps in phylogenetic and ontogenetic cortical development. Work in non-human primates identifies three belts of cortices following a concentric plan of organization, centred on the olfactory cortex (Mesulam and Mufson, 1982a, Mesulam and Mufson, 1982b). A gradual and sequential increase in laminar differentiation is observed: the first belt is composed periallcortex (i.e. agranular) characterized by three layers of cortex and absence of granular cells (fused layers II/III; VI/V); the second belt is composed of proisocortex (i.e. dysgranular) where six layers (including granular layers) are progressively distinct; the third belt is composed of isocortex (i.e. granular) characterized by clear separation between the six cortical layers (see Figure 1.3; Mesulam and Mufson, 1982a). Further fine-grained analyses can differentiate fifteen distinct architectonic subregions of the insula (Evrard *et al.*, 2014). Neurons within the middle short gyrus are generally more complex in terms of dendritic/spine extent than neurons in the posterior short or anterior long gyri. This observation is consistent with a posterior-anterior gradient of dendritic complexity, suggesting a functional differentiation of insula, with the anterior insula more similar to 'high-integration' cerebral regions (such as heteromodal and supramodal cortices), and the posterior insula more similar to 'low-integration' cerebral regions (such as primary and unimodal cortices;

Anderson *et al.*, 2009). The presence of an integration gradient is supported by the location of von Economo neurons within agranular insular cortex in humans, great apes and in other primates and mammals (Evrard *et al.*, 2012). These neurons are large projection neurons characterized by a narrow dendritic arborisation and symmetrical dendrites that suggests a specialized role in the comparison and integration of information (Allman *et al.*, 2011). Putatively, these neurons (also present within ACC, see section 1.1.1.3) thereby support self-referential processes underlying consciousness through embodied mechanisms dependent upon predictive control of internal physiological interoceptive state (Critchley, 2004, Critchley, 2005). Functionally, insular cortex is implicated in interoception, receiving visceral afferent inputs at its posterior extent, and re-representing this sensorial information in higher-level integrated representations of bodily state in more anterior regions (Craig, 2004). Correspondingly, High Angular Resolution Diffusion Imaging reveals this insular cortical-cortical structural connectivity. Moreover, in-vivo probabilistic tractography differentiates those ventral pathways connecting anterior insular cortex to rostral regions (including orbital/inferior prefrontal, anterior/polar temporal cortex) from pathways connecting posterior insular cortex to posterior temporoparietal regions (Cloutman *et al.*, 2012). Insula is also widely connected to subcortical structures including thalami, amygdala, basal ganglia, and brainstem (Mesulam and Mufson, 1982a, Flynn *et al.*, 1999, Guenot *et al.*, 2004, Saleh *et al.*, 2017) but also to cortical ones (Ghaziri *et al.*, 2017), such as the ACC.

1.1.1.3 The Anterior Cingulate Cortex

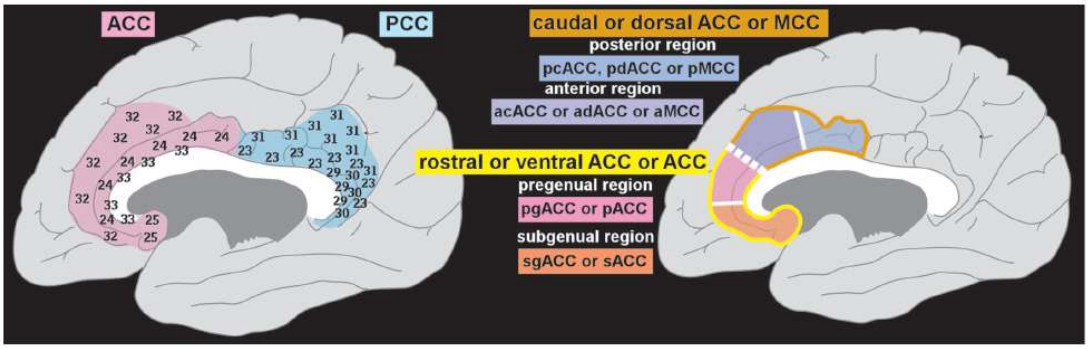


Figure 1.4 Subdivision of the cingulate cortex.

(Left) The anterior part of the cingulate (ACC) is represented in pink and the dorsal part of the cingulate cortex is represented in blue (PCC). (Right) The ACC is subdivided in two sections: the rostral or ventral ACC or ACC outlined in yellow and the caudal or dorsal ACC or MCC outlined in orange.

The cingulate cortex (*to surround, encompass* in Latin) is a medial belt of cortex wrapped around the corpus callosum. Initially, Brodmann divided into two parts: the anterior and posterior cingulate cortices (Brodmann, 1909, see Figure 1.4). Roughly, the ACC is distinguishable from the PCC by the absence of neurofilament protein-immunoreactive neurons in layer III and the presence of large spindle pyramidal neurons, the von Economo neurons, in layer Vb (Nimchinsky *et al.*, 1995, Vogt *et al.*, 1995). However, a much more complex cytoarchitecture and transitional laminar organisation of the cingulate cortex has been described (for a detailed description see Wise, 2008, Vogt, 2009). Indeed, as many as 40 cytologically unique areas have been observed across the cingulate (Vogt and Vogt, 1919, von Economo 1929).

The ACC is classically divided between its dorsal and ventral portions (see Figure 1.4). The dorsal part (dACC), also called the Middle Cingulate Cortex (MCC) is the part which is on the top of the corpus callosum and is composed of Brodmann areas 24a', 24b', 24c', 24d, 32' and 33. This portion can also be divided into an anterior (aMCC) and posterior part (pMCC). The ventral portion (vACC) is more anterior and ventral to the corpus callosum. This portion is composed of Brodmann areas 24a, 24b, 24c, 25, 32 and 33 and can be divided into the pregenual (pACC) and the subgenual (sACC) part (Vogt, 2004, Vogt, 2005, Vogt *et al.*, 2005, Vogt, 2009, Shackman *et al.*, 2011).

Structural connectivity using diffusion tensor imaging found that sACC and the pACC were mainly sharing connections with limbic structures such as the amygdala, the hippocampus, the ventral striatum, but also with the medial orbitofrontal cortex (Johansen-Berg *et al.*, 2008). Concerning the MCC, main connections were observed with more cognitive and motor areas such as the dorsal prefrontal cortex or the premotor cortex, but also with the spinal cord, whereas the PCC has main connections with the precentral cortex, the parietal cortex and the hippocampus (Beckmann *et al.*, 2009, Stevens *et al.*, 2011).

Coherent with the structural connectivity findings, neuroimaging techniques show that the ACC is also involved in emotional regulation (Etkin *et al.*, 2011), in self-conscious emotional reactivity (Sturm *et al.*, 2013) and autonomic regulation (Critchley *et al.*, 2003). In humans, stimulation of the pACC provokes laughter (Caruana *et al.*, 2015). Moreover, electric stimulation of the sACC and pACC modulate autonomic aspects such as respiration, cardiac output, blood pressure, gastric mucosa erosion, or facial blushing (Escobedo *et al.*, 1973, Talairach *et al.*, 1973, Henke, 1983). Ventral ACC and MCC are activated during social pain whereas self-report of distress involves the sACC and pACC (Rotge *et al.*, 2015). On the other hand, MCC is involved in emotional response inhibition (Albert *et al.*, 2012), valence-specific emotional processing (Levar *et al.*, 2017), risk-taking (Critchley et al., 2001) and cognitive reappraisal (Giuliani *et al.*, 2011). Also, in humans, electric stimulation of the MCC leads to context-dependent gestures (Escobedo *et al.*, 1973, Talairach *et al.*, 1973).

To sum up, sACC and pACC seems to be involved in affective processing whereas the aMCC seems to be involved in more cognitive and sensorimotor processing (Margulies *et al.*, 2007, Yu *et al.*, 2011). However, both these regions react to unpleasant/noxious stimuli (Vogt, 2005, Shackman *et al.*, 2011). For example, in a task, participants were watching short video clips depicting a noxious object (e.g., a sharp knife) or an innocuous object (e.g., a butter knife) striking a person's hand and were required to execute an action. Participants' reaction times were faster when faced with a potentially noxious challenge and this phenomenon was associated with increased bold signal in MCC (Morrison *et al.*, 2007). Indeed, both pACC and MCC encode the

affective and motivational dimension of pain, preparing behavioural responses to pain and other negatively-valenced stimuli (Morrison *et al.*, 2004, Morrison *et al.*, 2007, Perini *et al.*, 2013).

1.1.1.4 Coactivation of both insular and anterior cingulate cortices

Interestingly, the ACC is usually coactivated with the AI cortex, during several types of cognitive, stress or exercise related-tasks (Critchley *et al.*, 2000, Dosenbach *et al.*, 2007) (for review see, Medford and Critchley, 2010). In rhesus monkeys, the cingulate cortex receives afferent input from the insular cortex (Vogt and Pandya, 1987). The AI is functionally connected with the pACC/aMCC and the pMCC, while the mid/posterior insula is only connected with the pMCC (Taylor *et al.*, 2009). These data are in line with several aspects described in the next paragraphs.

A large body of literature supports the crucial role of insular cortex and ACC/MCC in pain processing (Petrovic *et al.*, 1999, Craig, 2009, Nakata *et al.*, 2014, Zunhammer *et al.*, 2016, Orenius *et al.*, 2017), visceral monitoring (Craig *et al.*, 2000, Critchley *et al.*, 2000, Critchley *et al.*, 2001, Critchley *et al.*, 2003, Critchley *et al.*, 2004) and empathic feelings (Gu *et al.*, 2013, Klumpp *et al.*, 2013). As discussed in section 1.1.1.1, the mid-posterior insula receives nociceptive inputs which are then re-represented in the AI, conferring a conscious access to subjective affective state (Craig, 2002). In the meantime, projections from the thalamus are sent to the ACC (Craig, 2008). The ACC encodes the affective and motivational dimension of sensations and prepares the body for action (Sewards and Sewards, 2002, Morrison *et al.*, 2004, Fuchs *et al.*, 2014).

For Craig, via a representation of predicted bodily state, the insula provides the feeling whereas the ACC/MCC provides the motivational aspect and the sense of agency of an action (Craig, 2002). Knowing that both AI and ACC/MCC are involved in the representation of physical pain and generation of subjective feelings, it is not surprising that these structures are also activated in the empathic processing of another's pain (i.e. empathy for pain;

for review Lamm *et al.*, 2011) (Singer *et al.*, 2004, Jackson *et al.*, 2005, Jackson *et al.*, 2006, Morrison *et al.*, 2007).

Furthermore, AI and ACC/MCC also play a crucial role in salience attribution. Indeed, as part of the “salience network” (Seeley *et al.*, 2007), the AI is hypothesized to detect the most relevant bottom-up events among internal and external environments, and to temporarily initiate attentional control inputs, which then are sustained by ACC (Menon and Uddin, 2010). Moreover, switching between central-executive and default-mode networks involves specifically the right AI (Sridharan *et al.*, 2008).

As mentioned, the AI and the ACC are crucial for interoception and visceral monitoring. In the next section, different ways of measuring interoception and brain/body axis aspects will be described.

1.1.2 How to measure or manipulate brain/body interactions?

Interoception classically refers to the signalling, representation, and perception of internal bodily sensations coming from the viscera, for example, heartbeats, gastric distension, or visceral pain (Cameron, 2001). Interoceptive processes are the safeguards of homeostatic control and contribute to motivational and affective behaviours (Tsakiris and Critchley, 2016). Advance has been made in the conceptualisation of interoception as measured by laboratory tasks, notably the three dimensional model, which distinguishes between subjective, objective (accuracy), and metacognitive aspects of interoceptive task performance (Garfinkel *et al.*, 2015).

1.1.2.1 Classic measurements of interoception

The first dimension is a subjective measure of interoceptive belief called *Interoceptive Sensibility*. Typically, researchers use a self-report questionnaire to assess the frequency at which bodily sensations occur, and examples of such questionnaires include the Body Perception Questionnaire (Porges, 1993) or the Multidimensional Assessment of Interoceptive Awareness (Mehling *et al.*, 2012).

The second dimension is an objective measure of interoception called *Interoceptive Accuracy*. Different interoceptive channels can be objectively measured. For example, the water load test is usually used to measure gastric interoception; participants have to drink until satiation and then, until maximum stomach fullness (Herbert *et al.*, 2012, van Dyck *et al.*, 2016). Respiratory interoception can be measured using an inspiratory resistance detection task in which participants breathe through an open breathing circuit and judge whether resistance to the airflow is present on targeted trials (Garfinkel *et al.*, 2016a). In addition, manipulation of rectal or oesophageal distension using a balloon can be used to access other dimensions of interoception (Nozu and Kudaira, 2009, Zaman *et al.*, 2016). However, the cardiac interoceptive channel (i.e. cardioception) is arguably the easiest to measure and, therefore, is a classic way to assess interoceptive processes.

The most popular tasks measuring cardioception are the heartbeat tracking and the heartbeat discrimination task (Schandry, 1981, Katkin *et al.*, 1983, Garfinkel *et al.*, 2015, Brener and Ring, 2016).

During the heartbeat tracking task, participants are asked to silently count their own heartbeats during a given period of time. Meanwhile, a pulse oximeter attached to their index finger tracks the participants' actual heartbeat. The greater the overlap between the participant's subjective heartbeat count and the objective recording, the higher the interoceptive accuracy.

In the heartbeat discrimination task, participants hear a series of sets of ten sounds, some of which are played synchronously with their heartbeat and others are played with a delay in their heartbeat (for more details, see Chapter 2, section 2.2.4.2.5). Participants are asked to detect whether or not the

sounds were played in sync with their heartbeat and higher accuracy in this task suggests better cardiac interoception (For more details on these two tasks, see Chapter 2).

While these two tasks are often used interchangeably in the objective measurement of interoception, heartbeat discrimination and heartbeat tracking tasks involve different cognitive mechanisms (Garfinkel *et al.*, 2015, Garfinkel *et al.*, 2016b). Heartbeat tracking requires the participant to focus only on his/her internal cardiac sensations, whereas performance on the heartbeat discrimination task requires the participant to flexibly switch perception between, and to integrate, external (e.g. sound) and internal (e.g. actual heartbeat) cues. Therefore, in the discrimination task, this integration of both internal and external sensorial information is crucial for making accurate judgments of synchronicity.

In the functional Magnetic Resonance Imaging scanner (fMRI), a modified version of the discrimination task is usually used in order to generate contrasts that can be subtracted. Participants are asked to attend to their own heartbeats and indicate at the end of a sequence of 10 notes if the feedback was synchronous or delayed. In the control trial, participants attend to the quality of the feedback notes and signal at the end whether the notes were all the same or if one was different. Attention toward heartbeat classically elicits enhanced activity in insula, somatomotor and cingulate cortices, compared to attention toward notes. Moreover, the right AI activity positively predicts subjects' interoceptive accuracy on the task. In addition, right AI grey matter volume correlates with both interoceptive accuracy and sensibility, suggesting that this brain region supports a representation of body states, accessible to awareness (Critchley *et al.*, 2004).

A third dimension of interoception can be assessed by asking participants to provide confidence ratings in the task measuring interoceptive accuracy (i.e. heartbeat counting and heartbeat discrimination). Confidence is specified on a scale from zero (total guess) to 100 (complete confidence). The degree to which participants' accuracy and confidence align provides a metacognitive measure of interoception, also called *Interoceptive Awareness*. This

metacognitive measure indicates the participant's insight into their own performance.

One question frequently asked is whether cardioception is a good measure of 'general' interoception. Even if further research is needed to clarify the relationship between different interoceptive channels (e.g. is it possible to be impaired in only one channel?), cardioceptive and gastric interoception accuracy are positively correlated (Herbert *et al.*, 2012, van Dyck *et al.*, 2016). On the other hand, respiratory and cardiac accuracy does not correlate significantly, nevertheless, interoceptive awareness scores on the two tasks do. Finally, neither interoceptive accuracy nor metacognitive awareness for cardiac and respiratory measures are related to touch acuity, an exteroceptive sense (Garfinkel *et al.*, 2016a). Sensitivity to affective touch is not correlated with cardiac accuracy (Crucianelli *et al.*, 2017). This evidence suggests that accuracy might not align across all interoceptive channels but individuals might have a general insight in their abilities to perceive signals from different interoceptive channels. If the generalisation to exteroceptive senses does not seem to be true, in tasks using cardiac timing manipulation, interoceptive signals have been shown to modulate exteroceptive perception. Research supporting this statement will be described in the following section.

1.1.2.2 Cardiac timing manipulations

Visceral interoceptive signalling, for example, the phasic firing of arterial baroreceptors with each heartbeat, influences our behavior (Łukowska *et al.*, 2018). One can explore the impact of visceral inputs on perception or cognition by time-locking stimulation to certain points of the cardiac cycle. Indeed, within the brain, the magnitude of heartbeat-evoked cortical responses (reflecting the cortical representation of baroreceptor activation), preceding a near-threshold visual stimulus, predicts whether or not the stimulus is then successfully detected (Park *et al.*, 2014). In some instances though, visual stimuli are harder to detect if they mirror the phasic timing of the heartbeats (Salomon *et al.*, 2016). Insular activation mediates this effect, showing reduced activation to stimuli presented at the cardiac frequency. In other words, there can be a cancelling out of external stimuli that appear 'self-related,' in this case

predicted by heartbeat frequency. These same cardiac signals appear to enhance the attribution of self: in the Rubber Hand Illusion, where individuals are induced to perceive an artificial hand as their own, the effect is strengthened if the artificial hand pulses in synchrony with the participant's heartbeat (Suzuki *et al.*, 2013).

Interestingly, both these effects appear to be related to higher-order cortical processing. However, a model dating back to the 1970s suggests that baroreceptor activation has a lower-level inhibitory influence on sensory processing and cortical excitability (Lacey and Lacey, 1970, Walker and Sandman, 1979, Walker and Sandman, 1982, Droste *et al.*, 1994, Mini *et al.*, 1995, Angrilli *et al.*, 1997, Edwards *et al.*, 2001). For example, the autonomic and insular responses to electrocutaneous shocks are suppressed if the shocks are delivered at cardiac systole (between 100 to 500ms after the electrocardiography (ECG) R wave), coincident with the peak of baroreceptor signaling (Eckberg and Sleight, 1992, Gray *et al.*, 2009, Gray *et al.*, 2010). In the brain, this effect is associated with reduced activation within the insula and pons and increased activation of the amygdala. Moreover, on an individual basis, these activation and deactivation patterns are correlated with parasympathetic vagal reactivity (Gray *et al.*, 2009). Smaller amplitude of pain evoked P2 component was observed for expected electric shock delivered on the T wave compared to stimuli delivered on the R wave (Gray *et al.*, 2010).

Emotional processing is also affected by cardiac timing. Emotional facial expressions of disgust and fear in particular are rated as more intense when presented at systole (during natural baroreceptor activation), potentially mediated by the periaqueductal grey or ventral frontal cortex and, for fear, by the amygdala (Gray *et al.*, 2012, Garfinkel *et al.*, 2014). Interestingly, sustained artificial baroreceptor activation *via* neck suction, particularly on the right side, attenuates emotional ratings of fearful faces and inhibits activations within the insula, amygdala, hippocampus, thalamus, and brainstem (Makovac *et al.*, 2015, Makovac *et al.*, 2017).

Taken together, these data link cerebral excitability to baroreceptor signalling, meaning that the modulation of autonomic output (e.g. blood pressure) and its

subsequent repercussion on behaviour, could give us insight into the brain/body interactions.

1.1.2.3 Affective priming paradigm

As we saw in section 1.1, emotional feeling states originate in part through subjective experience and cerebral representation of peripheral physiological reactions to affective stimuli (James, 1884, Lange, 1912). Bodily changes also inform and influence perceptual experience, allocation of attentional resources, emotional processing and decision-making (Bechara *et al.*, 1996, Gray *et al.*, 2010, Park *et al.*, 2014, Makovac *et al.*, 2015, Garfinkel *et al.*, 2016c, Makovac *et al.*, 2017, Łukowska *et al.*, 2018). One experimental way of testing how autonomic changes influence cognition is the use of an affective priming paradigm. Here, the emotional valence of a briefly presented stimulus (i.e. the prime) affects the processing of subsequent stimuli. Typically, if the prime and the target are of the same valence, a facilitator effect (e.g. reduced reaction times) is observed. If the valence is different, an inhibitory effect is observed (e.g. increased reaction times) relative to a control condition. In some forms of this paradigm, the prime is followed by a stimulus that prompts performance of a task, e.g. making a lexical decision (Hull *et al.*, 2002). For example, the subliminal presentation of the word “ANGER” (vs “RELAX”) as a prime, just prior to rapid judgments of letter-strings, increases systolic blood pressure in healthy individuals. Interestingly, the magnitude of this increase predicts RT prolongation on the task (Garfinkel *et al.*, 2016c). Compared to “RELAX” primes, “ANGER” primes activated the dorsal pons/parabrachial nucleus - a main node for sympathetic efferences and interoceptive afferences, as described in section 1.1.1.1. Increased systolic blood pressure is also observed in priming studies using emotional faces (Gendolla and Silvestrini, 2011, Silvestrini and Gendolla, 2011b, Silvestrini and Gendolla, 2011c, Lasauskaite *et al.*, 2013) (for review see van der Ploeg *et al.*, 2017). Such physiological changes are proposed to activate mental representations of affective states (*via* interoceptive pathways) that inform behavioral responses (Gendolla, 2012). Within the brain, positive priming scores are correlated with nucleus accumbens activation to happy faces. Sad face primes lead to a negative priming effect characterized by activations in medial, middle,

and superior frontal and middle temporo-occipital areas, amygdala, as well as in insula. (Suslow *et al.*, 2013).

By its involvement in a wide range of low to high levels cognitive and affective functions, the insular cortex has been proposed to play an important role in predictive coding models of interoception.

1.1.3 Interoceptive predictive coding and emotion

The concept of predictive coding has arisen (since Helmholz in the1860s) to understand how the brain makes sense of continuous barrage of sensory information. It is proposed that the brain is a 'prediction engine' that generates hypotheses about the sources of sensory information then tests these predictions against incoming data. The process generates prediction errors which are used to update the accuracy of the predictive model or to motivate actions/behaviours to resample and/or change the source of the sensory data, a notion called active inference. Predictive coding, error correction and active inference are proposed to be part of a more general Free Energy Principle (Friston *et al.*, 2006). This principle stipulates that dynamics of any system, which does not dissipate over time, will change of states to maximize Bayesian model evidence. In other words, an organism is soliciting information from the environment and is modelling this information to maximise evidence of its own existence. The human brain is hypothesised to generate predictions about the world that are based on the learning of stable characteristics of the environment. These predictions inform the interpretation of sensory inputs, by either changing the source of the information by acting on environment (e.g. moving) or by changing predictions itself.

Predictive coding models are composed of descending (top-down) predictions arising from generative models. In addition, these generative models are constantly updated by ascending (bottom-up) prediction errors. The main goal is to suppress predictions errors, meaning that the predicted signal should be as similar as possible to the received input in order to maximise the predictability of the environment. This kind of framework has been widely applied to perception and action (Rao and Ballard, 1999, Hosoya *et al.*, 2005, Spratling, 2008, Hakonen *et al.*, 2017). Daniel Wolpert and others extended

predictive motor control framework to social interactions (Wolpert *et al.*, 2003). Recently, the predictive coding has been applied to the understanding of emotional and interoceptive states (Seth *et al.*, 2011, Seth, 2013, Apps and Tsakiris, 2014, Ainley *et al.*, 2016, Seth and Friston, 2016). In the context of interoception, it is proposed that subjective feelings are defined through interoceptive predictions of bodily states. This proposition is in line with the theories of emotion we reviewed in section 1.1, proposed by James-Lange, Schachter and Singer or Damasio (James, 1884, Lange, 1912, Schachter and Singer, 1962, Damasio, 1996).

1.1.3.1 In the context of interoception

In the interoceptive context, brain and body can be considered as two distinct systems. In the Embodied Predictive Interoception Coding (EPIC) model, proposed by Barrett and Simmons, all agranular visceromotor cortices (including cingulate, posterior ventral medial prefrontal, posterior orbitofrontal and ventral AI cortices) are hypothesized to issue visceromotor predictions which are sent to the hypothalamus, brainstem, and spinal cord nuclei to preserve homeostasis. Agranular cortices are also hypothesised to predict changes in interoceptive signals which will be induced by such homeostatic changes (interoceptive prediction). In response to the afferent signals, primary interoceptive granular cortices (insula) generate interoceptive predictions (errors) back to visceromotor cortices and efferent organs (Barrett and Simmons, 2015).

Given its posterior-anterior gradient of dendritic complexity, the insular cortex seems particularly suited to receive interoceptive prediction errors in its mid-posterior part (low integration region), which will be compared to interoceptive predictions generated in the more anterior part (high integration region) (Anderson *et al.*, 2009, Gallay *et al.*, 2012). Consistent with the potential prediction encoding, the crucial role of AI in risk-taking, uncertainty, error processing and error awareness has been documented in healthy and psychiatric samples (Paulus *et al.*, 2003, Sarinopoulos *et al.*, 2010, Harsay *et al.*, 2012, Orr and Hester, 2012, Klein *et al.*, 2013, Allen *et al.*, 2016). As mentioned in section 1.1.1.4, the AI and ACC/MCC play a crucial role in

salience attribution. In fact, as part of the “salience network” (Seeley *et al.*, 2007), the AI is hypothesized to detect the most relevant events among (internal or external) environment, and to temporarily initiate attentional control inputs, which, then are sustained by ACC (Menon and Uddin, 2010). Large projection neurons (von Economo neurons) have been hypothesised to optimise communication between these two structures (Craig, 2002, Allman *et al.*, 2011, Critchley and Seth, 2012). Taking together, the insular cortex seems to integrate prediction errors and predictions in order to modulate salience given to prediction errors. This will then allow the mobilisation of sustained attention, via the involvement of other regions. Subjective feelings may arise from the specific salience attribution to relevant bodily signals.

1.1.3.2 In the context of empathy

Empathy (i.e. understanding the affective states of other people) is crucial to social cognition and is expressed for (higher-order) thoughts and emotional feeling states but also for lower-level physical sensations, e.g. pain (Decety and Jackson, 2004, Singer *et al.*, 2004, Kanske *et al.*, 2017). In the context of empathy, self and other can be considered as two distinct systems. Interestingly, good interoceptive abilities are associated with stable body representations, greater emotional Theory-of-Mind processing, better ability to describe one’s own emotions and increased recognition of emotional facial expressions (Tsakiris *et al.*, 2011, Terasawa *et al.*, 2014, Shah *et al.*, 2017). This dovetails with the hypothesis of a predictive coding model of emotion in which the understanding of affective states of others (empathic feelings) arises from the top-down simulation (interoceptive prediction) of likely bodily state and the integration of subsequent interoceptive afferent signals into affective representation of both self and other (Singer *et al.*, 2009, Ainley *et al.*, 2014). However, this association needs to be learnt.

An interesting theoretical model integrating psychoanalysis, developmental neuroscience and predictive coding has been proposed by Fotopoulou and Tsakiris (2017). In their paper, the authors argue that empathy arises from mentalization of bodily states, which is based on an intrinsically social process. Given the immaturity of infants’ motor and sensorial systems, by touching,

holding, feeding, producing emotional expressions, the caregiver will modify interoceptive states of the infant and allow the generation of interoceptive predictions. In other words, caregiver's actions will shape the infant's perception of affective states (satisfaction, pain, pleasure etc), contributing to infant's mentalization of bodily states *via* associative learning. The mentalization of bodily states will then inform the development of empathic feelings (Sagi and Hoffman, 1976, de Haan and Gunnar, 2009). Finally, innate social attachment is proposed as an important mechanism to favour caregiver-infant interactions, allowing associative learning to take place (Bowlby, 1969). In this context, alexithymia, a deficit in affect regulation, is characterized by poor attachment style and deficits in mentalization as well as in self-awareness (Lemche *et al.*, 2004, Moriguchi *et al.*, 2006, Besharat and Khajavi, 2013, Lane *et al.*, 2015, Koelen *et al.*, 2016).

1.2 Alexithymia and emotional processing

Alexithymia is a personality construct classically defined by difficulties in describing and identifying ones' own emotional feelings (Apfel and Sifneos, 1979, Taylor *et al.*, 1985). Early interpersonal trauma is hypothesized as a cause of alexithymia development (Berenbaum, 1996). For example, the relationship between child maltreatment and risky sexual behaviour, in undergraduate students, is partially mediated by alexithymia (Hahn *et al.*, 2015). Moreover, a meta-analysis focusing on parenting styles shows a strong relationship between maternal care and characteristics of alexithymia (Thorberg *et al.*, 2011). Avoidant attachment style is a strong predictor of alexithymia in alcohol dependent subjects (De Rick and Vanheule, 2006). In addition, alexithymia is also related to maladaptive coping strategies - such as emotional inhibition and immature defensive styles- which are factors of risky behaviours development (Helmes *et al.*, 2008).

Alexithymic subjects show abnormal affective regulation characterized by reduced emotional face recognition, delayed automatic rapid facial reactions, reduced empathy, reduced emotional awareness, abnormal emotional remapping and higher body representation malleability (Moriguchi *et al.*, 2007, Grynberg *et al.*, 2012, Grynberg and Pollatos, 2015, Lane *et al.*, 2015,

Scarpazza *et al.*, 2015, Georgiou *et al.*, 2016, Lyvers *et al.*, 2017, Scarpazza *et al.*, 2017). Moreover, in alexithymia, the representation and integration of interoceptive bodily responses appear to be impaired.

1.2.1 Alexithymia and interoception

Alexithymia has been previously linked to impaired bodily regulation and interoception through notions of 'Apraxithymia' (Helling, 2009) and 'Alexisomia' (Ikemi and Ikemi, 1986), terms that conceptualize abnormalities in the expression and awareness of bodily feelings. A multi-dimensional failure of interoception is suggested to be a very important contributor to alexithymia (Brewer *et al.*, 2016, Murphy *et al.*, 2017). Correspondingly, alexithymia is associated with poorer cardiac interoceptive accuracy (Herbert *et al.*, 2011, Shah *et al.*, 2016a, Shah *et al.*, 2016b), yet an over-reporting of subjective physical symptoms including a hypersensitivity to touch (Sivik, 1993, Lumley *et al.*, 1996, Nyklicek and Vingerhoets, 2000, Nakao *et al.*, 2002, Lumley *et al.*, 2005, Kano *et al.*, 2007, Lumley *et al.*, 2007, Kojima, 2012, Kojima *et al.*, 2014). These latter findings demonstrate a mismatch between objective and subjective aspects of interoception, possibly impacting emotional processing and 'sense of self'. In alexithymic subjects, morphological, functional and neurochemical abnormalities of interoceptive cortices are observed.

1.2.2 Alexithymia and neural correlates of interoception

Neuroimaging studies show a decreased activation of the ACC during emotion judgment tasks (Jongen *et al.*, 2014), of the MCC during face recognition for anger (Kano *et al.*, 2003), of the left ACC during empathy for pain (Moriguchi *et al.*, 2007), and of the sACC during heartbeat detection (Wiebking and Northoff, 2015). Although the majority of studies point to an ACC hypoactivity as a main abnormality in alexithymia, there is no real consensus concerning the abnormal functioning of the insula in alexithymic subjects. Some studies find that the insula is hypoactivated (Kano *et al.*, 2003, Silani *et al.*, 2008, Bird *et al.*, 2010, Reker *et al.*, 2010, Jongen *et al.*, 2014) whereas others find the opposite trend (Moriguchi *et al.*, 2007, Wiebking and Northoff, 2015, Enzi *et al.*, 2016). In studies using voxel-based morphometry, alexithymia is associated with reduced grey matter volume in the left posterior insula and

increased grey matter volume in the sACC (Goerlich-Dobre *et al.*, 2015, Xu *et al.*, 2018). However, in a study involving a large sample of brain-injured subjects, anterior insula damage, but not ACC damage, predicted alexithymia (Hogeveen *et al.*, 2016).

Using magnetic resonance spectroscopy (MRS), it is possible to measure the concentration of metabolites, *in vivo*. For example, in the left insula, the concentration of glutamate, which is the main excitatory neurotransmitter, is positively associated with alexithymia (Ernst *et al.*, 2014). In addition, in the ACC, the concentration of gamma-Aminobutyric acid (GABA), which is the main inhibitory neurotransmitter, is positively associated with alexithymia (Ernst *et al.*, 2014). Moreover, glutamate levels in the left insula, but not in the ACC, were positively correlated with the awareness of autonomic nervous system reactivity. The authors postulate that increased glutamate concentration in the insula could lead to higher unspecific interoceptive arousal in alexithymia. This hypothesis is in line with the suggested hypersensitivity toward bodily sensations observed in alexithymic individuals. Furthermore, the authors interpret the GABA concentration in ACC as an effort to regulate insula hyperactivation. Results from a second study by the same team further support their earlier findings (Wiebking and Northoff, 2015). However, another study targeting GABA concentration levels in the left AI, rather than in the ACC, in healthy subjects, found a positive correlation between insula-related GABA and empathy (Wang *et al.*, 2014). As described in section 1.1.1.1, the insular cortex is connected with important homeostatic regions of the brainstem. Accordingly, alexithymia is also characterized by an abnormal autonomic profile.

1.2.3 Alexithymia and autonomic system

Alexithymia is typically associated with anxiety problems (Lyvers *et al.*, 2014) and poor stress-management skills (Fukunishi and Rahe, 1995), which are reflected in lower-level psycho-physiological abnormalities (Bogdanov *et al.*, 2013).

Conversely, peripheral abnormal autonomic abnormalities are documented in alexithymic subjects and have been related to increased risk of premature

death and cardiovascular disease (Kauhanen *et al.*, 1994, Kauhanen *et al.*, 1996, Helmers and Mente, 1999, Tolmunen *et al.*, 2010). Dampened autonomic reactivity to emotional challenges or stress supports a hypoarousal model of alexithymia (Neumann *et al.*, 2004, Pollatos *et al.*, 2011, Constantinou *et al.*, 2014, Peasley-Miklus *et al.*, 2016, Cecchetto *et al.*, 2018). Nevertheless, a consensus is still missing as hyperactivity of the sympathetic system has also been described in alexithymia (Infrasca, 1997, Lumley *et al.*, 2007, Nandrino *et al.*, 2012). This abnormality is characterized by higher basal and longer habituation time of electrodermal activity (Stone and Nielson, 2001, Bogdanov *et al.*, 2013). Moreover, alexithymia is typically associated with diminished heart rate variability (HRV), suggestive of reduced parasympathetic tone (Fukunishi *et al.*, 1997, Fukunishi *et al.*, 1999). However, there is yet no conclusive evidence for an association between alexithymia and abnormal baroreflex sensitivity (Virtanen *et al.*, 2003). Alexithymic individuals report higher self-reported anxiety and show greater systolic blood pressure reactivity during the stress of blood donation (Byrne and Ditto, 2005). During anger recall, alexithymic individuals have attenuated cardiac responses (Neumann *et al.*, 2004) but then show hampered recovery (of diastolic blood pressure recovery and cardiac pre-ejection period), compared to non-alexithymic individuals (Neumann *et al.*, 2004). Nevertheless, alexithymia is more prevalent among hypertensive patients than in the general population (Gage and Egan, 1984, Todarello *et al.*, 1995, Jula *et al.*, 1999).

Observations in hypertensive patients can inform our understanding of how interoceptive abnormalities may underlie the expression of alexithymia. In hypertension, abnormalities of the baroreflex mechanism are observed, including reduced baroreflex sensitivity (Mussalo *et al.*, 2002). Hypertensive patients are also reported to show impaired interoceptive abilities during a heartbeat detection task (Yoris *et al.*, 2017). This deficit is observed independently of heart rate or HRV (an index of parasympathetic cardiac control) and exteroceptive abilities remain preserved (Yoris *et al.*, 2017). This disruption of interoceptive processing in hypertensive patients is likely to contribute to the association between raised resting systolic blood pressure and impaired emotional processing (Pury *et al.*, 2004, McCubbin *et al.*, 2011).

These findings suggest importance of blood pressure on aspects of interoception.

Alexithymia is characterized by disrupted emotional and interoceptive processing, in body and brain. Interestingly, alexithymia is highly prevalent in people suffering from addictive disorders, particularly in alcohol use disorders.

1.2.4 Alexithymia and alcohol use disorder (AUD)

A strong association between alexithymia and AUD is found showing that 60 to 70 % of alcohol-dependent patients suffer from alexithymia (Uzun *et al.*, 2003), while only 10% suffer from this emotional impairment in the general population (Rybakowski *et al.*, 1988, Loas *et al.*, 1995). High alexithymia scores predict earlier age of alcohol consumption, duration of alcohol misuse and amount of alcohol consumed in people with alcohol dependence (Kopera *et al.*, 2015). Moreover, alexithymia is negatively related to an ability to remain abstinent (Loas *et al.*, 1997) and is inversely correlated with measures of emotional intelligence (Fukunishi *et al.*, 2001). Thus, alexithymia may specifically increase the likelihood of alcohol use disorders (Uzun *et al.*, 2003, for review see: Thorberg *et al.*, 2009).

1.3 AUD and emotional processing

Difficulties in emotion regulation seem to predispose to AUD (Williams and Clark, 1998). Drinking as a coping strategy ("escape drinking") is described alongside enhancement ("social drinking") as a key motivator and driver of alcohol-related problems (Lyvers *et al.*, 2010, Bradley *et al.*, 2011). Childhood deficits in emotional and interpersonal skills are associated with risky alcohol consumption and drug use in adolescence (Hessler and Katz, 2010), while lower measures of emotional intelligence increase the likelihood of relapse in detoxified patients (Kopera *et al.*, 2015). Impairments in recognising emotional expressions (Kornreich *et al.*, 2002, Townshend and Duka, 2003) alongside deficits in empathy and emotional awareness (Maurage *et al.*, 2011) are reported in alcoholic patients. Even after cognitive behavioural therapy, emotion regulation skills still significantly predict future alcohol use in alcohol-dependent patients (Berking *et al.*, 2011). Moreover, emotional impairments are linked to interpersonal problems and thus represent a relapse factor in

alcoholism (Kornreich *et al.*, 2002). Consequently, emotional dysregulation is proposed to be a major factor in both the development and maintenance of AUD (Loas *et al.*, 1997, Kun and Demetrovics, 2010, Kopera *et al.*, 2015). As interoception is a crucial facet of emotional regulation, one could expect disruption of interoceptive processes in AUD. Given the lack of data on interoception in alcohol use disorder, the next two sections will be broadened to include both alcohol and drug abuse disorders.

1.3.1 Addiction and interoception

In the context of drug addiction, indirect evidence suggests that interoceptive processes underpin urges to take a drug, also known as drug craving (Naqvi and Bechara, 2010). For example, lesion studies implicate the insular cortex, a brain region supporting interoceptive states (see section 1.1.1.1), in the phenomenon of craving (Gray and Critchley, 2007). Neuroimaging studies further reveal abnormalities in the morphometry, functional activity and connectivity of insular cortex in alcohol, cannabis and, also, tobacco users (Berk *et al.*, 2015, Maria *et al.*, 2015, Grodin *et al.*, 2017). In addition to lesion and neuroimaging investigations, more direct evidence concerning the relationship between interoceptive ability and craving can be drawn from behavioural studies. Interestingly, the interoceptive accuracy of alcohol-dependent subjects and drug users has only been tested using the heartbeat tracking task. Diminished interoceptive abilities are observed in these populations and this deficit is positively associated with both subjective craving sensations and alexithymia (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). However, the effective integration of interoceptive inputs is crucial for both subjective experience and social skills (Park and Tallon-Baudry, 2014, Shah *et al.*, 2017); this might explain why those abilities are typically impaired in alcohol and drug use disorders, as described in section 1.3 (D'Hondt *et al.*, 2014, Verdejo-Garcia, 2014). Moreover, as mentioned in section 1.1.1.2, the insular cortex plays a crucial role in interoceptive processes.

1.3.2 Addiction and interoceptive cortex integrity

The insular cortex is involved in the neurocircuitry of addiction: interoceptive components of drug seeking (notably craving states) are thought to originate within the insula (Gray and Critchley, 2007). Correspondingly, impairments in interoceptive bodily sensation are reported in 'addicts', including methamphetamine users (Stewart *et al.*, 2014), adolescent cannabis users (Berk *et al.*, 2015), and in internet gaming disorder (Zhang *et al.*, 2016). Damage to insula cortex can change addictive behaviours (Naqvi *et al.*, 2014). For example, focal insular lesions reduce nicotine craving in smokers and reduce the occurrence of distorted cognitive appraisals that drive compulsive betting (Clark *et al.*, 2014) in individuals with gambling disorder. The volume of bilateral insulae (and related striatal 'reward' regions) is reduced in alcohol use disorder, in the context of more diffuse grey matter shrinkage (Yang *et al.*, 2016). The volume and thickness of anterior insular cortex are negatively correlated to impulsivity and compulsions in alcohol-dependent individuals (Grodin *et al.*, 2017). The integrity of major cerebral white matter tracts is also reduced in heavy drinkers. This structural disruption of interregional connectivity correlates negatively with functional reactivity of the insular cortex to alcohol cues. Thus, structural changes may underpin exaggerated sensitivity to cues regulating alcohol consumption (Monnig *et al.*, 2014). The functional interaction between insular cortex and prefrontal regions is also reduced during emotional processing in individuals with alcohol dependence, indicating a more generalised impact upon the regulation of motivational and affective processes (O'Daly *et al.*, 2012). Functional abnormalities in alcohol use disorders may be ascribed to changes at the neurochemical level.

1.3.3 AUD and interoceptive cortex neurochemistry

Alcohol interacts with glutamatergic neurotransmission, suppressing excitatory synaptic signalling, particularly through inhibition of N-methyl-D-aspartate (NMDA) receptors (Lovinger *et al.*, 1989). This impacts synaptic plasticity by reducing long-term potentiation (Stephens *et al.*, 2005). In compensation, the number and sensitivity of NMDA receptors increase proportionally to the amount and frequency of alcohol intake (Trujillo and Akil, 1995). A sharp reduction or cessation of alcohol consumption can induce rebound neuronal hyperexcitability, leading to excitotoxicity (Tsai *et al.*, 1995). In rats, ethanol increases glutamate concentration within striatal reward circuitry (Ding *et al.*, 2012). In humans, it is possible to quantify neurochemicals *in vivo*, including glutamate, using MRS. In alcohol-dependent patients, glutamate concentration is increased within the ventral striatum following detoxification and glutamate concentration in the left dorsolateral prefrontal cortex correlates with rated intensity of alcohol craving (Bauer *et al.*, 2013, Frye *et al.*, 2016). Moreover, the combined concentration of glutamate and glutamine (Glx) within the ventral striatum and ACC correlates positively with compulsions to drink alcohol (Bauer *et al.*, 2013). A reduction of glutamate and an increase of glutamine concentration within anterior cingulate cortex relates to the severity of alcohol use disorder; suggesting a perturbation of cingulate glutamate-glutamine cycle dynamics in alcohol addiction (Thoma *et al.*, 2011). Lower concentrations of glutamate and N-acetylaspartate (NAA; a neuronal integrity marker) within ACC are observed in the early stages of stopping drinking; these concentrations normalize after five weeks of abstinence (Mon *et al.*, 2012). Similarly, glutamate and NAA concentrations within ACC decrease after a bout of heavy drinking in individuals with alcohol dependence (Prisciandaro *et al.*, 2016). Finally, lower glutamate concentration within neighbouring prefrontal white matter predicts loss of control and severity of alcohol dependence in heavy drinkers (Ende *et al.*, 2013).

1.4 Alcohol use, interoception and alexithymia

As mentioned earlier, people suffering from substance and alcohol use disorders show higher prevalence of alexithymia and impaired social cognition. Also, behavioural and neuroimaging evidence suggests that the processing of bodily sensations is disrupted in people with substance use disorders (May *et al.*, 2013, Berk *et al.*, 2015). Moreover, poorer interoceptive accuracy correlates with higher alexithymia scores (Sönmez *et al.*, 2016) and an enhanced craving for alcohol (Ates Çöl *et al.*, 2016) in alcohol-dependent individuals. Finally, neurochemical, structural or functional abnormalities of important nodes of interoceptive pathway, such as the insular cortex, are observed in both alcohol use disorders and alexithymia (Ernst *et al.*, 2014, Hogeveen *et al.*, 2016, Grodin *et al.*, 2017, Reza *et al.*, 2017, Xu *et al.*, 2018). Nevertheless, despite the growing literature highlighting the association between addictions and interoceptive impairments, the relationship between abnormal sensitivity to bodily sensations and alexithymia has never previously been investigated, in alcohol users.

Taken together, interoceptive processes are involved in alexithymia and addictions. As already suggested by some authors, future therapies should target interoceptive processes in order to try to modulate emotional regulation in addictions (Paulus *et al.*, 2013, Naqvi *et al.*, 2014). The neuropeptide oxytocin could be one of the candidates.

1.5 Oxytocin and emotional processing

Oxytocin (OT) is a neuropeptide hormone that is mostly synthesised in the paraventricular and the supraoptic nuclei of the hypothalamus and released into the bloodstream through the posterior pituitary gland (Sokol and Valtin, 1967). OT receptors are present in central and peripheral tissues, including the brain, heart, gastrointestinal tract, and uterus. (Gimpl and Fahrenholz, 2001). OT's essential contribution to labour and childbirth is well known but additionally, OT plays a key role in the social behaviour of mammals (Carter, 2003, Parker *et al.*, 2005). The importance of OT in mother-child bonding is established across animal species and in human (Kendrick *et al.*, 1987, Young and Wang, 2004). The victims of parental neglect, abuse and, more generally,

stressful early emotional interactions, show lower baseline OT concentrations than people reporting a less stressful early development (Meinlschmidt and Heim, 2007, Heim *et al.*, 2009). Across individuals, endogenous (salivary) OT levels positively correlate with rated levels of emotional competence and interpersonal skills (Koven and Max, 2014). However, underlying mechanisms remain unclear, since rodent ligands for OT receptors are not selective for human OT receptors (Paloyelis *et al.*, 2014, Leng and Ludwig, 2015, Quintana *et al.*, 2015, Valstad *et al.*, 2016, Valstad *et al.*, 2017).

Nevertheless, OT administration can increase trust and enhance the detection of others' emotional signals (Domes *et al.*, 2007a, Keri and Kiss, 2011, Schulze *et al.*, 2011, Lischke *et al.*, 2012, Van and Bakermans-Kranenburg, 2012, Perry *et al.*, 2013, Kanat *et al.*, 2015). Relatedly, OT administration can also improve capacity for "mind reading" (mentalization) (Guastella *et al.*, 2010), empathy (Hurlemann *et al.*, 2010, Panksepp and Panksepp, 2013) and mimicry of angry faces (Korb *et al.*, 2016). One proposed mechanisms by which OT impacts emotional regulation is via the modulation of attentional resources. Indeed, intranasal OT will preferentially increase attention toward social cues, such as emotional faces, compared to neutral or non-social cues (Tollenaar *et al.*, 2013, Clark-Elford *et al.*, 2014, Dal Monte *et al.*, 2014a, Domes *et al.*, 2016, Kanat *et al.*, 2017, Pfundmair *et al.*, 2017). There is emerging interest in whether the modulation of interoceptive processing underpins the impact of the OT system on social cognition.

1.5.1.1 Oxytocin and interoception

OT is hypothesized to enhance attention toward interoceptive signals (i.e. precision of central interoceptive representations), which can inform generative models of emotional and selfhood (Quattrocki and Friston, 2014). In other words, OT would modulate associative learning mechanisms described in the predictive coding model of empathy in section 1.1.3.2 (Fotopoulou and Tsakiris, 2017). Intranasal OT administration may not markedly influence performance on heartbeat discrimination in healthy human participants (Yao *et al.*, 2017), yet electrophysiological studies show that OT gates the transmission of viscerosensory afferent information (Peters *et al.*,

2008). Correspondingly, OT has a direct impact on neurons within the nucleus of the solitary tract (NTS), the main visceroceptive relay within the brainstem (Craig, 2002, Karelina and Norman, 2009).

Also, it has been recognised that attachment quality is dependent on the endogenous levels of OT, which is also associated with the risk of developing addictive behaviours (Buisman-Pijlman *et al.*, 2014).

1.5.1.2 Oxytocin and alcohol use

Interestingly, a new wave of translational research suggests that OT administration can inhibit alcohol consumption. One clinical trial showed that intranasal OT attenuated alcohol withdrawal symptoms in alcoholic patients (Pedersen et al., 2013). In rodents, the overexpression of OT receptors in mice reduces the rewarding proprieties of ethanol (Bahi, 2015) and OT injections decrease ethanol consumption, ethanol preference and ethanol-triggered dopamine release within the accumbens nucleus (Peters *et al.*, 2013, MacFadyen *et al.*, 2016, King *et al.*, 2017, Peters *et al.*, 2017). Such observations further implicate the OT system in the development and maintenance of addiction (McGregor and Bowen, 2012, Buisman-Pijlman *et al.*, 2014, Lee *et al.*, 2016). Indeed, in adolescent animals, OT exposure will reduce the expression of anxiety and protect against development of adult alcohol and drug seeking behaviours (Bowen *et al.*, 2011, Hicks *et al.*, 2016). These effects are bidirectional: alcohol injection is known to inhibit endogenous OT release (Fuchs *et al.*, 1967). Again, interoception appears to be an important mediator: the processing of bodily sensations is impaired in alcohol-dependent individuals and the degree of this impairment correlates with emotional impairments, including alexithymia. OT can improve the emotional skills of alexithymic individuals (Luminet *et al.*, 2011), most likely through its impact on brain centres supporting both interoception and emotion (Crockford *et al.*, 2014, Lancaster *et al.*, 2015, Strauss *et al.*, 2015).

In addition, alcohol seems to inhibit endogenous release of OT. For example, intravenous alcohol infusion was used to avoid premature labour (Lynn, 1970). In addition, two studies report greater plasma levels of OT in alcohol-dependent patients in recent alcohol abstinence (at 1, 4, 7, 15 and 28 days

after alcohol withdrawal; Legros *et al.*, 1983, Marchesi *et al.*, 1997). The authors postulate that this may be due to a “rebound effect” due to the ethanol consumption cessation (Legros *et al.*, 1983, Marchesi *et al.*, 1997). Taken together, this supports the potential involvement of the oxytocinergic system in addiction, which might also be associated with aberrant interoceptive processes.

1.6 Summary

Interoceptive abilities are crucial for both emotional processing and sense of self integrity (Seth, 2013, Ainley *et al.*, 2014, Barrett and Simmons, 2015, Critchley and Garfinkel, 2017). An emergent theory suggests that alexithymia is, in fact, the outcome of an interoceptive failure (Brewer *et al.*, 2016, Shah *et al.*, 2016a, Shah *et al.*, 2016b, Murphy *et al.*, 2017). Moreover, alexithymia, alongside, emotional and interoceptive impairments have been observed in alcohol use disorders (Rybakowski *et al.*, 1988, Ziolkowski *et al.*, 1995, Townshend and Duka, 2003, Maurage *et al.*, 2009, Thorberg *et al.*, 2009, Charlet *et al.*, 2014, Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). Within the brain, evidence from lesion and neuroimaging studies seems to highlight the potential involvement of a common cerebral region, the insular cortex (Critchley *et al.*, 2004, Naqvi *et al.*, 2007, Naqvi *et al.*, 2014, Hogeveen *et al.*, 2016, Gorka *et al.*, 2017, Xu *et al.*, 2018). However, to date, no study explored the relationship between the three variables. Finally, the neuropeptide oxytocin facilitates empathy and enhances interoceptive afferent transmission (Domes *et al.*, 2007b, Peters *et al.*, 2008, Bartz *et al.*, 2010, Leknes *et al.*, 2013, Aoki *et al.*, 2014). Recent data in human and rodents suggest that oxytocin could decrease alcohol withdrawal symptoms, alcohol consumption and alcohol preference (Pedersen *et al.*, 2013, Hicks *et al.*, 2016, MacFadyen *et al.*, 2016, King *et al.*, 2017, Peters *et al.*, 2017). Therefore, one could think that oxytocin reduces stress-responses and craving sensations by impacting interoceptive processes *via* modulation of insular activity. We hope to clarify the relationships between interoception, alexithymia, alcohol use and oxytocin which might lead to a better mechanistic understanding of some aspects of addiction, and, thus, might inform the development of new therapeutic strategies.

1.7 Aims of the project

The overall research plan of this PhD thesis was to characterise the relationship between interoception, alcohol use and alexithymia. To do so, I designed a set of five studies to dissect systematically this relationship through the measurement of experiential (subjective), behavioural, physiological and neural data, in order to address the aims listed below.

In Chapter 3, I sought first to characterise how subjective measures of alexithymia, sensitivity to bodily sensations and alcohol consumption were interrelated, using mediation analyses to infer likely causality.

In Chapter 4, my aim was to find a potential mediator, within the brain that could account for the interoceptive impairment associated with alcohol misuse that I observed in Chapter 3. Combining MRS with behavioral and psychometric ratings, I tested the relationship between alcohol-related measures (i.e. severity of alcohol use, craving, and compulsion) and middle insular cortex neurochemistry (glutamate, glutamine and TNAA metabolite concentrations) in alcohol users. To gain deeper insight, I also tested for associations between alcohol-related measures and insular morphology (volume and surface gyrification) and used voxel and surface-based morphometry.

Pre-existing literature and the findings of my investigations described in the Chapters 3 and 4, strengthened evidence of an association between interoceptive impairments and alcohol misuse. In Chapter 5, my aim was to explore the interaction between interoception, alcohol use and oxytocin. Indeed, oxytocin has been shown to improve emotional regulation. Moreover, interoception is hypothesised to underpin emotional processes. Therefore, I sought to characterize the impact of intranasal OT on interoceptive processing in alcohol users, using classical behavioural interoceptive tasks.

In Chapter 6, I sought to explore in more depth the mechanisms through which OT was acting in order to produce the behavioural modulations observed in Chapter 5. Therefore, my aim was to identify changes in the functional cerebral activity for empathy-for-pain (using multiband fMRI), while combining technical

(using cardiac timing presentation) and pharmacological (through oxytocin administration) manipulations of visceral input.

Finally, in Chapter 7, my aim was to better understand the relationship between bodily sensations and decision-making during emotional processing. I sought to identify how physiological changes (indexed by beat-to-beat systolic blood pressure) evoked by emotional primes (using an affective priming paradigm) influence cognition, specifically the accuracy and speed of decision-making. A second aim was to test whether alexithymia contributed to the relationship between bodily sensations and decision-making during emotional processing.

1.8 Hypotheses to test

In Chapter 3, I hypothesised that alexithymia, sensitivity to bodily sensations and alcohol consumption will all be positively correlated and that alexithymia will mediate the relationship between bodily sensations and alcohol consumption.

In Chapter 4, based on the evidence of alcohol-induced glutamatergic excitotoxicity, I hypothesised that insular glutamate plus glutamine concentration would be lower in individuals with higher scores on alcohol use measures. I also predicted, based on previous reports, that insular grey matter volume and probably cortical gyrification index would be negatively correlated with these alcohol use measures.

In Chapter 5, I hypothesised that interoceptive skills are negatively correlated with alcohol use severity and that OT administration improves interoception and hence can reduce the impairments observed in heavy alcohol users.

In Chapter 6, within the predicted activation of the empathy-for-pain matrix when viewing of painful pictures compared to non-painful pictures, I expected that attenuation at ventricular systole (during baroreceptor activity) relative to diastole (baroreceptor inactivity).

Also, I hypothesized that OT would decrease the activation of the empathy-for-pain matrix. However, taking into account the hypothesis that OT attenuates interoceptive precision, we expected that intranasal OT would attenuate the degree of cortical inhibition induced by baroreceptor activity at systole.

In Chapter 7, I predicted that affective priming with brief anger stimuli would be associated with increases in systolic blood pressure that influence accuracy and response times during a short-memory task. I hypothesized that alexithymia may partly mediate the relationship between physiological changes and decision-making through its association with increased systolic blood pressure at rest.

Chapter 2 Materials and Methods

2.1 Study 1 (Chapter 3)

In this first study, I sought to characterise relationships between subjective measures of alexithymia, sensitivity to bodily sensations and alcohol consumption, using bootstrapping mediation analyses to infer likely causality. To do so, I designed and undertook an online survey (study 1).

2.1.1 Participants

Participants were recruited from students and staff at the Universities of Brighton and Sussex via posters, social networks, and via online advertisements. The study was a computerised survey, distributed via an online data collection platform (Qualtrics, Provo, UT, USA; http://www.qualtrics.com). A total of 779 participants consented and 600 individuals completed all questions and provided full data. To avoid the pitfalls of missing datasets, I used a conservative approach (case deletion), and confined all analyses to the 600 individuals who provided full data (Kang, 2013). The study was approved by the local research ethics committee (Brighton and Sussex Medical School Research Governance and Ethics Committee BSMSRGEC). Participation was encouraged by the chance to win a £20 prize.

2.1.2 Measures and procedure

Potential participants were given a link to the online platform. This provided information about the study and what would be expected of them. Participants consented by agreeing to the first statement of the survey and 'clicking' continue. The online data collection platform did not allow block randomisations, therefore all participants completed the following measures in the same order:

2.1.2.1 Socio-demographic information and questionnaires

This collected information including age, gender, and level of education.

2.1.2.1.1 Toronto Alexithymia Scale (TAS-20)

The TAS-20 (Bagby *et al.*, 1994a) consists of 20 items rated on a five-point Likert scale (from 1 "strongly disagree" to 5 "strongly agree"). The TAS-20 is a

widely used index of alexithymia. It is a self-report scale in which participants rate their perceived ability to identify emotions. This naturally raises questions about the accuracy with which someone lacking all emotional language can accurately make such judgement. Correspondingly care has been taken in validating the use of the scale in different populations. Exploratory factor analysis and confirmatory factor analyses demonstrated that TAS-20 had a good internal consistency (Cronbach's α=0.81), a good test-retest reliability (0.77, p <0.01) and a three-factor structure, in both clinical and non-clinical populations (Haviland *et al.*, 1988a, Haviland *et al.*, 1988b, Taylor *et al.*, 1988, Taylor *et al.*, 1992, Bagby *et al.*, 1994a, Bagby *et al.*, 1994b, Loas *et al.*, 2001, Taylor *et al.*, 2003, Bagby *et al.*, 2006). The first factor measures *difficulties in identifying feelings* (DIF), the second factor measures *difficulties in describing feelings* (DDF) and the third factor measures the way the participant uses *externally oriented thoughts* (EOT). The total alexithymia score is the sum of responses across all 20 items.

The cut-offs of the TAS-20 have been developed using methods assessing its sensitivity, specificity and predictive value, in order to discriminate between individuals with and without alexithymia (Taylor *et al.*, 1988, Bagby *et al.*, 1994b, Taylor *et al.*, 1997, Bagby *et al.*, 2006). People with an alexithymia score lower or equal to 51 are considered as "Non alexithymic" (NAL), people with an alexithymia score higher or equal to 61 are considered as "Alexithymic"(AL); people with a score between the 2 cut-offs are considered as "Intermediate" (INT).

Cronbach's α=0.722 indicated acceptable internal consistency in the current sample. However, I only considered the total score in the mediation analysis.

2.1.2.1.2 Alcohol Use Questionnaire (AUQ)

The AUQ (Mehrabian and Russell, 1978) is a 15-item scale measuring the frequency and quantity of alcohol consumption (a*lcohol units drunk per week)*. Participants were asked to estimate the number of drinking days, the usual quantity consumed and drinking pattern, taking into account their alcohol consumption over the previous six months. The AUQ is a reliable measure of drinking quantity and drinking pattern (Townshend and Duka, 2002).

2.1.2.1.3 Body Perception Questionnaire (BPQ)

Individual differences in *sensitivity to bodily sensations* were assessed using the BPQ (Porges, 1993). Participants completed the *awareness* subscale as it is the most relevant and widely used subscale to assess subjective sensitivity to bodily sensations ('sensibility'; Garfinkel *et al.*, 2015). The *awareness* subscale (BPQ_A) incorporates 45 statements about different bodily sensations (e.g. stomach and gut pains, facial twitches, mouth being dry, urge to urinate) and participants indicated their awareness of each sensation, using a five-point scale ranging from 'never' to 'always' (1 = never; 2 = sometimes; 3 = often; 4 = very often; 5= always). The BPQ is a widely used instrument. The internal consistency within the current sample was very good with Cronbach's α= 0.974.

2.1.3 Statistical Analyses

My strategy for statistical analyses was to ensure that I employed the most robust means to identify effects, by mitigating both Type 1 and 2 errors. Approaches were selected on their appropriateness of their sensitivity, distribution of the data and hypothesis. Mediation approaches were used in order to have insight into casual interactions between related variables.

A database of the anonymised scores of each participant was compiled for subsequent analysis. The normality of the data distribution was checked for each variable. The data were examined for multivariate outliers using Mahalanobis distance ($p<0.001$; Tabachnick and Fidell, 2012). Ten cases were thus identified and removed from the data set.

2.1.3.1 Correlations

Exploratory non-parametric correlations were initially conducted due to the non-normality of data distributions.

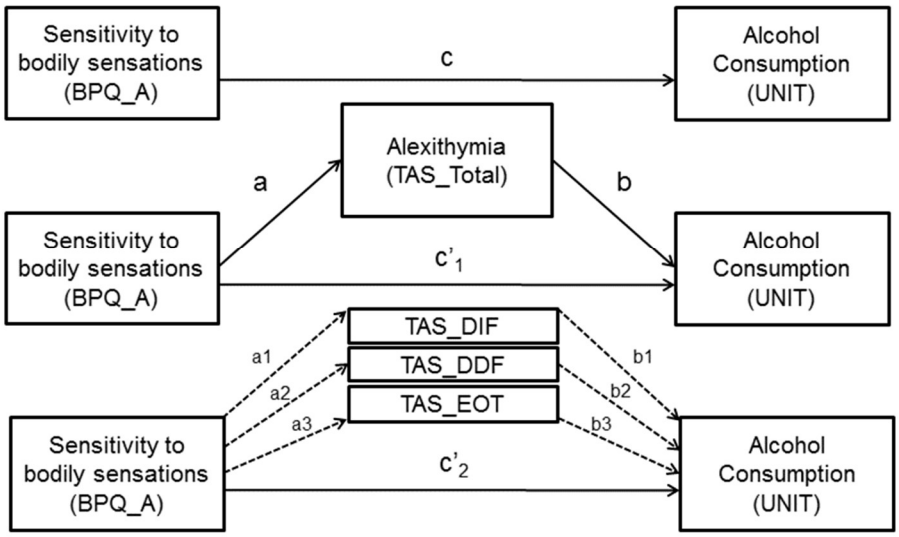


Figure 2.1 Schematic representations of the mediation models of interest.

The top panel shows the total effect of sensitivity to bodily sensation on alcohol consumption. The intermediary panel depicts indirect and direct effects of model "TAS_Total" (i.e. testing for mediation effect of TAS-20 total score on the relationship between sensitivity to bodily sensations and alcohol consumption).The bottom panel depicts indirect and direct effects of model "TAS_Subscales" (i.e. testing for mediation effect of TAS-20 subscale scores on the relationship between sensitivity to bodily sensations and alcohol consumption).

2.1.3.2 Mediation Analysis

The two models of interest were computed (Figure 2.1). The first model tested whether the total alexithymia score on the TAS-20 questionnaire score ("TAS_Total") mediated the relationship between sensitivity to bodily sensations on alcohol consumption. A second model investigated the mediating effect of the TAS-20 three subscales ("TAS_Subscales") on the same relationship.

Analyses estimated: (1) the total effect of sensitivity to bodily sensations on alcohol consumption (path c; figure 1); (2) the indirect effect of model "TAS_Total" (path ab); (3) the direct effect of model "TAS_Total" that was mediated by the Tas-20 total score (path c'_1); (4) the indirect effect of model "TAS_Subscales" (paths a1b1, a2b2, a3b3); and (5) the direct effect of model "TAS_Subscales" that was mediated by the Tas-20 subscales scores (path c'_2).

Models were tested using the approach proposed by Preacher and Hayes that allows simple and multiple mediators to be included in the analysis (Preacher and Hayes, 2008). The model was specified and estimated using the PROCESS macro in SPSS 22 (Hayes, 2013). First, classic mediation criteria were tested: *(1) The predictor predicts the outcome - path c; (2) The predictor predicts the mediator - path a; (3) The mediator predicts the outcome while controlling for the predictor - path b* (Baron and Kenny, 1986). Finally, statistical significances of the indirect effects were estimated using a bootstrapping method. To avoid biased estimations under conditions of non-normality, bias-corrected confidence intervals (95%) were obtained with 5000 bootstrap resamples. Models were corrected for age, gender and education.

2.2 Study 2 (Chapters 4, 5, 6 and 7)

2.2.1 Aims of study 2

This second study was divided into four sub-studies. In the experiment presented in Chapter 4, my aim was to find a potential mediator, within the brain, explaining interoceptive impairment observed in alcohol use disorders, combining MRS, SBM and VBM with behavioral and psychometric ratings.

In Chapter 5, my aim was to explore the interaction between interoception, oxytocin and alcohol use, using classical behavioural interoceptive tasks.

In Chapter 6, my aim was to further identify changes in the functional cerebral activity for empathy-for-pain (using multiband fMRI), while combining technical (using cardiac timing presentation) but also pharmacological (through oxytocin administration) manipulations of visceral input.

In Chapter 7, my aim was to better understand the relationship between bodily sensations (indexed by beat-to-beat systolic blood pressure) and decision-making during emotional processing, using an affective priming paradigm. A second aim was to test whether alexithymia contributed to this relationship.

2.2.2 Summary of study 2 sessions

Therefore, to test all these questions, I designed a set of studies (that I called Study 2). This set of studies was composed of three sessions.

Session 1 was a baseline session during which participants gave blood, filled in psychometric questionnaires and performed the affective priming task.

Session 2 was composed of administration of oxytocin or placebo nasal spray followed by a structural scan, a functional scan while participants were doing an empathy for pain paradigm and, then, heartbeat tracking and discrimination tasks outside the scanner.

Session 3 was similar to session 2, but a spectroscopic scan was added at the end of the scanning session.

We choose to use a mixed experimental design that involved testing all participants in both oxytocin and placebo conditions, to disentangle mechanisms of interaction between the variables of interest. This kind of study design has a number of strengths: 1) This design has greater power to detect effects of interest on the dependent outcome variable; 2) This has the additional benefit of reducing the total number of participants that need to be recruited (and hence the overall burden to this community of the study).

Furthermore, it also has a number of potential disadvantages, the most important of which is the increased burden on each individual participant. Each participant will need to be tested twice. A potential alternative, was the use of independent groups design (e.g. participants tested only for oxytocin or placebo). However, this would have necessitated substantially bigger groups to achieve acceptable statistical power. Other study designs were discussed with a medical statistician. Overall we considered the current study design to offer the best balance between power to detect effects of interest and participant burden.

2.2.3 Participants

Thirty-two male participants (mean age 25.1 years; range 18–36years; mean education 16.9 years; SD = 2.6) were recruited from study 1 and from advertisements placed around the University and Medical School campus, as well as from advertisements by emails and on social networks. The project was approved by the Brighton and Sussex Medical School (BSMS) Research Governance and Ethics Committee. All participants gave written informed consent and were compensated £7 per hour for their time.

The sample size selected followed discussion with, and the advice of, a professional statistician. A sample size of 30 was determined on the basis that it gave sufficient power to generate parameter estimates for the target measures that can be used alongside existing datasets. Moreover, equivalent group sizes were used in published studies from which we derived comparative data (Luminet *et al.*, 2011, de Haan *et al.*, 2014) facilitating comparison and inference across literature.

We calculated that with n=30, assuming a correlation of 0.6 for within-subject measurements, the equivalent sample size for a parallel group trial would be 150 (i.e. 75 per condition). The minimum effect size we could detect with 80% power at $p<0.05$ significance would be 0.46. Assuming a standardized difference of 12.9, as per the De Haan's paper, the minimum difference we could detect would be 6 points on the TAS-20. Therefore, with 30 patients in a within-subject design, we can detect a 6 point difference in TAS-20 with 80% power at $p<0.05$ significance assuming a standardized difference of 13 points and a within-subject correlation of 0.6.

One of the main limitations of this approach was that we considered two behavioural studies in order to compute the sample size of a multimodal project (i.e. including neuroimaging, electrophysiology and behavioural measures). This potentially explains why results of Chapter 6 (involving neuroimaging) and Chapter 7 (involving electrophysiology) are underpowered. To compute different power calculations for each different measure (i.e. leading to different sample sizes of each study) would have been more appropriate.

2.2.3.1 Inclusion and exclusion criteria

In study 2, I took the decision to recruit only male participants as plasma oxytocin levels fluctuate during the natural menstrual cycle of women (Mitchell *et al.*, 1981). As study 2 is composed of 3 sessions, it would have been necessary to recruit women on the same menstrual cycle day for all sessions. In the interest of time and for the sake of simplicity, I decided to focus on men. This limitation is specified in the discussion (see section 0).

Participants needed to be healthy social drinkers aged from 18 to 40 years-old, able to understand and speak English, without a history of hyper/hypotension, MRI compatible and able to provide written informed consent to take part in the study. All participants were healthy individuals with no history of psychiatric or neurological diseases. During the screening, participants were directly asked if they had any history or received any diagnosis of alcohol or drug use disorders.

2.2.3.2 Recruitment

The recruitment of the thirty participants was based on alcohol use. To be eligible, participants were required to be social drinkers, meaning that they should be drinking, at least, one unit of alcohol per week. The recruitment was based on the number of alcohol units drunk by week, derived from the AUQ. (Mehrabian and Russell, 1978).

2.2.4 Procedure

Study 2 was composed of three sessions, taking place on three different days. Session 2 and 3 needed to be separated by two to three days. Participants were asked to abstain from drinking alcohol 24h before each session. Prior to any session, participants were breathalysed to test for alcohol use and a urinary sample was collected to test for drug use. The urinary drug test was undertaken to confirm the absence of drug use to exclude drug use disorder. The alcohol test was undertaken to ensure that participants abstained before the sessions. In the case of positive results, the participant would be excluded. The results were obtained at the beginning of each session. No participant failed. Sessions 1 – 3 are delineated in more detail in the following paragraphs. Participants were told that the goal of the study was to explore the impact of oxytocin on emotional regulation.

2.2.4.1 Session 1

In session 1 (see Figure 2.2), participants were asked to sign the consent form and the inclusion criteria were checked. A blood and a urinary sample were collected. Next, participants were asked to fill in psychometric questionnaires (see section 2.2.4.1.2) and to perform a computer-based task (see section 2.2.4.1.3). The order of task and questionnaires was counter-balanced for each participant.

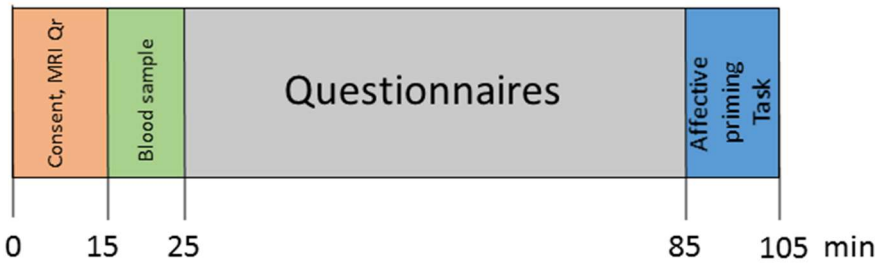


Figure 2.2 Diagram illustrating the experimental procedure the session 1 of the complex Study 2

MRI Qr = MRI safety questionnaire.

2.2.4.1.1 Plasma oxytocin (OT) measure

For the OT radioimmunological measurements, 10 ml blood was drawn into ethylenediamine tetraacetic acid vial. I failed to collect blood from two participants. Samples were mixed briefly and were then centrifuged in a refrigerated centrifuge at 4° C for 10 min at 1300 g. A quantity of 0.8 ml plasma was pipetted into 2 ml Eppendorf vials and samples were kept at -80° C until shipping. OT specific radioimmunoassays were conducted by Professor Rainer Landgraf's team at the University of Munich (http://www.riagnosis.com). Assays analyses were standardised and validated in animal and human studies across different physiological states (hypertonicity, parturition, lactation, stress, etc.) to reliably detect the bioavailable neuropeptide in peripheral (plasma) compartments (for more details on the procedure see Landgraf, 1985, Wotjak *et al.*, 1998, Kagerbauer *et al.*, 2013, Striepens *et al.*, 2013).

2.2.4.1.2 Questionnaires

Participants filed in psychometric questionnaires, assessing:

2.2.4.1.2.1 Alcohol Use Questionnaire (AUQ)

The AUQ is described in section 2.1.2.1.2. For the purpose of my project, I focused only drinking quantity (i.e. alcohol units per week). Following United Kingdom guidelines, alcohol consumption of 14 or less units of alcohol per week is considered as mild social drinking. Alcohol consumption of more than 14 units of alcohol per week is considered as harmful drinking or moderate-to-heavy drinking (https://www.nhs.uk). This questionnaire was used in Chapters 3,4,5, 6 and 7.

2.2.4.1.2.2 Obsessive Compulsive Drinking Scale (OCDS)

The OCDS (Anton *et al.*, 1995) is a 14-item scale measuring the obsessive and compulsive aspects of craving (e.g. drinking-related thoughts, urges to drink, and the ability to resist those thoughts and urges). The scores for 'obsessions' and 'compulsive drinking' subscales were calculated based on the methodology proposed by Anton (Anton *et al.*, 1995). This questionnaire was used in the Chapter 4.

2.2.4.1.2.3 Alcohol Use Disorders Identification Test (AUDIT)

The AUDIT (Babor *et al.*, 2001) is a 10-item screening tool developed by the World Health Organization (WHO) to assess severity of alcohol use (e.g. alcohol consumption, drinking behaviours, and alcohol-related problems). Each question is scored from 0 to 4, with higher numbers indicating a greater level of risk for having or developing an alcohol use disorder. This questionnaire was used in the Chapter 4.

2.2.4.1.2.4 Toronto Alexithymia Scale-20 items (TAS-20)

The TAS-20 is described in section 2.1.2.1.1. This questionnaire was used in Chapters 3, 5, 6 and 7.

2.2.4.1.2.5 Trait Anxiety (STAI)

Trait anxiety was assessed using the Trait version of the Spielberger State/Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1983). This questionnaire is composed of 20 questions, assessing trait anxiety with questions such as “I lack self-confidence” and “I have disturbing thoughts”. Participants were asked to answer each statement using a response scale (which runs from 1 “Almost never” to 4 “Almost always”) in order to capture a stable dispositional tendency (trait) for anxiety. This questionnaire was used in Chapters 5, 6 and 7.

2.2.4.1.2.6 Beck Depression Index II (BDI)

Symptoms and severity of depression were evaluated using the BDI (Beck *et al.*, 1996). Participants responded to 21 questions designed to assess the individual’s level of depression (e.g. Sadness, pessimism, past failure etc.). The BDI items are scored on a scale from 0–3. All items were then summed for a BDI total score. This questionnaire was used in Chapters 5, 6 and 7.

2.2.4.1.3 Affective priming task

2.2.4.1.3.1 Stimuli

Ninety-six strings of seven letters were selected (e.g. KOPLTFV, IZTNLDS). Each presentation of a letter-string was followed by a visual mask and, then, by the presentation of a target letter. The target letter was present in half of the letter-string trials. During the experiment, the letter-string was preceded by a visual prime: a very short presentation of an image displaying an emotional

facial expression (EFE) of sadness, anger or neutrality (see 2.2.4.1.3.3). For each of these affective conditions, there were 32 trials (32 letter-strings preceded by an EFE of sadness, 32 letter-strings preceded by an EFE of anger, 32 letter-strings preceded by an EFE of neutrality). The EFE, coloured and from front perspective, were selected from the Karolinska Directed Emotional Faces battery based on gender and emotions (for list of stimuli codes see table 2.1; Lundqvist *et al.*, 1988).Trials were presented in blocks of four consecutive trials of the same EFE, balanced and randomised for male/female gender. This cognitive task was run using Matlab 2014a and Cogent 2000.

AF01ANS	AF12ANS	AM07ANS
AF01NES	AF12NES	AM07NES
AF01SAS	AF12SAS	AM07SAS
AF02ANS	AF13ANS	AM08ANS
AF02NES	AF13NES	AM08NES
AF02SAS	AF13SAS	AM09ANS
AF03ANS	AF14ANS	AM09NES
AF03NES	AF14NES	AM09SAS
AF03SAS	AF14SAS	AM10ANS
AF04ANS	AF15ANS	AM10NES
AF04NES	AF15NES	AM10SAS
AF04SAS	AF15SAS	AM11ANS
AF05ANS	AF16ANS	AM11NES
AF05NES	AF16NES	AM11SAS
AF05SAS	AF16SAS	AM12ANS
AF06ANS	AM01ANS	AM12NES
AF06NES	AM01NES	AM12SAS
AF06SAS	AM01SAS	AM13ANS
AF07ANS	AM02ANS	AM13NES
AF07NES	AM02NES	AM13SAS
AF07SAS	AM02SAS	AM14ANS
AF08ANS	AM03ANS	AM14NES
AF08NES	AM03NES	AM14SAS
AF08SAS	AM03SAS	AM15ANS
AF09ANS	AM04ANS	AM15NES
AF09NES	AM04NES	AM15SAS
AF09SAS	AM04SAS	AM16ANS
AF10ANS	AM05ANS	AM16NES
AF10NES	AM05NES	AM16SAS
AF10SAS	AM05SAS	AM17SAS
AF11ANS	AM06ANS	
AF11NES	AM06NES	
AF11SAS	AM06SAS	

Table 2-1 List of stimuli from the Karolinska Directed Emotional Faces resource

With Letter 1: Session A = series one; Letter 2: Gender F = female, M = male; Letter 3 & 4: Identity number 01 – 35; Letter 5 & 6: Expression; AN= angry, NE = neutral, SA = sad; Letter 7: Angle S = straight.

2.2.4.1.3.2 Physiological recording

Blood pressure was recorded using non-invasive, beat-to-beat monitoring via photoplethysmographic technology (Finometer PRO, Finapres medical systems, Amsterdam, The Netherlands). An inflatable finger cuff and infrared plethysmograph were fitted to the middle finger of the participant's left hand, allowing measurements of beat-to-beat systolic blood pressure. The heart level electrode was attached to participant's clothing in the mid-clavicular line at the level of the heart. Physiological data was recorded using Spike software 2 6.08.

2.2.4.1.3.3 Paradigm

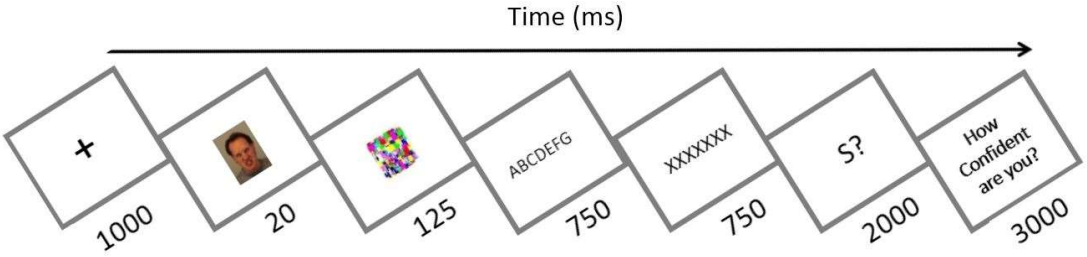


Figure 2.3 Paradigm of the affective priming task

After a 5 min calibration period, the participant was invited to start the task (Figure 2.3). Each trial began with a 1000 ms fixation cross, followed by the EFE (20 ms) and a backward mask (125 ms). This rapid series of events was immediately followed by a string of seven letters that remained on screen for 750 ms, followed by a backward mask of seven "X" letters (750 ms). Then a target letter appeared at the centre of the screen until participants made a decision (max 2000 ms), denoting whether or not the target letter was present in the string by pressing the right or the left arrow key respectively. The letter X was never the target letter. Next, a visual analogue scale (VAS) allowed participants to rate their confidence for each trial, from "zero" to "extreme" (3000 ms). The question "How confident are you?" was asked. The scale length was 18 cm. The VAS cursor was presented in the middle of the scale by default. In case of non-response to the target-letter task, the message "Please answer more quickly" was presented during 3000 ms (e.g. instead of the VAS to keep the same length for each trial). Participants were encouraged to answer as quickly and accurately as possible. Reaction times (RT) and

accuracy were recorded. The order in which these blocks were presented was randomized. Based on prior study data, participants are unaware of the emotional nature of the prime stimulus (Gendolla and Silvestrini, 2011). This was not formally confirmed for each individual trial in the present study. .This cognitive task was programmed and run using Matlab 2014a and Cogent 2000.

2.2.4.2 Session 2

In session 2 (Figure 2.4), participants were asked to sign the second sheet of the consent form and the inclusion criteria were checked. The Pulse Transit Time (PTT) was calculated and a urinary drug test was performed. All task-instructions were explained to the participants, as well as safety MRI guidelines. Next, participants were asked to inhale oxytocin or placebo prior going in the MRI-scanner. Functional MRI data were collected on the empathy-for-pain task, which was followed by a high resolution structural scan. After scanning, participants were sent to the physiology laboratory to do two interoceptive computer-based tasks outside the scanner.

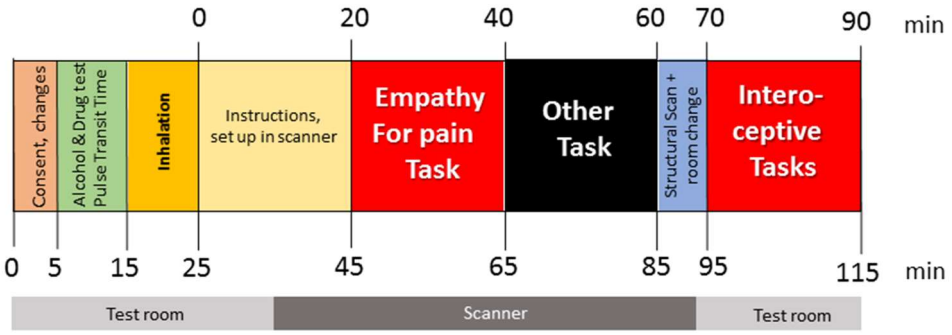


Figure 2.4 Diagram illustrating the experimental procedure on session 2 of the complex Study 2

The empathy-for-pain task began ~20 min after the drug administration, the interoceptive tasks began ~70 min after inhalation.

2.2.4.2.1 Oxytocin dosage

Synthetic oxytocin is administered *via* a nasal spray to manipulate the levels of oxytocin in the brain (Parker *et al.*, 2005, Domes *et al.*, 2007a, Domes *et al.*, 2007b, Guastella *et al.*, 2008, Buchheim *et al.*, 2009, Di Simplicio *et al.*, 2009, Bartz *et al.*, 2010, Fischer-Shofty *et al.*, 2010, Gamer *et al.*, 2010, Guastella *et al.*, 2010, Hurlemann *et al.*, 2010, Labuschagne *et al.*, 2010,

MacDonald *et al.*, 2011, Norman *et al.*, 2011, Schulze *et al.*, 2011, Lischke *et al.*, 2012, Leknes *et al.*, 2013). Previous studies demonstrate that the intranasal administration of oxytocin can be sensed by certain groups of neural cells in the central nervous system, up to 78 min after onset of treatment onset (Paloyelis *et al.*, 2014). Almost all human studies have administered synthetic oxytocin via the intranasal route (MacDonald *et al.*, 2011). By 2010, intranasal oxytocin had been administered to 1529 participants (79% male; of which 8% were participants with developmental or mental health difficulties), in doses of 18 to 40 IU per dose, without observing any adverse effects or reliable side effects (MacDonald *et al.*, 2011). Following an established protocol (Paloyelis *et al.*, 2014, Paloyelis *et al.*, 2016), the current study administered a single dose of 40IU of intranasal oxytocin/placebo. Dr. Paloyelis, a co-investigator in this project, has safely used this dosage on more than 100 male and female participants over the past three years.

2.2.4.2.2 Nasal spray administration

A within-subject design was used; the drug sequence was counter-balanced and allocated to each participant for the second and third session. Each participant self-administered 40 IU of oxytocin (OT) nasal spray (Syntocinon; Novartis, Basel, Switzerland) or placebo (same composition as Syntocinon except for OT) in the presence of the experimenter in the psychophysiology laboratory, and subsequently performed the tasks 20 minutes after the administration.

2.2.4.2.3 Instructions related to the nasal spray administration

1) Instructions related to the administration: the participant was required to take 10 puffs of nasal spray (one ‘puff’ containing 4 IU): 5 in each nostril, alternating between right and left nostril. The examiner gave a signal to the participant every 30 seconds, to deliver the puff, thereby ensuring they were appropriately distinct in time.

2) Instructions related to the nasal spray squeezing: before administration, participants were given a spray bottle full of water and were trained to deliver strong and reliable puffs in the air.

3) Instructions related to the nasal spray sniffing: the participant was instructed to self-adminstier the oxytocin puff, giving a quick and strong squeeze while simultaneously quickly sniffing. The experimenter gave an example demonstration of what was required.

4) Instructions related to the head position: afterward, the examiner showed the participant the head position for self-administering the nasal spray and gave the instructions “Put the nozzle in the nostril as far as you can. Do not touch the septum or the walls of your nose. Your head needs to be slightly back. The nozzle needs to be in line with your nose, no angle.”

When the participant felt ready, administration began and lasted for 5 minutes in the presence of the experimenter. Nostril side sequence (left or right side first) was randomized across participants.

2.2.4.2.4 Empathy-for-Pain task

2.2.4.2.4.1 Stimuli and Design

In the empathy task, the participant viewed brief presentations of sixty-four pictures, all illustrating a hand, either in a painful or in a non-painful context (32 pictures of each condition; Jackson *et al.*, 2005). Each picture was presented twice, once at cardiac diastole and once at systole. Correspondingly, the experiment took the form of a 2x2x2 repeated measures design with 2 levels of emotion (pain, no pain), 2 levels of cardiac cycle (systole/diastole), and 2 levels of drug (oxytocin/placebo). In total: 128 trials; 32 trials per condition; 4 conditions (pain/systole; pain/diastole; no pain/systole; no pain /diastole).

2.2.4.2.4.2 Paradigm

The empathy for pain task involved the presentation of: (1) a fixation cross (3000 ms), (2) a picture on or off the heartbeat (150 ms), a jitter of 1, 3 or 5s, and then (3) a visual analogue scale (3000 ms; see Figure 2.5). During the time of presenting the visual analogue scale (VAS), participants were asked to rate “How painful was the picture?”, using a scale of 0 (not painful at all) to 100 (very painful).

During the experiment, real-time cardiovascular timing information was obtained via an MRI-compatible pulse oximeter (8600Fo; Nonin Medical Inc.,

MN, USA) with the sensor attached to the participant's left index finger to obtain information. The peak of the finger pulse oximetry waveform (POW) reflects the R-wave delayed by PTT. By considering three previous interpulse intervals (generally called interbeat interval; IBI), I predicted the occurrence of the next finger pulse (R-wave + PTT) with validated accuracy (Gray *et al.*, 2010, Gray *et al.*, 2012). I subtracted the PTT from the predicted time of the next peak of POW, to derive the R-wave timing (coincident with diastole, i.e. baroreceptor quiescence). Similarly, subtracting the PTT from the predicted time of the next peak of POW and adding 300 ms, predicted the timing of ventricular systole (i.e. high baroreceptor activation). Stimuli were delivered using Cogent2000 v1.32 in MATLAB R2012b (The MathWorks, Inc., Natick, MA). Pulse oximetry and event-related information were extracted from recordings in Spike.

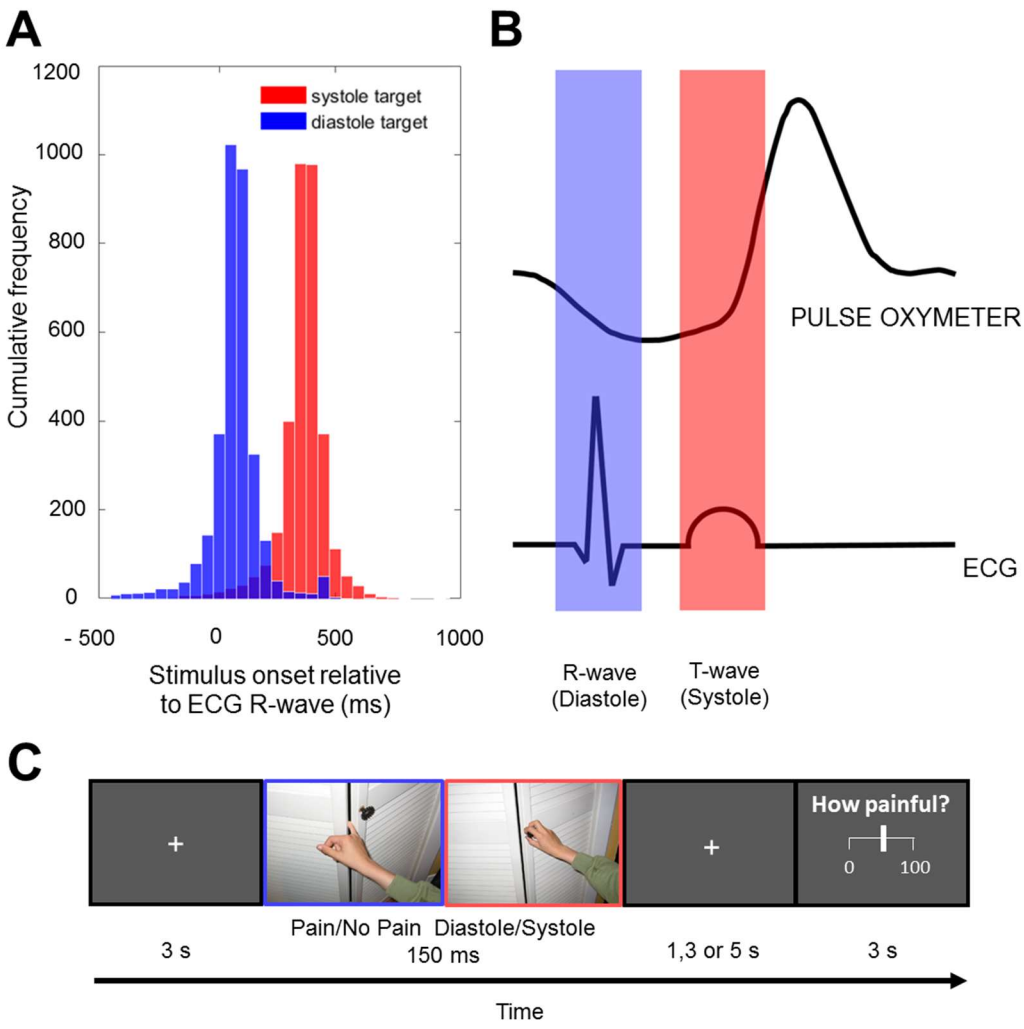


Figure 2.5 Empathy-for-pain paradigm and cardiac timing manipulation.

(A) Histogram detailing pictures presentation timing in relation to cardiac cycle within the MRI scanner. (B) Stimulus presentation was time-locked to coincide with two distinct points of the cardiac cycle: cardiac diastole in blue (ECG R-wave) and cardiac systole in red (ECG R-wave + 300ms, equivalent to ECG T-wave). (C) For the empathy-for-pain task, pictures of hand (painful context or non-painful context) were time-locked to diastole or systole. Participants made subsequent trial-by-trial pain intensity rating using a VAS.

2.2.4.2.4.3 Structural Magnetic Resonance Imaging (MRI) data acquisition

Structural Magnetic resonance imaging was performed using a 1.5 Tesla Siemens Magnetom Avanto MRI scanner with a 32-channel phased-array head coil, tuned to 63.6 MHz. A whole-brain, high-resolution T_1-weighted 3D structural image was obtained using a magnetisation-prepared gradient-echo sequence, consisting of 192 contiguous axial slices (TR=2730 ms, Echo Time (TE)=3.57 ms, flip angle = 7˚, matrix = 256x256, field of view (FoV) = 256x256

mm, 1.0 mm isotropic voxel size, GRAPPA acceleration factor = 2; acquisition time = 5 min 58 s). The T1-weighted image was used as an anatomical reference for each participant.

2.2.4.2.4.4 Functional MRI data acquisition

A T2*- weighted multiband echo-planar imaging (EPI) sequence was used, with a slice acquisition acceleration factor of 2. Each volume consisted of 36 axial slices oriented 30 degrees relative to the AC-PC line, covering the whole brain. Slices were acquired in an ascending interleaved order. The following functional imaging parameters were used: TR=1379 ms, TE=42 ms, flip angle 90˚, matrix= 64x64, FoV=192x192 mm, slice thickness=3.0 mm with a 20 % gap, resulting in 3.0 mm isotropic voxels. The exact number of fMRI volumes acquired depended on participants' heart rate (mean volumes acquired: 950). The first ten volumes were discarded to allow for steady-state magnetisation. A T1 MPRAGE structural was acquired for registration (TR=2730 ms, TE=3.57 ms, 1x1x1 mm resolution).

2.2.4.2.5 Interoceptive tasks

Interoceptive accuracy was gauged by the participants' ability to detect their own heartbeats using a heartbeat tracking task (Schandry, 1981) and a heartbeat discrimination task (Whitehead *et al.*, 1977, Katkin *et al.*, 1983). During each task, heartbeats were indexed and recorded using sensitive pulse oximetry (standard for our laboratory). A soft finger sensor was used to avoid pressure-induced pulsatile sensation at the fingertip and to gain accurate timing of pulse onsets from oximetric waveform output (Nonin4600 pulse oximeter, Nonin Medical Inc. Plymouth MN USA). Participants' heartbeats were monitored and recorded with the pulse oximeter sensor mounting attached to their index finger.

2.2.4.2.5.1 Heartbeat Tracking Task

For the heartbeat tracking task, participants were required to count their heartbeats during six randomized time windows of varying length (25, 30, 35, 40, 45 and 50 s) and, at the end of each time window, to report the number of heartbeats detected to the experimenter. To derive measures for interoceptive accuracy, heartbeat tracking scores were calculated on a trial-by-trial basis

based upon the ratio of perceived to actual heartbeats (Hart *et al.*, 2013, Garfinkel *et al.*, 2015):

$$1 - |n\text{beats}_{real} - n\text{beats}_{reported}|/((n\text{beats}_{real} + n\text{beats}_{reported})/2)$$

This measure calculates interoceptive accuracy, independent of the number of heartbeats in the trial by normalising the absolute error in perceived heartbeats as a function of the overall number of heartbeats. Mean interoceptive accuracy was computed by averaging accuracy for all trials. In addition, a measure of heart rate was computed for each trial.

2.2.4.2.5.2 Heartbeat Discrimination Task

For the heartbeat discrimination task, each trial consisted of ten tones presented at 440 Hz and having 100 ms duration, which were triggered by the heartbeat. Under the synchronous condition, stimuli were presented at the start of the rise of the pulse oximetry signal (i.e. indicator of cardiac systole O'Rourke *et al.*, 2001). Under the asynchronous condition, a delay of 300 ms was inserted (i.e. diastolic phase). At the end of each trial, participants signaled to the experimenter whether they believed the tones to be synchronous or asynchronous with their heartbeats. Therefore, the outcome of each trial was binary (1= Accurate, 0= Inaccurate). Mean interoceptive accuracy for the heartbeat discrimination task was calculated as a ratio of correct to incorrect synchronicity judgments, for each participant. Moreover, a measure of heart rate was computed for each trial. The task was composed of 20 trials.

2.2.4.3 Session 3

Session 3 was similar to session 2. The only difference was the addition of a Magnetic Resonance Spectroscopy (MRS) scan after the structural scan (see Figure 2.6 Diagram illustrating the experimental procedure on session 3 of the

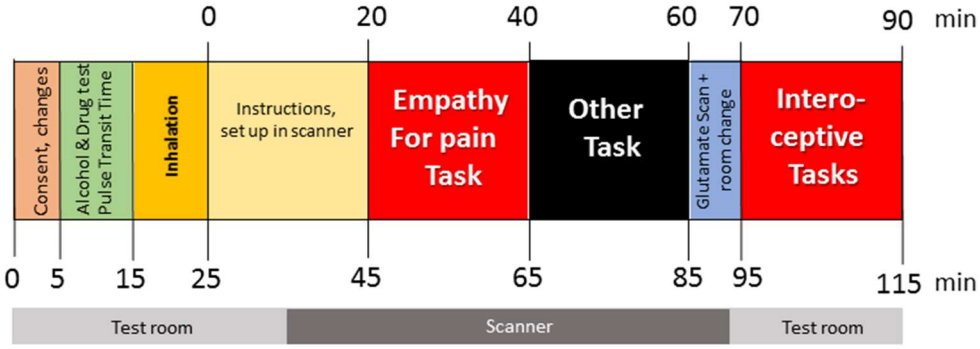


Figure 2.6 Diagram illustrating the experimental procedure on session 3 of the complex Study 2

complex Study 2

2.2.4.4 Magnetic Resonance Spectroscopy (MRS) data acquisition

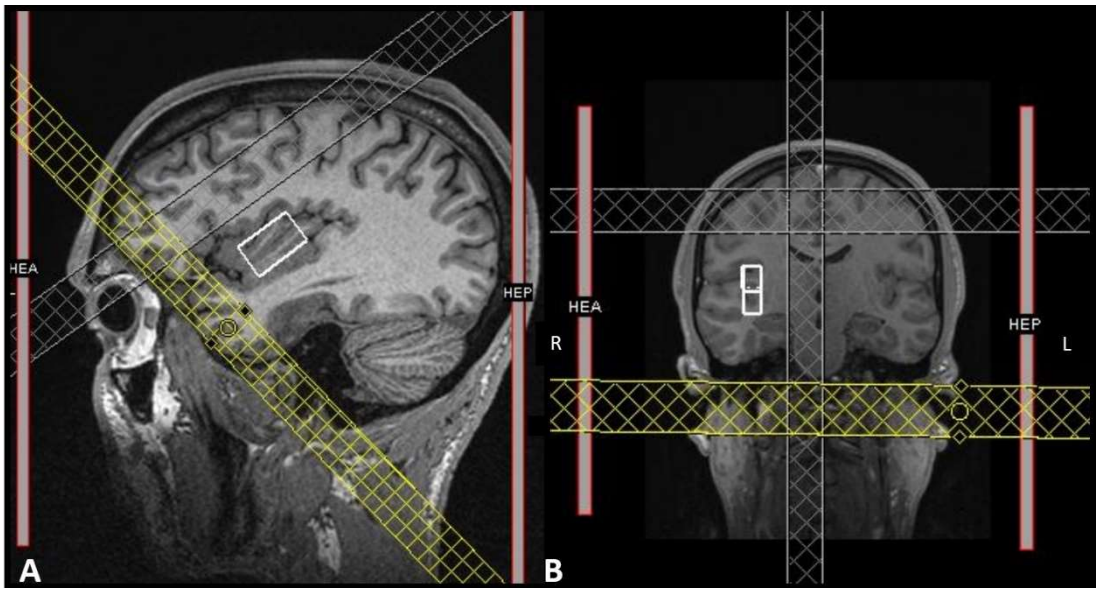


Figure 2.7 MRS Voxel placement screenshot.

A. Sagittal and B. coronal views of MRS Voxel placement in the right mid-insula of one participant.

Magnetic resonance imaging and ^{1}H-MRS were performed using the same 1.5 Tesla Siemens Magnetom Avanto MRI scanner. Using these structural images, a single ^{1}H-MRS voxel was positioned in the right mid insula (Figure

2.7). A point-resolved spectroscopy sequence (TR = 2 s, TE = 40 ms, voxel size = 10x15x25 mm, averages = 128, flip angle =90°; acquisition time = 4 min 24 s) was collected, using a short TE of 40 ms to enhance the discrimination of glutamate(Jang *et al.*, 2005). A shim box of the size of the voxel was used and manual shimming was performed to minimize linewidth. An unsuppressed water sequence for use as a concentration reference was collected with 4 averages and other identical parameters.

2.2.5 Data analysis

2.2.5.1 Chapter 4 (Measure of insular glutamate concentration)

2.2.5.1.1 MRS and Structural MRI data analyses

Raw time-domain ^{1}H-MRS data in the spectral dimension were analysed using TARQUIN Version 4.3.5 (Wilson *et al.*, 2011) with the unsuppressed water scan as concentration reference.

The TARQUIN algorithm is composed of three parts: pre-processing, basis set stimulation and the solution of a non-linear least squares fitting problem. Residual water is removed using the Hankel singular values of dynamic system methods. After being re-phased using zero and first order correction terms, the signal is automatically referenced: the water signal resonates at the centre of the spectrum (0Hz). Then, based on a prior knowledge of Chemical shift and J-coupling, TARQUIN simulates basis sets for each metabolite using a Lorenzian lineshape. Finally, TARQUIN fits experimental data to the modified simulated basis signals, using a linear combination, under some constraints (e.g. by minimising the difference between model and acquired signal).

The quality of the model fit was manually verified. Any spectrum presenting at least one of the following characteristics was rejected from the analyses: (1) fit residuals containing significant signal; (2) unstable baseline; (3) a Cramer-Rao lower bound (CRLB) of the fit to the peak of interest greater than 20%. CRLB describes the lowest possible standard deviations of all unbiased model parameter estimates (Cavassila *et al.*, 1999).

Metabolite concentrations in molality units of mmol / kg of tissue water were computed for glutamate (Glu), glutamine (Gln), total glutamate plus glutamine (Glx) and total N-acetylaspartate plus N-acetyl-aspartylglutamate (TNAA). Despite technical and methodological advances, the separation of glutamate from glutamine spectral peaks is constrained by homogeneity of the magnetic field at 1.5 Tesla. As already mentioned, I specifically used a TE of 40 ms to increase precision of glutamate concentration estimation (Jang *et al.*, 2005). Moreover, I computed the correlation coefficient C between derived glutamate and glutamine concentration (C = 0.107). Since the absolute value of the

correlation coefficient between the two metabolites |C|, was low (< 0.5), then the two metabolites can be considered sufficiently uncorrelated to permit separation (Near, 2014). Thus, individual metabolite concentrations can be plausibly reported. However, I also report the sum of the two metabolite concentrations. Using the same approach, the observed high correlation coefficient between N-acetylaspartate and N-acetyl-aspartylglutamate (|C|=0.756) justifying only reporting the sum of the two metabolite concentrations, as they appeared insufficiently separable.

The T_1-weighted structural MR images were segmented into grey matter (GM), white matter (WM) and cerebrospinal liquid (CSF) using Statistical Parametric Mapping 12 (SPM12; Wellcome Department of Imaging Neuroscience, University College London, U.K.). To calculate the tissue content of the MRS spectroscopic voxel, a binary mask of the region in each participant was created and registered to the segmented T_1-weighted structural images. The proportion of each tissue type (tissue fractions) was calculated for the spectroscopic voxel (i.e. volume of interest) by summing the 'structural' voxels of each tissue type and dividing by the total number of 'structural' voxels within the volume of interest. Metabolite concentrations were then calculated and corrected (partial volume, T_1 and T_2 relaxation) using Gasparovic's methods (Gasparovic *et al.*, 2006, Gasparovic *et al.*, 2009).

2.2.5.1.2 Water and metabolites concentrations' corrections

2.2.5.1.2.1 TARQUIN corrections

In summary, TARQUIN is computing:

[Met]_TQ = (signal amplitude * water concentration * water attenuation *2) / water amplitude

where [Met]_TQ is the metabolite concentration from Tarquin and 2 is the number of water protons. This can be rewritten:

[Met]_TQ= (S_M_Obs * water concentration * R_H2O * 2) / SH2O_obs

where S_M_Obs is the observed metabolite signal, water concertation is the default value of 35880 mM, R_H2O is an assumed relaxation effect on water and SH2O_obs is the observed water signal.

With:

R_H2O = (exp(-TE/T2_H2O)) / exp(-TE/T2_M)

Where TE is the echo time, T2_H2O is theT2 relaxation of water and T2_M is theT2 relaxation of metabolites.

2.2.5.1.2.2 Gasparovic corrections (papers 2006,2009)

Gasparovic suggests (Gasparovic *et al.*, 2006, Gasparovic *et al.*, 2009):

[MET]_G = (S_M_Obs * 2 * [H2O] / SH2O_GM/WM_R * R_M)

where [Met]_G is the metabolite from the Gasparovic equations, [H2O] denotes the molar concentration (mM) of MR-visible water in the metabolite solution of the parenchyma, assumed to be pure water (55510 mM), SH2O_GM/WM_R is the expression for the concentration reference intensity in the parenchyma, and R_M is relaxation effects for the metabolite.

As we know that the expression for the concentration reference intensity can be derived from the observed water signal SH2O_obs as below:

SH2O_GM/WM_R = SH2O_obs * (1 - fCSF) / fGM * RH2O_GM + fWM * RH2O_WM + fCSF *RH2O_CSF

where various relaxation attenuation factors are given by

RH2O_y = exp[-TE/T2H2O_y](1 – exp[–TR/T1H2O_y])

where T1H2O_y and T2H2O_y are the T1 and T2 relaxation times of water in tissue y, TE is the echo time, TR is the repetition time; fGM, fWM, and fCSF are the fractions of water attributable to grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF), respectively as:

fGM = fGM_vol * d_GM / (fGM_vol * d_GM + fWM_vol * d_WM + fCSF_vol * d_CSF)

fWM = fWM_vol * d_WM / (fGM_vol * d_GM + fWM_vol * d_WM + fCSF_vol * d_CSF)

fCSF = fCSF_vol * d_CSF / (fGM_vol * d_GM + fWM_vol * d_WM + fCSF_vol * d_CSF)

Where fGM_vol, fWM_vol and fCSF_vol are the volume fractions of GM, WM, and CSF determined by T1 image segmentation and d_GM, d_WM, and d_CSF are the relative densities of MR-visible water in GM, WM, and CSF, respectively. Then, it can be written:

[MET]_G = S_M_Obs * 2 * [H2O] / ((SH2O_obs *(1- fCSF)) / (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) * R_M

which becomes:

[MET]_G = ((S_M_Obs* 2 * [H2O] * (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) * R_M / (SH2O_obs *(1- fCSF))

which you can be re-written:

[MET]_G = ((S_M_Obs * 2 * [H2O] / SH2O_obs) * (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) * R_M) * 1 / (1- fCSF))

2.2.5.1.2.3 Comparison between the two types of correction

When we compare [MET]_TQ and [MET]_G:

[MET]_G = ((S_M_Obs * 2 * [H2O] / SH2O_obs) * (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) *R_M) * 1/(1- fCSF))

[Met]_TQ= (S_M_Obs * water concentration * H2O_R * 2) / SH2O_obs

The differences are about:

(1) the water concentration value used (water concentration for water in white matter 35880 versus [H2O] for pure water 55510 mM),
(2) the attenuation correction factor used (R_H2O versus (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) *R_M),
(3) and the correction for the CSF fraction (to get the parenchyma vs the entire voxel concentration).

As the water attenuation and the water concentration are factors which can be set manually in TARQUIN; if I set H2O_R to 1 and water concentration to 55510 mM, we have:

[MET]_TQ = S_M_Obs*[H20]/SH2O_obs

Which means that to apply partial volume correction proposed by Gasparovic (Gasparovic *et al.*, 2006, Gasparovic *et al.*, 2009), TARQUIN metabolites concentrations (with H2O_R = 1 and water concentration = 55510 mM) need to be multiplied by the term T below:

T = (fGM * Rh2O_GM + fWM * Rh2O_WM + fCSF * Rh2O_CSF)) *R_M) * 1/(1-fCSF)).

2.2.5.1.3 Voxel and Surface-Based morphometry (VBM)

Voxel and Surface-Based Morphometry were used to identify focal differences in brain tissue composition and structure, to account for anatomical inter-individual differences (Ashburner and Friston, 2000, Dahnke *et al.*, 2013). VBM and SBM were performed using the Computational Anatomy Toolbox (CAT 12 r1165, http://dbm.neuro.uni-jena.de/cat/).

T1-weighted structural images were realigned to the Anterior Commissure-Posterior Commissure. Image pre-processing followed established (default) settings in accordance with details described in the manual of CAT 12 toolbox (http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf). In brief, T_1-weighted 3-D images were coregistered to MNI space. Tissue probability maps derived from 452 healthy adults were used in affine registration and affine regularization, referencing ICBM space template-European brains. The affine processing parameter was set as default ('rough'). Medium strength correction

for inhomogeneity was applied. Images were segmented into cerebrospinal fluid (CSF), grey matter (GM) and white matter (WM), using the 'Adaptive Maximum A Posterior' technique. Mean estimates of grey matter volume (prior to normalization) were extracted for the region of interest (ROI): right insular cortex. The ROI was defined referencing the Neuromorphometrics Inc atlas provided within CAT 12 under academic subscription (http://neuromorphometrics.com/).

Global gyrification index was estimated during the tissue segmentation step (Luders *et al.*, 2006). Local cortical gyrification index was computed within CAT12 using a high-resolution parametric mesh-based approach. This approach allows estimation of the mean curvature of the brain at different spatial scales. Large positive values (expressed in degrees) for local maxima correspond to gyri. Large negative values for local minima correspond to sulci. Values are then converted to positive values by step incorporating the absolute mean curvature. Finally, the data are smoothed using a surface-based heat kernel filter of 25 mm resulting in reveal higher values for areas with pronounced gyrification.

Mean estimates of gyrification index were extracted (prior to normalization) for the ROI the right insula. For each participant, ROI was labeled using the Desikan-Killiany Atlas which is included in CAT 12 (Desikan *et al.*, 2006). Analyses were undertaken to determine the extent to which any spectroscopic concentration might relate to a reduction in insular structure.

2.2.5.2 Chapter 6 (Empathy-for-Pain Paradigm)

2.2.5.2.1 Functional MRI data analysis

I used SPM12 for data preprocessing. Preprocessing of functional images was carried out for each session separately. The first ten images were discarded to allow steady-state magnetisation. Images were converted from dicom to nifti format and re-aligned to anterior commissure. Images were slice-time corrected, spatially realigned to the first image, and unwarped using the acquired field and magnitude maps. The T1-weighted structural image was co registered to the mean functional image and subsequently segmented to obtain normalisation parameters based on the standard MNI template. The

segmentation parameters were used to transform each subject's functional images and the bias-corrected structural image into MNI space. Voxel sizes of the functional and structural images were retained during normalisation, and the normalised functional images were spatially smoothed using an 8mm Gaussian kernel (full-width-half-maximum). For each participant and session, normalised functional images were averaged and mean images were visually checked for artefacts.

2.2.5.3 Chapter 7 (Affective Priming Task)

In Chapter 7, the 32 male participants were primed by an emotional face (anger, sadness or neutral) prior a short-term memory task. For each trial, we measured beat-to-beat systolic blood pressure, reaction time and accuracy.

2.2.5.3.1 Behavioural data processing

For each trial, reaction time on the lexical decision and accuracy of response were recorded. One participant was excluded due to a depression score above three standard deviations of the mean. Very fast reaction times (< or = 100ms) were deleted (Whelan, 2008). Missing data were quantified. Seventy-six reaction times data points were missing from a total of 1240 observations. Rather than deleting these cases, we handled missing data by performing multiple imputation using predictive mean matching. Predictive mean matching (PMM) is a semi-parametric imputation approach which imputes missing values by means of the nearest-neighbour donor with distance based on the expected values of the missing variables conditional on the observed covariates (Little, 1988) To do so, I used the Multiple Imputation by Chained Equations (MICE) package in R, with PMM as method of imputation and the number of imputations set at 5 (Van Buuren and Groothuis-Oudshoorn, 2011). Imputed data were checked and included in the dataset.

2.2.5.3.2 Physiological data processing

Inter-beat-Interval (IBI, ms), beat-to-beat values of systolic blood pressure (SBP, mmHg) and event-related information were extracted from recordings in Spike. Physiological data were smoothed using a Gaussian function (set to 1) to create a constant signal over systolic peaks and average across potential spike artefacts. Events data were aligned and binned at 100 Hz. All data were

exported to Matlab (MATLAB and Statistics Toolbox Release 2016a, The MathWorks, Inc., Natick, Massachusetts, United States). Trial by trial systolic blood pressure levels were derived and systolic peaks values were averaged over each trial.

2.2.6 Statistical analyses

My strategy for statistical analyses was to ensure that I employed the most robust means to identify effects, by mitigating both Type 1 and 2 errors. Approaches (e.g. mixed-effect linear models) were selected with condsideration of their sensitivity the data distributions, and the hypotheses. Where relevant, participants were treated as random factors within the statistical models, following a data driven approach (Barr *et al.*, 2013) Some analyses were conducted on platforms such as SPM12, built upon consensus approaches to optimal neuroimaging analyses grounded upon general linear models.

2.2.6.1 Chapter 4 (Measure of insular glutamate concentration)

2.2.6.1.1 Impact of intranasal oxytocin on metabolites

Spectroscopy data were acquired 1 hr and 20 min post-inhalation of the drug (OT or placebo) To check that the administrated drug did not have an effect on metabolite concentration before collapsing the data together I ran independent samples t-tests.

2.2.6.1.2 Analyses between psychometric measures, metabolites concentrations, VBM and SBM parameters

Correlations and regressions were used to explore the relationship between psychometric measures and metabolite concentrations (as metabolite concentrations were already corrected for the VOI tissue content). Partial correlations, controlling for intracranial volume and age, and regressions were used to test relationships between insular (ROI) volumetric/surface parameters, metabolite concentrations and psychometric measures. For regressions, effect sizes (Cohen's f^2) were calculated (Cohen, 1988). All analyses were done using IBM SPSS, version 24.

2.2.6.2 Chapter 5 (Tracking and Discrimination Heartbeat Tasks)

2.2.6.2.1 Interoceptive tasks

One participant specified he was experiencing a hangover on one of the sessions; his data were discarded from the analyses.

Analysis of interoceptive tracking accuracy used linear mixed-effects models as the outcome was continuous. I analysed interoceptive discrimination accuracy using generalized linear mixed models as the outcome was binary (Inaccurate =0; Accurate =1; binomial family function), using the lme4 package (Bates *et al.*, 2015) in the R environment (version 3.4.2; RCoreTeam, 2013). P values were computed using lmerTest package (Kuznetsova *et al.*, 2014).

Both types of interoceptive accuracy were analysed with drug (2 levels: Placebo=0; OT=1), units of alcohol per week (continuous predictor), their interaction and control variables (heart rate, anxiety, depression, alexithymia and drug sequence) as fixed factors. Participants were treated as a random factor. In order to specify the random effect structure, we used a data-driven approach for both tasks (Barr *et al.*, 2013). Models including (1) random intercepts, (2) random uncorrelated intercepts and slopes and (3) random correlated intercepts and slopes were run. Models' goodness of fits were compared using likelihood ratio tests. The relative quality of the models was estimated using Akaike information criterion (AIC). The lower AIC is associated with the best fit (Posada and Buckley, 2004).

For the tracking task, the model including random correlated intercepts and slopes explained more variance than the two other models (Model 1: AIC =-293.91; Model 2: AIC =-354.91; Model 3: AIC =-385.46; Models 1-2 comparison: $\chi^2_{(11)} = 61.00$; $p < 0.001$; Models 2-3 comparison: $\chi^2_{(13)} = 34.55$; $p < 0.001$; Models 1-3 comparison: $\chi^2_{(13)} = 95.55$; $p < 0.001$). For the discrimination task, the model including random uncorrelated intercepts and slopes explained more variance than the model including random intercept alone (Model 1: AIC =1687.4; Model 2: AIC =1684; $\chi^2_{(10)} = 3.33$; $p < 0.001$). The model including random correlated intercepts and slopes did not adequately converge, even after rescaling and optimization. All continuous predictor variables were centred. For the tracking task model, the intercept

reflected the interoceptive accuracy value in the placebo condition. For the discrimination task model, the intercept reflected the log of the odds ratio of interoceptive accuracy value in the placebo condition. Log odds value $\hat{Y}$ can be converted back into proportions using the inverse logit formula, $\frac{e^{\hat{Y}}}{1+e^{\hat{Y}}}$.

Plasma OT levels were missing for two participants from whom I was unable to collect blood samples. Generalized and linear mixed models omit cases with missing data. Therefore, to check the effect of plasma OT levels on accuracy, I ran the final models, restricted to the participants with plasma OT information (e.g. N=30). Thereafter, I ran a second model, similar to the first one (e.g. N=30), but including plasma OT levels as predictor (fixed factor). For both tasks, I compared the two models using likelihood ratio tests.

2.2.6.3 Chapter 6 (Empathy-for-Pain Paradigm)

2.2.6.3.1 Behavioral data analysis

Analysis of pain ratings used a linear mixed-effects model, since the outcome was continuous, conducted with the lme4 package (Bates *et al.*, 2015) in R (version 3.4.2; RCoreTeam, 2013). P values were computed using lmerTest package (Kuznetsova *et al.*, 2014).

Ratings were analysed with drug (2 levels: Placebo=0; OT=1), emotion (2 levels: No Pain =0; Pain=1), Cardiac Timing (2 levels: Diastole=0; Systole = 1) and their interactions, as fixed factors. The intercept reflected the average pain rating in the placebo no pain diastole condition. Participants were treated within the analysis as a random factor. We used a data-driven approach to specify the random effect structure (Barr *et al.*, 2013). Models were run that included (1) random intercepts and (2) random correlated intercepts and slopes. The goodness of fit of these models were compared using likelihood ratio tests. The model including random correlated intercepts and slopes explained more variance than the other models (Model 1: AIC =-72 202; Model 2: AIC =-72 103; Models 1-2 comparison: $\chi^2_{(2)} = 103.03$; $p < 0.001$).

This basic model was then compared to a similar model that also included anxiety, depression, alexithymia, alcohol intake, sequence, interbeat interval,

fMRI jitter and plasma OT levels to test confounding effects of these variables. I contrasted the goodness of fit of these models using likelihood ratio tests.

To test for possible Interbeat interval (IBI) changes due to OT administration, a mixed-effect linear model was fitted to the data, with IBI as outcome and drug as predictor. Participants were treated as a random factor, with random correlated intercepts and slopes, in function of the drug.

2.2.6.3.2 Functional MRI data analysis

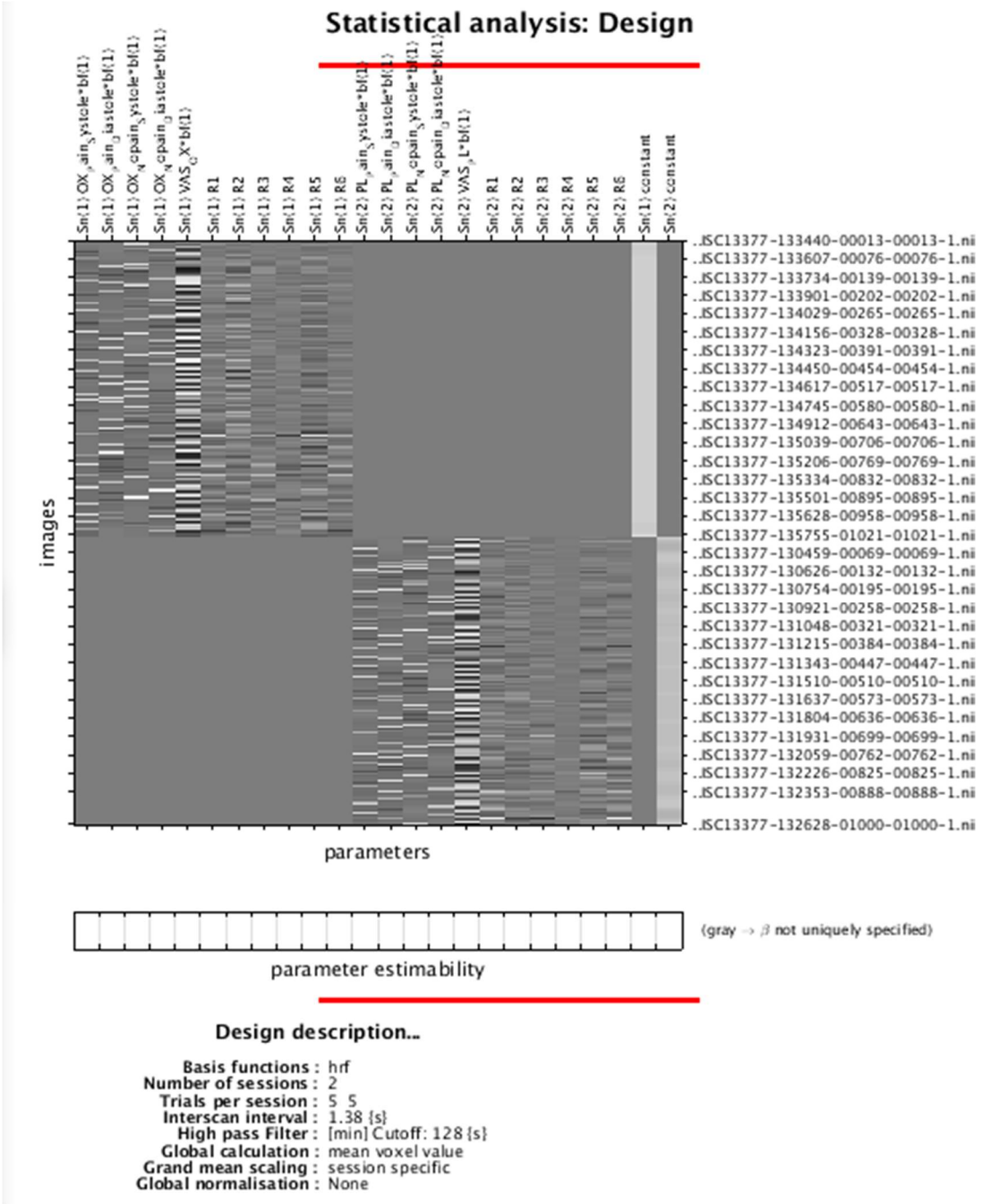


Figure 2.8 Example of first level design matrix from one subject.

The 22 regressors (left-to-right) are (1) Oxytocin Pain Systole; (2) Oxytocin Pain Diastole; (3) Oxytocin No Pain Systole; (4) Oxytocin No Pain Diastole; (5) VAS presentations for the oxytocin session (6-11) movement translations and rotations for the oxytocin session; (12) Placebo Pain Systole; (13) Placebo Pain Diastole; (14) Placebo No Pain Systole; (15) Placebo No Pain Diastole; (16) VAS presentations for the placebo session (17-22) movement translations and rotations for the placebo session.

Task events were analysed in a general linear model composed of two sessions (OT and Placebo; see Figure 2.8). Each session included four regressors representing the onset and duration of the presentation of (1) pain images at systole, (2) pain images at diastole, (3) no pain images at systole, and (4) no pain images at diastole respectively. In addition, for each session, a further regressor, comprising the onsets and durations of VAS presentation, was added to the general linear model to separate the BOLD signal relating to pain rating from stimulus presentation. Movement regressors were included as confounds (6 head movement parameters calculated from scan volume realignment). Given our short TR, the FAST covariance structure was chosen to model serial correlations. Single-regressor T-contrasts were generated for each condition ((1) Oxytocin Pain Systole; (2) Oxytocin Pain Diastole; (3) Oxytocin No Pain Systole; (4) Oxytocin No Pain Diastole; (5) Placebo Pain Systole; (6) Placebo Pain Diastole; (7) Placebo No Pain Systole; (8) Placebo No Pain Diastole). These were entered into a full factorial second-level analysis, with drug, emotion and cardiac timing as non-independent (repeated measures) factors. Given that control variables (i.e. anxiety, depression, alexithymia, alcohol intake, sequence, interbeat interval, fMRI jitter and plasma OT levels) did not improve the model's fit at the behavioural level, I did not model them within the final full factorial design.

Contrasts were generated to test for (1) all experimental effects (F contrast: [all 8 experimental conditions]), (2) drug effects for both emotion and cardiac timing (Oxytocin Pain Systole; Oxytocin Pain Systole; Oxytocin No Pain Systole; Oxytocin No Pain Systole; Placebo Pain Systole; Placebo Pain Systole; Placebo No Pain Systole; Placebo No Pain Systole), (3) overall drug effect (Oxytocin < Placebo), (4) overall emotion effect (Pain> No Pain), and (5) overall cardiac effect (Diastole>Systole ; T contrasts). In addition, the following three contrasts were tested for interactions (PL(PvsNP)>OT(PvsNP); PL(PDvsNPD)>PL(PSvsNPS); OT(PSvsNPS)>OT(PDvsNPD)). Statistic images were thresholded at an initial cluster-forming threshold of $p<0.001$ for cluster-wise False Discovery Rate (FDR) correction for multiple comparisons at $p<0.05$ (Chumbley and Friston, 2009). Significant clusters were localised according to the Anatomy toolbox (v 2.2b, Eickhoff *et al.*, 2005) (see Chapter

6 Table 6-4). Contrast estimate effect size plots were generated in SPM for each condition, at the peak coordinate of significant (empathy-for-pain related) regions according to the F test for all effects (see Chapter 6, Figure 6.3 & Figure 6.5).

2.2.6.4 Chapter 7 (Affective Priming Task)

2.2.6.4.1 Correlations

Mean and standards deviations were computed for reaction time, accuracy and systolic blood pressure. Physiological and behavioral measures were correlated to psychometric data, using 2-tailed nonparametric correlations.

2.2.6.4.2 Mixed effects linear models

I used mixed-effects modeling to test effects of the variables (accuracy, reaction times (RT) and systolic blood pressure (SBP)), measured on trial by trial basis, (Barr *et al.*, 2013).

Accuracy was analyzed using a generalized linear mixed model as the outcome was binary (binomial family; Inaccurate =0; Accurate =1). To satisfy normality assumption, reaction times were also analyzed using a generalized linear mixed-effects model (Lo and Andrews, 2015). After fitting different density to the observed reaction times distribution, the relative quality of the models was estimated using AIC. The lower AIC (e.g. best fit) was observed when a Gamma distribution was fitted to the observed reaction time distribution.

The same basic model was tested for each of the two outcomes (i.e. accuracy and reaction time). The basic model included systolic blood pressure, emotion (3 levels: Neutrality=0; Sadness=1; Anger=2), TAS score and the interactions terms as predictors. Therefore intercept reflected the outcome value in the neutral condition. Given the established influence of age on blood pressure reactivity, age was included in the basic model as a control variable. Finally, participants were specified as a random (subject) factor, allowing for random intercepts.

The basic model was then compared to a similar model that also included anxiety, depression and alcohol intake to test confounding effects of these

variables, contrasting the goodness of fit of the models using likelihood ratio tests.

All continuous predictors were mean-centered prior being entered in models. Analyses were undertaken using the lme4 package (Bates *et al.*, 2015). For models including a random term, the default lme4 optimizer was used. Finally, *p* values were computed using lmerTest package (Kuznetsova *et al.*, 2014). All analyses were run in the R environment (version 3.4.2; RCoreTeam, 2013).

2.2.6.4.3 Post hoc analyses: Heart rate variability

In order to explore if accuracy, reaction times and systolic blood pressure were related to a deceleration at the heart rate level, I analysed the heart rate variability in the frequency domain. To do so, I used the software HRVAS (Ramshurhttp://sourceforge.net/projects/hrvas/?source=navbar). The Lomb-Scargle method was preferred as this method provides power spectral density estimates of unevenly sampled data (Laguna *et al.*, 1998). For each participant, I computed mean interbeat interval, low cardiac frequencies percentage (0.04Hz to 0.15Hz), high cardiac frequencies percentage (0.15Hz to 0.4Hz) and the ratio low-to-high cardiac frequencies. Finally, mean and standards deviations were computed for each variable. Physiological measures were correlated to behavioural data, using 2-tailed nonparametric correlations.

Chapter 3 How do self-assessment of alexithymia and sensitivity to bodily sensations relate to alcohol consumption?[1]

[1] As first author I designed the study and undertook all stages of data collection analyses and interpretation with the guidance of my senior colleagues and the assistance of Dr Pfeifer. In the text that follows, as in the published paper, this group contribution is acknowledged in my use of plural personal pronouns.
This chapter led to a scientific publication in an international journal:
Betka, S., Pfeifer, G., Garfinkel S., Prins, H., Bond, R., Sequeira, H., Duka, T., Critchley H. (2018) How Do Self-Assessment of Alexithymia and Sensitivity to Bodily Sensations Relate to Alcohol Consumption? *Alcoholism: Clinical and Experimental Research* 42 (1), 81-88 DOI: 10.1111/acer.13542

3.1 Abstract

Background Alexithymia describes an abnormality of emotional experience that is commonly expressed among individuals with addiction and alcohol abuse disorders. Alexithymic individuals are characterized by difficulties in identifying and describing their emotions. This impairment is linked to the development and maintenance of addiction. Moreover, an emergent theory suggests alexithymia is itself secondary to a failure of interoception (sensitivity to internal bodily signals, including physiological arousal states).

Methods The present study tested for hypothesized contributory roles of alexithymia and dysfunctional interoception in the expression of binge drinking. Alexithymia, subjective sensitivity to bodily sensations, and alcohol consumption scores were quantified using the Toronto Alexithymia Scale, the Body Perception Questionnaire and the Alcohol Use Questionnaire respectively, in a normative sample (N=600). Regression and bootstrapping mediation analyses were used to test the hypothesis that alexithymia mediated the association between sensitivity to bodily sensations and alcohol consumption.

Results Alexithymia was positively correlated with sensitivity to bodily sensations and with alcohol consumption. Mediation analysis revealed that alexithymia, and more precisely, difficulty in identifying feelings, mediated the relationship between sensitivity to bodily sensations and alcohol consumption, such that the predictive effect of sensitivity to bodily sensations on alcohol intake became non-significant when controlling for alexithymia.

Conclusions These results indicate that alexithymia is associated with subjective hypersensitivity to bodily sensations. Moreover, our findings support the theoretical proposal that alexithymia is an expression of impaired processing of bodily sensations including physiological arousal, which underpins the development of maladaptive coping strategies, including alcohol use disorders. Our observations extend a growing literature emphasizing the importance of interoception and alexithymia in addiction, which can inform the development of new therapeutic strategies.

Key words: Addiction, Alcohol Consumption, Alexithymia, Interoception, Bodily Sensations

3.2 Background

Alexithymia, an emotional dysfunction characterized by difficulties to describe and identify one's own emotional feelings (Taylor, 2000), is highly prevalent in patients suffering from alcohol use disorders (AUD; Rybakowski *et al.*, 1988). Correspondingly, an established literature describes alexithymia as a contributing factor to the development and maintenance of AUD (Loas *et al.*, 1997, Kopera *et al.*, 2015).

Interestingly, an emergent theory suggests that interoception, i.e. the ability to feel visceral bodily signals, is central to alexithymia (Brewer *et al.*, 2016, Murphy *et al.*, 2017). Indeed, alexithymia is associated with poorer interoceptive accuracy in cardioception (Herbert *et al.*, 2011, Shah *et al.*, 2016b) and in respiratory interoception (Murphy *et al.*, 2017), yet an over-reporting of subjective physical symptoms (Nakao *et al.*, 2002) including a hypersensitivity to touch (Sivik, 1993). These latter findings demonstrate a mismatch between objective and subjective aspects of body awareness, possibly impacting emotional processing and 'sense of self'. Indeed, alexithymic subjects show reduced emotional awareness (Lane *et al.*, 2015) and higher malleability of body representation in illusions of body-ownership (Georgiou *et al.*, 2016). Also, this clinical picture suggests the involvement of the anterior part of the insular cortex (i.e. integrating bodily sensations into subjective feelings with declarative access) in alexithymia; this idea is corroborated by a study showing that subjects acquired alexithymia after anterior insula lesion (Hogeveen *et al.*, 2016).

As already mentioned, people suffering from AUD show higher prevalence of alexithymia and impaired social cognition. Moreover, in people with substance use disorders the processing of bodily sensations is disrupted (May *et al.*, 2013, Berk *et al.*, 2015). Poorer interoceptive accuracy correlates with higher alexithymia scores (Sönmez *et al.*, 2016) and an enhanced craving for alcohol (Ates Çöl *et al.*, 2016) in alcohol-dependent individuals.

It is therefore plausible that disturbed representation of bodily states can lead to difficulty in interpreting emotional states (i.e. conventional definition of alexithymia), which in turn may foster the expression of risky behaviours,

including heavy drinking. Nevertheless, despite the growing literature highlighting the association between addictions and interoceptive impairments, the relationship between abnormal sensitivity to bodily sensations and alexithymia in alcohol use has never previously been investigated.

3.3 Aim and hypotheses

We, therefore, sought to characterise relationships between subjective measures of alexithymia, sensitivity to bodily sensations and alcohol consumption, using mediation analyses to infer likely causality.

We hypothesised that alexithymia, sensitivity to bodily sensations and alcohol consumption will be positively correlated and that alexithymia will mediate the relationship between bodily sensations and alcohol consumption.

3.4 Methods summary

Full methodological details are described in Chapter 2, section 2.1.

3.4.1 Procedure and participants

To test our hypotheses, we created an online survey composed of questionnaires assessing alexithymia (TAS-20), sensitivity to bodily sensations (BPQ) and alcohol use (AUQ).

A total of 779 participants consented and 600 individuals completed all questions and provided full data. To avoid the pitfalls of missing datasets, we used a conservative approach (case deletion) and confined all analyses to the 600 individuals who provided full data (Kang, 2013).The data were examined for multivariate outliers using Mahalanobis distance ($p<0.001$; Tabachnick and Fidell, 2012). Ten cases were thus identified and removed from the data set.

3.4.2 Statistical Analyses

Exploratory non-parametric correlations were initially conducted due to the non-normality of data distributions.

Two models of interest were computed and tested using mediation analyses (see Chapter 2 Figure 2.1). The first model tested whether the total alexithymia score on the TAS-20 questionnaire score ("TAS_Total") mediated the relationship between sensitivity to bodily sensations (BPQ_A) on alcohol consumption (UNIT). A second model investigated the mediating effect of the TAS-20 three subscales ("TAS_Subscales") on the same relationship.

Models were tested using the approach proposed by Preacher and Hayes that allows simple and multiple mediators to be included in the analysis (Preacher and Hayes, 2008). First, classic mediation criteria were tested : *(1) The predictor predicts the outcome - path c; (2) The predictor predicts the mediator - path a; (3) The mediator predicts the outcome while controlling for the predictor - path b* (Baron and Kenny, 1986). Finally, statistical significances of the indirect effects were estimated using a bootstrapping method. To avoid biased estimations under conditions of non-normality, bias-corrected

confidence intervals (95%) were obtained with 5000 bootstrap resamples. Models were corrected for age, gender and education.

3.5 Results

3.5.1 Sample

Measure	Type	N (%) or Mean ± SD (Range)
Age (years)		27.44 ± 12.18 (18-69)
Gender	Male	151 (25.6%)
	Female	438 (74.2%)
	Other	1 (0.2%)
Education level	Less than high school	2 (0.3%)
	High School/GED	172 (29.2%)
	Some college	89(15.1%)
	2-year College Degree	81 (13.7%)
	4-year College Degree	58 (9.8%)
	Master Degree	86 (14.6%)
	PhD Degree	33 (5.6%)
	Professional Degree	10 (1.7%)
	Other	59 (10%)
TAS-20	Alexithymia total score (TAS_Total)	46.79 ± 10.57 (20-77)
	Difficulty Identifying Feelings (TAS_DIF)	16.20 ± 5.77 (7-34)
	Difficulty Describing Feelings (TAS_DDF)	13.43 ± 4.09 (5-25)
	External Oriented Thinking (TAS_EOT)	17.16 ± 4.03 (8-30)
AUQ	Drunk Alcohol units by week (UNIT)	18.32 ± 16.57 (0-101)
BPQ	Awareness Subscale (BPQ_A)	2.30 ± 0.82 (1-5)

Table 3-1 Socio-demographic characteristics and questionnaires scores of the sample

Five hundred and ninety participants (n= 438 females) were enrolled in the study. Means, standard deviations, absolute numbers and percentages were calculated for all the socio-demographic characteristics and questionnaire scores (Table 3-1).

3.5.2 Correlations

	1	5	6
1. Alexithymia (TAS_Total)	-	.102 ***	.199 ***
2. Difficulty Identifying Feelings (TAS_DIF)	.665***	.112***	.230***
3. Difficulty Describing Feelings (TAS_DDF)	.624***	.077*	.157***
4. External Oriented Thinking (TAS_EOT)	.437***	.057*	.035
5. Drunk Alcohol Units by week (UNIT)	.102***	-	.034
6. BPQ Awareness Subscale (BPQ_A)	.199***	.034	-

Table 3-2: Kendall's tau correlation (2-tailed) matrix for each variable

(Uncorrected p-value: *p < 0.05; **p < 0.01; ***p < 0.001)

Relationships between alexithymia, subjective sensitivity to bodily sensations and alcohol consumption were examined using Kendall's tau rank correlation coefficient (

Table 3-2). Alexithymia total score showed a significant positive correlation with both sensitivity to bodily sensations and alcohol consumption. All alexithymia subscales were positively correlated with alcohol consumption. However, sensitivity to bodily sensations was not correlated with alcohol consumption nor the "Externally Oriented Thinking subscale" of the TAS.

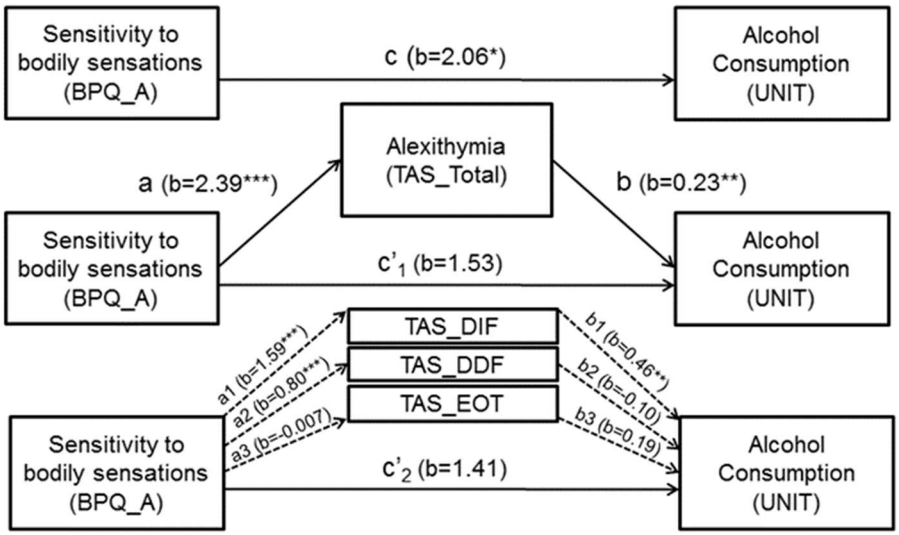


Figure 3.1 Schematic showing unstandardized regression coefficients for total, indirect and direct effects of models 1 and 2.

Age, gender and education level were used as covariates (p-value: *p < 0.05; **p < 0.01; ***p < 0.001).

3.5.3 Mediation analyses

A schematic representation of the results showing unstandardized regression coefficients is depicted in Figure 3.1.

3.5.3.1 Total effect

Prior to analysing the mediation model, the total effect of sensitivity to bodily sensations on alcohol consumption was estimated (i.e. path c). With no mediators in the model, the regression coefficient was statistically significant (path c; b= 2.06, *t* (585) = 2.46, *p* = 0 .014, 95% CI= 0.4191, 3.7098).

3.5.3.2 Model "TAS_Total" - Indirect and direct effects

Results indicated that sensitivity to bodily sensations was a significant predictor of alexithymia (path a; b= 2.39, *t* (585) = 4.60, *p* < 0 .001, 95% CI= 1.3675, 3.4053). Alexithymia was also a significant predictor of alcohol consumption, controlling for sensitivity to bodily sensations (path b; b= 0.23, *t* (585) = 3.39, *p* = 0 .007, 95% CI= 1.3675, 3.4053). The indirect effect was estimated (i.e., path ab) and was statistically significant (path ab; bootstrapped estimate = 0.5360, SE= 0.2135, 95% CI= 0.1993, 1.0779).

The direct effect of sensitivity to bodily sensations on alcohol consumption with alexithymia as mediator was also estimated (i.e., path c'_1). The regression coefficient was not statistically significant (path c'_1; b= 1.53, *t* (585) = 1.81, *p* = 0 .07, 95% CI= -0.1315, 3.1884).

These results support the mediational hypothesis; sensitivity to bodily sensations was no longer a significant predictor of alcohol consumption after controlling for total score of alexithymia, consistent with mediation. In order to explore the mediation role for each factor of alexithymia specifically, we included the three subscales of the TAS-20 as mediators in the "TAS_Subscales" model.

3.5.3.3 Model "TAS_Subscales" - Indirect and direct effects

Results indicated that sensitivity to bodily sensations significantly predicted the "Difficulty Identifying Feelings" subscale (path a1; b= 1.59, *t* (585) = 5.58, *p* < 0 .001, 95% CI= 1.0326, 2.1557), as well as the "Difficulty Describing Feelings" subscale (path a2; b= 0.80, *t* (585) = 3.92, *p* < 0 .001, 95% CI= 0.3985, 1.2001). The "Externally Oriented Thinking" subscale was not predicted by sensitivity to bodily sensations (path a3; b= - 0.007, *t* (585) = -0.03, *p*= 0.972, 95% CI= -0.4113, 0.3971).

Only the "Difficulty Identifying Feelings" subscale predicted alcohol consumption when controlling for sensitivity to bodily sensations (path b1; b= 0.46, *t* (585) = 3.24, *p* < 0 .01, 95% CI= 0.1811, 0.7369; path b2; b= -0.10, *t* (585) = -0.47, *p*= 0 .635, 95% CI= -0.4934, 0.3014; path b3; b= 0.19, *t* (585) = 1.12, *p* = 0.262, 95% CI= -0.1453, 0.5326)

Estimated indirect effects for path a1b1, a2b2 and a3b3 further demonstrated that the "Difficulty Identifying Feelings" subscale (path a1b1) was the only significant mediator between sensitivity to bodily sensations and alcohol consumption (path a1b1; bootstrapped estimate = 0.7317, SE= 0.2723, 95% CI= 0.2889, 1.3785; path a2b2; bootstrapped estimate = -0.0767, SE= 0.1629, 95% CI= -0.4495, 0.2153; path a3b3; bootstrapped estimate = -0.0014, SE= 0.0623, 95% CI= -0.1526, 0.1123).

We estimated the direct effect of sensitivity to bodily sensations on alcohol consumption, controlling for the three alexithymia subscales as mediators (i.e., path c'_2). The regression coefficient was not statistically significant (path c'_2; b= 1.41, *t* (585) = 1.12, *p* = 0 .099, 95% CI= -0.2642, 3.0859).

These results support a mediation effect of the “Difficulty Identifying Feelings” subscale; sensitivity to bodily sensations was no longer a significant predictor of alcohol consumption after controlling “Difficulty Identifying Feelings” subscale. No mediation effect was observed for the difficulty describing feelings and “Externally Oriented Thinking”.

3.6 Discussion

The present study examined the relationship between subjective measures of alexithymia, sensitivity to bodily sensations and alcohol consumption. Three key results were observed.

3.6.1 Main findings

First, alexithymia, and more precisely, difficulty in identifying feelings (e.g. "*I often don't know when I am angry*"), mediated the relationship between sensitivity to bodily sensations and alcohol consumption. This finding provides fresh insight into the possible causality of this relationship: sensitivity to bodily sensations might influence the ability to identify feelings, which can influence alcohol consumption. Although caution is required when discussing causation, recent research supports a causal interaction between interoceptive skills and alexithymia. Bornemann and Singer tested whether nine months of contemplative mental training could modulate interoceptive accuracy and emotional awareness (i.e. alexithymia, as measured by TAS-20), in healthy individuals (Bornemann and Singer, 2017). In the first three months of training, individuals were trained in breathing and body scan, which resulted in improved interoceptive accuracy and lowered alexithymia scores. Moreover, early changes in interoceptive accuracy predicted overall change (over the entire nine-month training period) in alexithymia, suggesting that a good reading of bodily sensations influences the ability to interpret one's emotion, rather than the opposite. Moreover, alcohol withdrawal in alcoholic patients does not affect alexithymia scores (de Timary *et al.*, 2008) whereas, alexithymia and poor emotional regulation ability predict relapse (Loas *et al.*, 1997, Berking *et al.*, 2011). Despite the difficulty to differentiate genetic from shared environmental impacts, a family history of alcohol dependence increases the risk of being alexithymic (Finn *et al.*, 1987, de Haan *et al.*, 2013). While alexithymia is not widely recognised as causal to addictive behaviours, it is interesting to note that alexithymic features such as "denial", "lack of insight" or "reduced self-awareness" are commonly described as underlying factors (Goldstein *et al.*, 2009). Taken together, our findings suggest that an inaccurate interpretation of bodily sensations (including bodily arousal) may

increase the propensity towards alexithymic characteristics (such as difficulty identifying feelings), which represent a risk factor for alcohol use disorders.

Our second main finding was that difficulties in identifying feelings, rather than difficulties describing feelings (e.g. *"It is difficult for me to find the right words for my feelings"*) or externally oriented thinking (e.g. *"I prefer to analyse my problems rather than just describe them"* - reversed item), mediated the relationship between subjective bodily sensations and alcohol intake. These results are coherent with other studies of alcohol and substance users indicating a specific relationship between interoceptive accuracy and difficulties in identifying emotions (Sönmez *et al.*, 2016). Moreover, poor interoceptive accuracy is associated with a reduced representation of other's affective mental states (Shah *et al.*, 2017) and a poorer recognition of emotional facial expressions (Terasawa *et al.*, 2014).

Our third main finding was that alexithymia was positively correlated with sensitivity to bodily sensations and alcohol consumption. We found that the more participants were alexithymic, the more they were drinking alcohol. This observation adds to growing evidence for the relationship between alexithymia and alcohol dependence (Uzun *et al.*, 2003, Craparo *et al.*, 2014) and social drinking (Bruce *et al.*, 2012). We additionally found that the more participants were alexithymic, the greater their subjective sensitivity to bodily states. These findings might appear contradictory, as it has been previously emphasized that alexithymic individuals have poor interoceptive accuracy. However, poor ability to feel or interpret bodily sensations, which is typically assessed using objective interoceptive measures (e.g. Sönmez *et al.*, 2016) could explain an overstatement at the subjective level. Indeed, interoceptive objective measures of accuracy (e.g. being accurate or inaccurate detecting heart rate) do not always align with interoceptive subjective measures of interoception as subjective data can be inaccurately overestimated or underestimated. Moreover, our data extends a previously observed association between subjective somatosensory overestimation and physical symptoms over-reporting in alexithymia (Nakao *et al.*, 2002). Finally, we found that sensitivity to bodily sensations was not correlated with alcohol consumption. This finding

is coherent with our mediation effect result suggesting no direct relationship between subjective report of body sensations and alcohol intake.

3.6.2 Limitations

We recognise limitations of our study. The main limitation of this study was our (pragmatic) use of self-report questionnaires to assess alexithymia, alcohol consumption, and especially sensitivity to bodily sensations: we postulate that alexithymia is characterized by a mismatch between subjective and objective dimensions of interoception; hence future studies need to quantify pure interoceptive sensibility and interoceptive accuracy together. However, a stable cohesion around the definition of interoception, and the development of a robust tool assessing the subjective dimension of interoception are still crucially needed. The measurement of alexithymia using self-report was not optimal either, given that alexithymic subjects, by definition, show biased insights into their bodily and emotional states. Future studies should lead to the development of an objective measure of alexithymia (e.g. inferred from multi-dimensional interoceptive accuracy). A second limitation was the use of a cross-sectional design, which restricted our interpretations in term of causation. Prospective cohort studies could clarify the nature of relationships between interoception, alexithymia and risk-taking behaviours such as alcohol use disorders.

3.6.3 Conclusion

Despite these limitations, our finding suggests that alexithymia is associated with subjective hypersensitivity to bodily sensations. Moreover, our results support the theoretical proposal that alexithymia is an expression of impaired processing of bodily sensations, which underpin the development of maladaptive coping strategies, including AUD.

Interestingly, the crucial role of the insular cortex in interoceptive processes is largely recognized (Craig, 2002, Critchley *et al.*, 2004, Craig, 2009). Insular lesions have been shown to lead to acquired alexithymia and to a reduction of drug seeking sensations (i.e. craving) (Naqvi *et al.*, 2007, Hogeveen *et al.*, 2016), supporting the importance of the interoceptive cortex in both emotional regulation and in addiction. Moreover, this also demonstrates that the

relevance of interoception to the concept of drug craving, as both phenomena share common neural correlates such as the insula (Gray and Critchley, 2007, Naqvi and Bechara, 2010, Naqvi *et al.*, 2014).

In the next experimental chapter, we will explore the morphological and neurochemical integrity of the insular cortex, in alcohol users, to find a potential mediator between interoception (indexed by craving sensations) and alcohol use.

Chapter 4 Neurochemical and structural correlates within insula cortex in alcohol use[2]

[2] As first author I designed the study and undertook all stages of data collection analyses and interpretation with the guidance of my senior colleagues and the assistance of Dr Pfeifer. In the text that follows, as in the submitted paper, this group contribution is acknowledged in my use of plural personal pronouns.
This chapter led to a scientific publication submission in an international journal:
Betka, S., Harris, L., Rae, C., Palfi, B., Pfeifer, G., Sequeira, H., Duka, T., Critchley, H. (submitted) Signatures of alcohol use in the structure and neurochemistry of insular cortex: a correlational study *Alcoholism: Clinical and Experimental Research*

4.1 Abstract

Background Insular cortex is implicated in supporting the representation of motivational feelings through the integration of interoceptive information concerning internal bodily physiology. Compromised insular integrity is implicated in alcohol and drug use disorders. Alcohol-associated insular dysfunction may arise through aberrant glutamatergic neurotransmission causing selective neuronal death and atrophy. In a sample of alcohol users, we combined magnetic resonance spectroscopy (MRS) with voxel and surface-based morphometry (VBM, SBM) to test the hypothesis that the neurochemical and structural properties of the insula relate to alcohol use.

Methods Thirty-two healthy individuals were characterized on measures of alcohol use and subjective craving. Right mid-insula Glutamate/Glutamine (Glx) and total N-acetylaspartate/N-acetyl-aspartylglutamate (TNAA) concentrations were measured using MRS. Right insular structure was quantified using VBM and SBM parameters. We tested for predictive associations between behavioral/psychometric and these neuroimaging measures.

Results Indicators of greater alcohol use were associated with reduced insular Glx concentration and lower levels of insular gyrification. Moreover, Glx concentration was significantly predicted from the insular gyrification index.

Conclusion This work is, to date, the first characterization of the neurochemical and morphological integrity of insular cortex in alcohol users. The data reveal a negative relationship between alcohol use and the neurochemical and structural integrity of the insula, a critical substrate for motivational behaviour. These neurobiological characteristics might contribute to loss of control toward compulsive drinking with prolonged and excessive alcohol use.

Key words: Addiction, Insula, Interoception, Magnetic Resonance Spectroscopy, Alcohol use, Voxel/Surface-Based Morphometry

4.2 Introduction

The insular cortex is involved in the neurocircuitry of addiction: interoceptive components of drug seeking (notably craving states) are proposed to originate within insula (Gray and Critchley, 2007). Correspondingly, impairments in interoceptive bodily sensations are reported in 'addicts', including dependent alcohol users (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016), methamphetamine users (Stewart *et al.*, 2014), adolescent cannabis users (Berk *et al.*, 2015), and people with internet gaming disorder (Zhang *et al.*, 2016). Moreover, patients with insular lesions are more likely to quit smoking easily, immediately, without relapse, and without persistence of the urge to smoke (Naqvi *et al.*, 2007).

Reduced insular grey matter volume, functional connectivity, structural connectivity and von Economo neurons prevalence are observed in alcohol use disorders (O'Daly *et al.*, 2012, Monnig *et al.*, 2014, Yang *et al.*, 2016, Grodin *et al.*, 2017). These abnormalities might be explained by the interaction of alcohol with the glutamatergic neurotransmission: alcohol use suppresses excitatory synaptic signalling, particularly through inhibition of N-methyl-D-aspartate (NMDA) receptors (Lovinger *et al.*, 1989). This impacts synaptic plasticity by reducing long-term potentiation (Stephens *et al.*, 2005). In compensation, the number and sensitivity of NMDA receptors increase proportionally to the amount and frequency of alcohol intake (Trujillo and Akil, 1995). A sharp reduction or cessation of alcohol consumption can induce rebound neuronal hyperexcitability, leading to excitotoxicity and atrophy (Tsai *et al.*, 1995).

In humans, it is possible to quantify metabolites *in vivo* using magnetic resonance spectroscopy (MRS). In alcohol dependent patients, increased glutamate and combined glutamate-glutamine concentrations are reported in left dorsolateral prefrontal cortex, anterior cingulate cortex (ACC) and striatal regions, and are associated with craving intensity and compulsions to drink alcohol (Hermann *et al.*, 2012, Bauer *et al.*, 2013, Frye *et al.*, 2016).

Interestingly, individuals suffering from alcohol dependence show lower concentrations of glutamate and N-acetylaspartate (NAA) within the ACC,

which is predicted by early abstinence, previous heavy drinking episodes and alcohol use severity (Thoma *et al.*, 2011, Mon *et al.*, 2012, Prisciandaro *et al.*, 2016). Finally, lower glutamate concentration within neighbouring prefrontal white matter predicts the tendency to lose control and the severity of alcohol dependence among heavy drinkers (Ende *et al.*, 2013).

Most neurochemical imaging (MRS) studies of alcohol use disorders focus on prefrontal or striatal reward-related areas. However, increasing evidence implicates 'interoceptive' insular cortex in specific aspects of drug seeking states (e.g. craving; Gray and Critchley, 2007). Together, these convergent observations motivate the current study, which tested the prediction that even the 'social' drinking of alcohol impacts the neurochemical and morphological (structural: volume and surface gyrification) integrity of insular cortex and related regions.

The majority of studies exploring alcohol-related changes in brain structure have used voxel-based morphometry (VBM) approaches. However, morphology of brain structure can also be appraised using complementary techniques for example, surface-based morphometry (SBM). SBM is proposed to be able to capture subtle grey matter changes (Hutton *et al.*, 2009, Kelly *et al.*, 2013). For example, extensive literature has explored the relationship between cortical folding (i.e. gyrification) and prenatal alcohol exposure in children and adolescents (De Guio *et al.*, 2014, Kuhn *et al.*, 2016, Hendrickson *et al.*, 2017, Hendrickson *et al.*, 2018). However, to our knowledge, differences in gyrification have not been previously explored in adult alcohol users.

4.3 Aims and hypotheses

Therefore, to test the relationship between insular neurochemistry (glutamate, glutamine and NAA metabolite concentrations, see Chapter 1, section 1.3.3), alcohol-related measures (i.e. severity, craving, and compulsion) and interoceptive ability in a non-clinical population of alcohol users, MRS measures were combined with behavioural and psychometric ratings. Moreover, associations between brain structure (brain volume and surface gyrification), alcohol-related measures, and interoception were also explored in this group.

Based on the evidence of alcohol-induced glutamatergic excitotoxicity, we hypothesised that insular glutamate-plus-glutamine concentration would be lower in individuals with higher scores on alcohol use measures. We also predicted, based on previous reports, that insular grey matter volume and cortical gyrification index would be negatively correlated with these alcohol use measures. Also, we expected that alcohol-related metabolites changes will be predicted by structural measures indicative of insular atrophy in alcohol users. Finally, we expected that alcohol-related psychometric measures would be positively interrelated.

4.4 Methods summary

Full methodological details are described in Chapter 2, section 2.2.

4.4.1 Procedure and participants

To test our hypotheses, thirty-two male alcohol users were recruited, who performed psychometric tests assessing alcohol use (AUQ), alcohol use severity (AUDIT), and alcohol craving (ACQ and OCDS). On the third (and last) session of the project, a structural scan and a MRS scan were acquired in order to quantify insular structure and neurochemistry.

Using TARQUIN, spectra were fitted and metabolites concentrations (Glutamate Glu; Glutamine Gln; Glutamate plus Glutamine Glx and total N-acetylaspartate plus N-acetyl-aspartylglutamate TNAA) were extracted and corrected. The quality of the model fit was manually verified and seven participants were discarded from the analyses.

Using CAT12 in SPM 12, estimates of grey matter volume and gyrification index of the right insula were extracted prior to normalization. Analyses were undertaken to determine the extent to which any spectroscopic concentration might relate to a reduction in insular structure.

4.5 Statistical Analyses

Pearson correlations and regressions were used to explore the relationship between psychometric measures and metabolite concentrations (as metabolite concentrations were already corrected for the VOI tissue content). Regressions, controlling for intracranial volume and age, and regressions were used to test relationships between insular (ROI) volumetric/surface parameters, metabolite concentrations and psychometric measures. For regressions, effect sizes (Cohen's f^2) were calculated (Cohen, 1988). All analyses were done using IBM SPSS, version 24.

4.6 Results

4.6.1 Psychometric measures

All means, standard deviations, and correlations with psychometric measures were computed (N=25,

Table 4-1). Twenty-four percent of the sample (n=6) scored between 0 and 7 on the AUDIT, suggesting absence or low level of alcohol-related problems. Forty percent of the sample (n= 10) scored between 8 and 15, suggesting a medium level of alcohol-related problems. Finally, thirty-six percent of the sample (n= 9) scored above or equal to 16 on the AUDIT, suggesting a high level of alcohol-related problems.

		UNIT OF ALCOHOL	AUDIT	OCDS OBSESSION SUBSCALE	OCDS COMPULSION SUBSCALE	OCDS
UNIT OF ALCOHOL	Pearson Correlation	-	**.506****	0.037	0.361	0.279
	Sig. (2-tailed)		**0.01**	0.861	0.077	0.178
AUDIT	Pearson Correlation	**.506****	-	**.512****	**.730****	**.758****
	Sig. (2-tailed)	**0.01**		**0.009**	**0**	**0**
OCDS OBESSION SUBSCALE	Pearson Correlation	0.037	**.512****	-	**.427***	**.754****
	Sig. (2-tailed)	0.861	**0.009**		**0.033**	**0**
OCDS COMPULSION SUBSCALE	Pearson Correlation	0.361	**.730****	**.427***	-	**.916****
	Sig. (2-tailed)	0.077	**0**	**0.033**		**0**
OCDS	Pearson Correlation	0.279	**.758****	**.754****	**.916****	-
	Sig. (2-tailed)	0.178	**0**	**0**	**0**	
	MEAN	24.40	13.24	3.00	7.92	10.92
	(SD)	19.00	7.51	2.61	4.29	5.90

Table 4-1 Means, standard deviations, Pearson coefficient correlations and p values for psychometric measures.

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed). OCDS OBS SUB: Obsession subscale of the OCDS; OCDS COMP SUB: Compulsion subscale of the OCDS

4.6.2 Insular magnetic resonance spectroscopy MRS

		UNIT OF ALCOHOL	AUDIT	OCDS OBSESSION SUBSCALE	OCDS COMPULSION SUBSCALE	OCDS	MEAN (SD)
GLUTAMATE (GLU)	Pearson Correlation	**-.475***	**-.400***	0.057	**-.411***	-0.274	8.64 (1.71)
	Sig. (2-tailed)	**0.016**	**0.048**	0.787	**0.041**	0.185	
GLUTAMINE (GLN)	Pearson Correlation	0.111	-0.175	-0.380	-0.199	-0.313	2.50 (1.65)
	Sig. (2-tailed)	0.599	0.404	0.061	0.340	0.128	
GLUTAMATE + GLUTAMINE (GLX)	Pearson Correlation	-0.257	**-.397***	-0.215	**-.422***	**-.402***	11.14 (2.47)
	Sig. (2-tailed)	0.216	**0.049**	0.301	**0.036**	**0.046**	
TNAA	Pearson Correlation	0.093	-0.141	0.131	0.124	0.148	5.59 (0.5)
	Sig. (2-tailed)	0.660	0.501	0.532	0.554	0.479	

Table 4-2 Means, standard deviations, Pearson coefficient correlations and uncorrected *p* values for psychometric measures and metabolites concentrations.

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

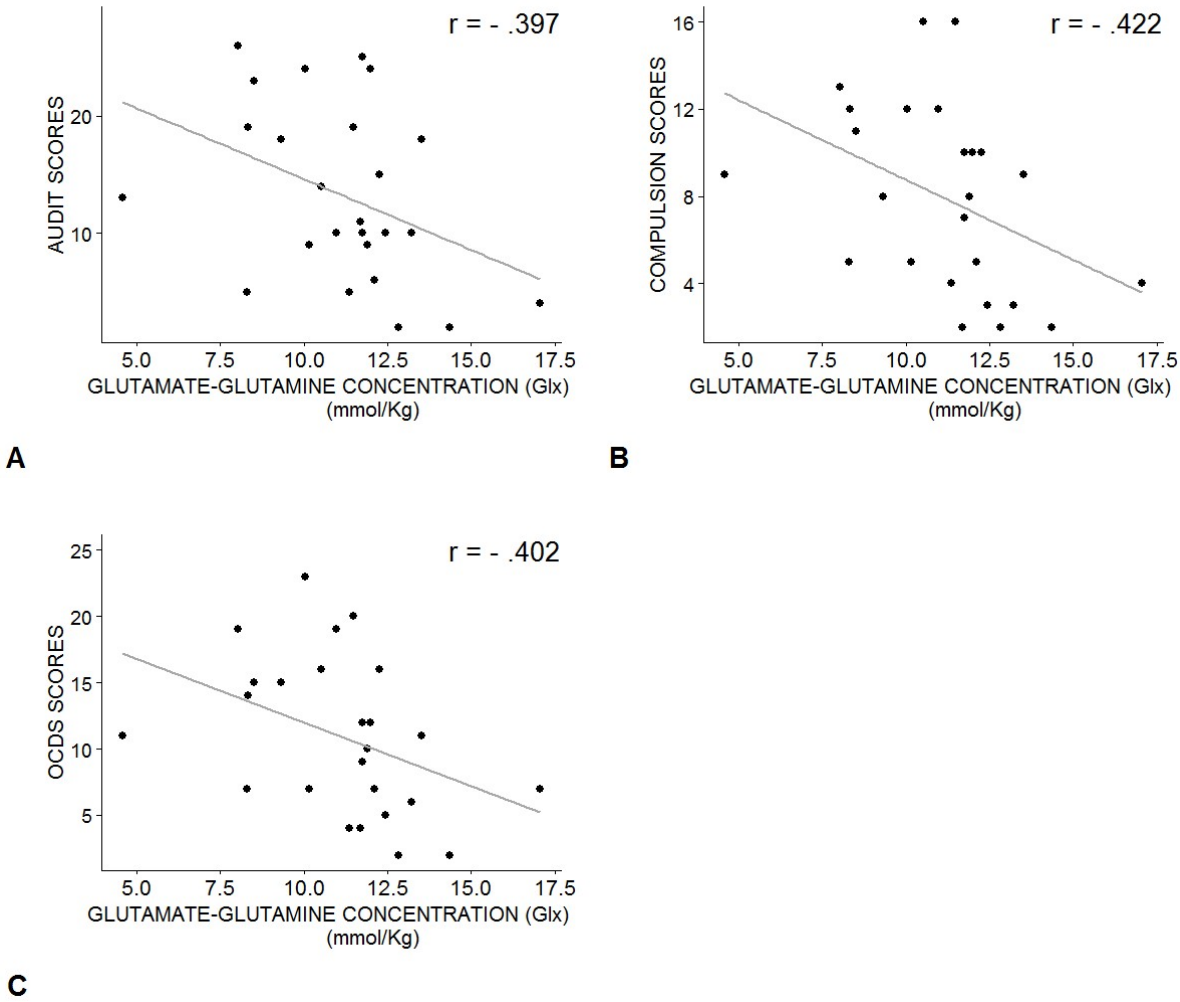


Figure 4.1 Correlation between metabolites concentrations and psychometric measures

A.; B. Negative correlation between insular Glx concentration and compulsion subscale of the OCDS; and C. Negative correlation between insular Glx concentration and OCDS scores.

All means, standard deviations and correlations between metabolites and psychometric measures were computed (Table 4-2). Glx concentration correlated negatively with AUDIT (Figure 4.1.A: $r = -0.397$, $p < 0.05$), the compulsion subscale of OCDS (Figure 4.1.B: $r = -0.422$, $p < 0.05$), and OCDS (Figure 4.1.C: $r = -0.402$, $p < 0.05$). Glu concentration correlated negatively with UNIT ($r = -0.475$, $p < 0.05$), AUDIT ($r = -0.400$, $p < 0.05$) and the compulsion subscale of OCDS ($r = - 0.411$, $p < 0.05$). No significant relationship was observed between TNAA concentration and psychometric measures.

	DRUG	N	MEAN	STD. DEV	STD. ERROR MEAN	T-TEST (P VALUE)
GLUTAMATE (GLU)	PLACEBO	12	8.83	1.73	0.50	0.596
	OXYTOCIN	13	8.46	1.73	0.48	
GLUTAMINE (GLN)	PLACEBO	12	2.70	1.77	0.51	0.561
	OXYTOCIN	13	2.31	1.58	0.44	
GLUTAMATE + GLUTAMINE (GLX)	PLACEBO	12	11.54	2.70	0.78	0.450
	OXYTOCIN	13	10.77	2.28	0.63	
TNAA	PLACEBO	12	5.42	0.57	0.16	0.107
	OXYTOCIN	13	5.75	0.39	0.11	

Table 4-3 Independent samples t-tests between oxytocin and placebo pools of participants.

Independent samples t-tests showing no effect of oxytocin on metabolite concentrations are presented below (Table 4-3).

4.6.3 Voxel and Surface-based Morphometry – ROI analyses of right insula

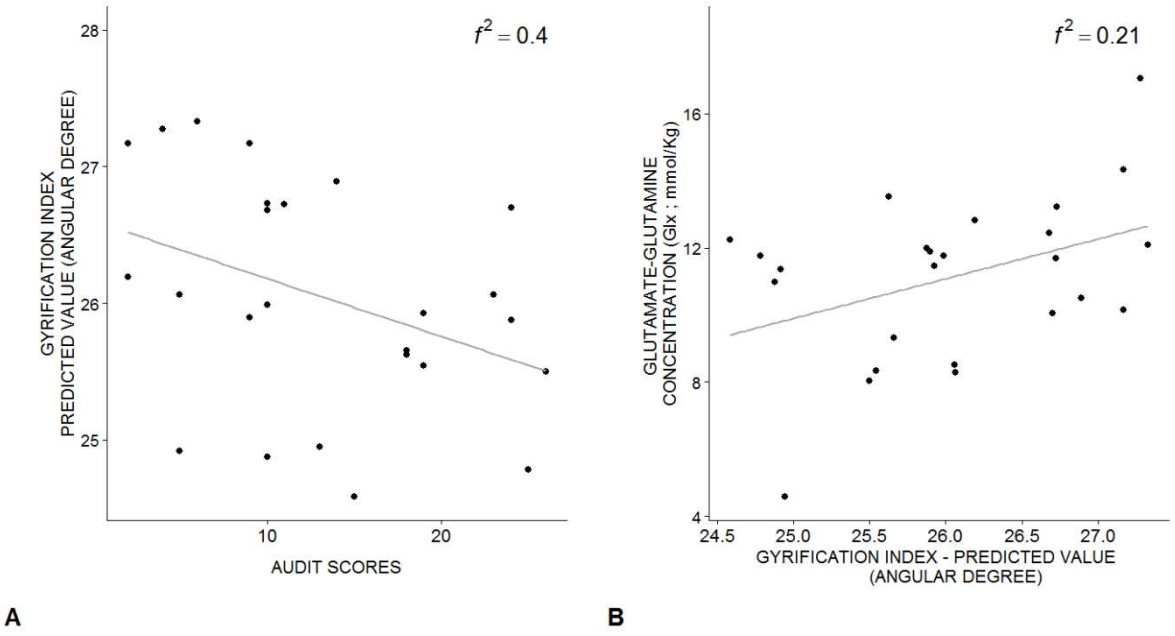


Figure 4.2 Regressions between predicted volume and surface parameters, metabolite concentrations and psychometric measures.

A. Insular Glx concentration correlated positively with right insula gyrification index, and B. Right insula gyrification index correlated negatively with AUDIT scores (controlled for age and intracranial volume)

Grey matter volume: Linear regressions, controlling for total intracranial volume (TIV) and age, did not find any significant relationship.

Gyrification index: Linear regression controlling for TIV and age, showed that decreased gyrification index within the right insula were predicted by alcohol use severity (AUDIT) scores (Figure 4.2.A: Model $R^2 = 0.53$, $p < 0.001$; $\beta = -0.38$, SE = 0.02, t = -2.43, $p < 0.05$; Cohen's $f^2 = 0.40$).

4.6.4 Relationship between MRS and SBM data

Interestingly, Glx concentration was positively related to the insular gyrification index (Figure 4.2.B: $R^2 = 0.17$, $\beta = 0.41$, SE = 0.41, t = 2.15, $p < 0.05$; Cohen's $f^2 = 0.21$).

4.7 Discussion

The present study examined the relationship between subjective measures of alcohol use, insular neurochemistry and insular structure. Two key results were observed: 1) reduced insular glutamate-glutamate concentration was associated with alcohol use severity and alcohol compulsions and, 2) alcohol use severity was associated with reduced insular cortical folding.

4.7.1 Main findings

Our results indicate lower combined glutamate-glutamine (Glx) concentration within the mid-insular cortex associated with the severity of alcohol use and the increased experience of alcohol-related compulsions. This same relationship was observed for glutamate concentration on its own, but not for glutamine concentration alone. Consequently, while the limitations of dissecting subcomponents of the Glx peak of nuclear magnetic resonance spectra acquired at 1.5 Tesla are acknowledged, it is reasonable to infer that the effect reflects a meaningful association between glutamate concentration and alcohol use. Alcohol intake and acute alcohol withdrawal are classically associated with an increased glutamatergic neurotransmission (Hwa *et al.*, 2017). However, glutamate concentration also may depend upon individual differences in drinking pattern (Ding *et al.*, 2012), including the consequences of binge drinking episodes. Thus, the findings might reflect the impact of recent heavy drinking within a subset of our participants. Indeed, others have observed that the number of heavy drinking episodes over a fortnight correlates with decreased glutamate concentration within the anterior cingulate cortex (Prisciandaro *et al.*, 2016). Moreover, in heavy drinkers, decreased glutamate in frontal white matter tracts (connecting insular and cingulate cortices) predicts subjectively-rated loss of control and a shift to alcohol dependency (Ende *et al.*, 2013). Dysfunction within these white matter tracts may also account for decrements in functional connectivity between insula and prefrontal/cingulate cortices, which may compromise motivational regulation in people with alcohol use disorders (O'Daly *et al.*, 2012). Our results also indicate a perturbation of the glutamate-glutamine metabolic cycle associated with alcohol use, also reported in clinical populations (Thoma *et al.*, 2011). This disruption is a potential neurobiological risk factor for alcohol use disorders, rather than an incidental consequence of alcohol drinking. In this context, abnormalities in the

glutamate/glutamine metabolic cycle within the anterior cingulate cortex correlate with higher impulsivity in youths with a family history of alcoholism (Cohen-Gilbert *et al.*, 2015). However, within the present study, glutamine concentration alone did not track increased alcohol intake. Speculatively, if MRS was conducted at a higher magnetic field, independent glutamate and glutamine concentrations may be differentiated with greater precision and confidence. Nevertheless, our findings indicate the potential presence of alcohol-related glutamate reduction within right insula, which may underlie a pathogenetic vicious cycle of alcohol craving and further drinking. Indeed, the disruption of interoceptive processes in alcohol-dependent individuals correlates positively with subjective craving ratings (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016) and more generally, impaired processing of bodily sensation is linked to insular cortex dysfunction in drug use disorders (Stewart *et al.*, 2014). However, it would be helpful to pursue neuroimaging studies to define functional neural correlates of pure visceral interoception (independently of other sources of bodily sensations such cutaneous touch or proprioceptive sensations of muscular effort), both in individuals with alcohol use disorders and those with non-clinical patterns of alcohol users.

Interestingly, the majority of neurochemical studies of alcohol use in animal or humans focus on the disruptive effect of alcohol within the ventral striatum/nucleus accumbens, where mesolimbic dopamine activity signals unpredicted reward (e.g. Rossetti and Carboni, 1995, Chen *et al.*, 2011, Carlson, 2018). Despite the role of insula in normal and dysfunctional motivational experience, there is a paucity of studies that have specifically tested the neurochemical integrity of the insular cortex in relation to alcohol use. One study reported no significant neurochemical differences within insular cortex of young alcohol-dependent patients compared to healthy subjects (Lee *et al.*, 2007). However, an increase in glutamate-to-creatine ratio within the anterior cingulate cortex was observed in the patients compared to the control subjects (Lee *et al.*, 2007). Another study, that focused on glutamate and glutamine levels in relation to pain processing, also measured alcohol consumption in social drinkers, but again did not find any significant relationship (Zunhammer *et al.*, 2016). Of technical importance, the majority of MRS studies used creatine concentration as an ‘external’ reference for quantification. However, this is likely to be problematic as the stability of creatine concentration in alcohol use disorders is questionable (Mon *et al.*, 2012).

Our second main finding was a negative relationship between the gyrification of the right insula and alcohol drinking severity. To our knowledge, the present study is the first study to quantify the relationship between insular gyrification with respect to alcohol use. Insular atrophy (with grey matter loss) is commonly observed in alcohol-dependent individuals (Yang *et al.*, 2016) and accounts within our sample for part of the relationship between reduced gyrification, decreased glutamate-glutamine concentration, and increased alcohol intake. Finally, no specific relationship was observed between alcohol-related measures and overall grey matter volume of the insular cortex. This finding suggests that surface-based morphometry parameters represent a more sensitive measure of early alcohol-associated decline in structural grey matter organization than voxel-based morphometry estimates.

4.7.2 Limitations

The results of the present study should be considered in light of several constraints. First, further information concerning aspects of alcohol consumption may have provided further mechanistic insight into pathoaetiological processes, including knowledge of starting age of alcohol intake, and the frequency, and precise quantity of alcohol intake over different stages of the lifespan. Moreover, family histories of alcohol use disorder, which also impacts neurochemical and morphological brain integrity (Cohen-Gilbert *et al.*, 2015), were not formally elicited. A detailed alcohol consumption history for the preceding two weeks before neuroimaging might have shed further light on differences in neurochemical levels, as recent heavy drinking episodes can perturb glutamatergic neurotransmission (Prisciandaro *et al.*, 2016). Also, while alcohol and drug abstinence were controlled for before the study, the findings might have been further enhanced by additionally controlling for tobacco smoking habits. Further studies should measure and control for tobacco use. Indeed, it is recognized that alcohol-dependent individuals who smoke show reduced N-acetylaspartate cerebral concentration when compared to alcohol-dependent non-smokers (Durazzo *et al.*, 2013). Lastly, a recognized technical limitation was the magnetic strength of the MRI scanner for neurochemical discrimination using MRS. A higher field strength can enable more direct, separate quantification of GABA, glutamate and glutamine concentrations.

4.7.3 Conclusions

The neurochemical and morphological integrity of the insular cortex was quantified in alcohol users. Together, these data provide evidence for disruption of insular glutamate-glutamine concentration and a modulation of brain surface parameters by alcohol use. The observed changes may underpin a loss of control over alcohol and shift toward compulsive drinking. In the next experimental chapter, we will explore if intranasal oxytocin, recognized to enhance empathy and reduced alcohol-withdrawal in humans, impacts interoceptive accuracy in alcohol users.

Chapter 5 Impact of intranasal oxytocin on interoceptive accuracy in alcohol users: An attentional mechanism?[3]

[3]As first author I designed the study and undertook all stages of data collection analyses and interpretation with the guidance of my senior colleagues and the assistance of Dr Pfeifer. In the text that follows, as in the published paper, this group contribution is acknowledged in my use of plural personal pronouns.
This chapter led to a scientific publication in an international journal:
Betka, S., Gould Van Praag, C., Paloyelis, Y., Bond, R., Pfeifer, G., Sequeira, H., Duka, T. & Critchley, H., (2018) Impact of intranasal oxytocin on interoceptive accuracy in alcohol users: An attentional mechanism? *Social Cognitive and Affective Neuroscience* DOI:10.1093/scan/nsy027

5.1 Abstract

Background Interoception, i.e. the perception and appraisal of internal bodily signals, is related to the phenomenon of craving, and is reportedly disrupted in alcohol use disorders. The hormone oxytocin influences afferent transmission of bodily signals and, through its potential modulation of craving, is proposed as a possible treatment for alcohol use disorders. However, oxytocin's impact on interoception in alcohol users remains unknown.

Methods Healthy alcohol users (N=32) attended two laboratory sessions to perform tests of interoceptive ability (heartbeat tracking: attending to internal signals and, heartbeat discrimination: integrating internal and external signals) after intranasal administration of oxytocin or placebo. Effects of interoceptive accuracy, oxytocin administration and alcohol intake, were tested using mixed-effects models.

Results On the tracking task, oxytocin reduced interoceptive accuracy, but did not interact with alcohol consumption. On the discrimination task, we found an interaction between oxytocin administration and alcohol intake: Oxytocin, compared to placebo, increased interoceptive accuracy in heavy drinkers, but not in light social drinkers.

Conclusions Our study does not suggest a pure interoceptive impairment in alcohol users but instead potentially highlights reduced flexibility of internal and external attentional resource allocation. Importantly, this impairment seems to be mitigated by oxytocin. This attentional hypothesis needs to be explicitly tested in future research.

Key words: Alcohol, Addiction, Alcohol use, Oxytocin, Interoception, Attention

5.2 Introduction

Interoception classically refers to the signaling, representation, and perception of internal bodily sensations coming from the viscera, for example, heartbeats, gastric distension, or visceral pain (Cameron, 2001). In the context of drug addiction, indirect evidence suggests that interoceptive processes underpin urges to take a drug, also known as drug craving (Naqvi and Bechara, 2010). As specified in the previous chapter, for example, lesion studies implicate the insular cortex, a brain region supporting interoceptive states, in the phenomenon of craving (Gray and Critchley, 2007). Neuroimaging studies further reveal abnormalities in the morphometry, functional activity and connectivity of insular cortex in alcohol, cannabis and, also, tobacco users (Berk *et al.*, 2015, Maria *et al.*, 2015, Grodin *et al.*, 2017). In addition to lesion and neuroimaging investigations, more direct evidence concerning the relationship between interoceptive ability and craving can be drawn from behavioural studies. Interoceptive ability (i.e. interoceptive accuracy) commonly measured using heartbeat tracking and heartbeat discrimination tasks (Garfinkel *et al.*, 2015, Brener and Ring, 2016). Diminished interoceptive abilities are observed in alcohol and drug user populations and this deficit is positively associated with both subjective craving sensations and alexithymia (e.g. difficulties in identifying and describing emotions) (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). We recently reported that alexithymia (a possible outcome of aberrant processing of bodily sensations) may play a role in alcohol use (Chapter 3 ; Betka *et al.*, 2017). Indeed, the effective integration of interoceptive inputs is crucial for both subjective experience and social skills (Park and Tallon-Baudry, 2014, Shah *et al.*, 2017); i.e. skills typically impaired in alcohol and drug use disorders (D'Hondt *et al.*, 2014, Verdejo-Garcia, 2014).

Oxytocin (OT) is a neuropeptide hormone that is mostly synthesised in the hypothalamus, and released into the bloodstream via the posterior pituitary gland (Sokol and Valtin, 1967). Intranasal OT has been hypothesized to facilitate empathy. Indeed, OT administration can increase trust and enhance the detection of emotional signals of others (Domes *et al.*, 2007a, Keri and Kiss, 2011, Schulze *et al.*, 2011, Lischke *et al.*, 2012, Van and Bakermans-Kranenburg, 2012, Perry *et al.*, 2013, Kanat *et al.*, 2015). Relatedly, OT administration can also improve capacity for "mind reading" (mentalization) (Guastella *et al.*, 2010), empathy (Hurlemann *et al.*, 2010, Panksepp and Panksepp, 2013) and mimicry of angry faces (Korb et al., 2015). As it is thought

that empathic abilities arise from mentalization of bodily sensations, one could postulate that oxytocin increases social cognition *via* the modulation of interoceptive accuracy. OT is hypothesized to enhance attention toward interoceptive signals (i.e. precision of central interoceptive representations), which can inform generative models of emotion and selfhood (Quattrocki and Friston, 2014). Intranasal OT administration may not markedly influence performance on heartbeat discrimination in healthy human participants (Yao *et al.*, 2017), yet electrophysiological studies show that OT gates the transmission of viscerosensory afferent information (Peters *et al.*, 2008, Karelina and Norman, 2009).

Finally, in rodents, the overexpression of OT receptors in mice reduces the rewarding proprieties of ethanol (Bahi, 2015) and OT injections decrease ethanol consumption, ethanol preference and ethanol-triggered dopamine release within the accumbens nucleus (Peters *et al.*, 2013, MacFadyen *et al.*, 2016, King *et al.*, 2017, Peters *et al.*, 2017). One clinical trial showed that intranasal OT attenuated alcohol withdrawal symptoms in alcoholic patients (Pedersen et al., 2013).

In summary, interoception is a crucial facet of emotional regulation, which seems to be impaired in alcohol-dependent individuals. OT is a facilitator of empathic emotional feelings and enhances afferent viscerosensory transmission. Moreover, OT may reduce alcohol withdrawal symptoms and diminish alcohol intake.

5.3 Aims and hypotheses

We therefore sought to characterize the impact of intranasal OT on interoceptive processing in alcohol users. We hypothesised that interoceptive skills are negatively correlated with alcohol use severity and that OT administration improves interoception and hence can reduce the impairments observed in heavy alcohol users.

Finally, higher levels of alexithymia, anxiety, and depression are usually observed in alcohol use disorder (Evren *et al.*, 2009). As these three conditions are associated with abnormal interoceptive profiles (Paulus and Stein, 2010, Barrett *et al.*, 2016, Garfinkel *et al.*, 2016b, Betka *et al.*, 2017), we thought it crucial to account for them in our analyses. However, we did not have any hypotheses regarding the influence of these variables.

5.4 Methods summary

Full methodological details are described in Chapter 2 section 2.2.

5.4.1 Procedure and participants

Thirty-two male volunteers (mean age 25.1 years; range 18–36 years; drinking at least 1 unit of alcohol per week) took part in the experiment. Participants attended the laboratory for three sessions. The first session was a baseline session during which psychometric/demographic information and a blood sample were collected. During the second and the third sessions, subjects performed the heartbeat tracking and discrimination tasks, after administration of 40 units (IU) of oxytocin or placebo intranasal spray. The drug sequence was counter-balanced for each participant, the researcher was double-blind.

In the heartbeat tracking task, participants silently count their own heartbeats, by focusing their attention on their internal cues. In the heartbeat discrimination task, participants attend to both internal and external cues, judging the synchrony between their own heartbeats and sequences of tones either presented in sync or out of sync with their own heartbeats.

In this task, participants flexibly switch attention between external and internal cues to integrate the perceptions for the synchronicity judgment. Interestingly, the interoceptive accuracy of alcohol-dependent subjects and drug users has only been tested using the heartbeat tracking task.

One participant specified he was experiencing a hangover on one of the sessions; his data were discarded from the analyses.

5.4.2 Statistical Analyses

Analysis of interoceptive tracking accuracy used linear mixed-effects models as the outcome was continuous. We analysed interoceptive discrimination accuracy using generalized linear mixed models as the outcome was binary (Inaccurate =0; Accurate =1; binomial family function), using the lme4 package (Bates *et al.*, 2015) in the R environment (version 3.4.2; RCoreTeam, 2013). P values were computed using lmerTest package (Kuznetsova *et al.*, 2014).

Both types of interoceptive accuracy were analysed with drug (2 levels: Placebo=0; OT=1), units of alcohol per week (continuous predictor), their interaction and control variables (heart rate, anxiety, depression, alexithymia and drug sequence) as fixed factors. Participants were treated as a random factor.

Plasma OT levels were missing for two participants from whom we were unable to collect blood samples. Generalized and linear mixed models omit cases with missing data. Therefore, to check the effect of plasma OT levels on accuracy, we ran a first version of our final models, restricted to the participants with plasma OT information. Thereafter, we ran a second model, similar to the first one, but including plasma OT levels as predictor (fixed factor). For both tasks, we compared the two models using likelihood ratio tests.

5.5 Results

5.5.1 Sample description, psychometric measures and correlations

	Mean	Std. Deviation	Range
TAS-20	55.42	9.76	36-74
Units per week	24.82	18.09	4.8-69.50
STAI	48.19	12.21	22-66
BDI	12.84	9.98	0-48
Basal plasma oxytocin level (pg/mL)	1.61	0.57	0.95-3.07
Mean discrimination Accuracy (Placebo)	0.53	0.13	0.30-0.85
Mean discrimination Accuracy (Oxytocin)	0.58	0.18	0.30-0.95
Mean heart rate during the discrimination task (Placebo; bpm)	64.07	9.97	47.20-88.80
Mean heart rate during the discrimination task (Oxytocin; bpm)	66.67	11.15	46-80-88.10
Mean tracking Accuracy (Placebo)	0.78	0.12	0.55-0.97
Mean tracking Accuracy (Oxytocin)	0.68	0.23	0.25-0.97
Mean heart rate during the tracking task (Placebo; bpm)	62.12	9.89	44.30-83.80
Mean heart rate during the tracking task (Oxytocin; bpm)	65.45	11.17	43.30-85.70

Table 5-1 : Mean and standard deviations of psychometric measures, basal plasma oxytocin (OT) level as well as interoceptive accuracy and heart rate for both tasks.

The data of thirty-one participants were analysed. Thirteen participants drank less than 14 alcohol units per week and were considered mild social drinkers. Eighteen participants drank more than 14 alcohol units per week and were considered mild to heavy drinkers. Means, standard deviations, and ranges of psychometric measures, basal plasma OT level as well as interoceptive accuracy and heart rate were computed for both tasks (

Table 5-1).

		TAS-20	Units per week	STAI	BDI
Units per week	Pearson Correlation	0.192	-		
	Sig. (2-tailed)	0.301			
STAI	Pearson Correlation	0.308	-0.179	-	
	Sig. (2-tailed)	0.091	0.337		
BDI	Pearson Correlation	0.354	-0.117	.496**	-
	Sig. (2-tailed)	0.051	0.531	0.005	
Basal plasma oxytocin level	Pearson Correlation	-0.149	0.113	-0.057	-0.179
	Sig. (2-tailed)	0.440	0.558	0.770	0.352

Table 5-2 Correlations coefficients for psychometric measures and basal plasma oxytocin (OT) level.

Correlations coefficients revealed no significant relationship between psychometric measures and basal plasma OT level (

Table 5-2).

5.5.2 Tracking Task

	β	Std. Error	z value	*p* value	
(Intercept)	0.794	0.025	32.254	0.000	***
Drug	-0.078	0.034	-2.287	0.030	*
Units per week	-0.002	0.001	-1.689	0.105	
HR	-0.005	0.002	-3.301	0.002	**
STAI	-0.005	0.002	-2.687	0.013	*
BDI	0.007	0.002	3.148	0.004	**
TAS-20	-0.001	0.002	-0.676	0.506	
Sequence	-0.050	0.030	-1.689	0.102	
Drug*Units	0.002	0.002	1.256	0.220	

Table 5-3 Mixed-effects regression model to explain accuracy on tracking task.

This regression is using predictors for oxytocin/placebo (drug), alcohol intake (units per week), and their interaction, with heart rate (HR), anxiety (STAI), depression (BDI), alexithymia (TAS-20) and drug sequence as control variables, including all participants. Formula: Accuracy ~ Drug * Units per week + HR + STAI + BDI + TAS-20 + Sequence + (1 + Drug|ID); signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Results of the mixed-effects regression model, for this task, are presented in

Table 5-3. We found a main effect of OT on accuracy, with reduced accuracy under OT compared to placebo (β = -0.08, SE = 0.03, *p* = 0.03). The main effect of heart rate was significant: participants with lower heart rate were more accurate than participants with higher heart rate (β = -0.01, SE = 0.01, *p* = 0.002). A main effect of anxiety as well as a main effect of depression was found; anxiety was associated with reduced accuracy whereas depression was associated with increased accuracy (anxiety: β = -0.01, SE = 0.01, *p* = 0.013; depression: β = 0.01, SE = 0.01, *p* =0.004). No main effect of units per week or interaction between OT/placebo and units per week was observed.

	β	Std. Error	z value	*p* value	
(Intercept)	0.800	0.025	31.959	0.000	***
oxytocin/placebo (drug)	-0.082	0.035	-2.322	0.028	*
Units per week	-0.002	0.001	-1.357	0.189	
HR	-0.005	0.002	-2.904	0.005	**
STAI	-0.006	0.002	-2.913	0.008	**
BDI	0.007	0.002	2.823	0.009	**
TAS-20	-0.001	0.002	-0.442	0.663	
Sequence	-0.064	0.030	-2.127	0.042	*
Drug*Unit	0.001	0.002	0.634	0.532	

Table 5-4 Mixed-effects regression model to explain accuracy on tracking task.

This regression is using predictors for oxytocin/placebo (drug), alcohol intake (units per week), and their interaction, with heartrate, anxiety (STAI), depression (BDI), alexithymia (TAS-20) and drug sequence as control variables, restricted to participants with plasma OT data.Formula: Accuracy ~ Drug * Units per week + HR + STAI + BDI + TAS + Sequence + (1 + Drug|ID); signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

	β	Std. Error	z value	*p* value	
(Intercept)	0.797	0.025	32.001	0.000	***
Drug	-0.080	0.036	-2.253	0.032	*
Units per week	-0.002	0.001	-1.555	0.134	
HR	-0.005	0.002	-3.171	0.002	**
STAI	-0.006	0.002	-2.967	0.007	**
BDI	0.007	0.002	3.030	0.006	**
TAS-20	-0.060	0.030	-2.007	0.054	.
Sequence	-0.001	0.002	-0.268	0.791	
Plasma	0.050	0.036	1.396	0.176	
Drug*Unit	0.001	0.002	0.647	0.523	

Table 5-5 Mixed-effects regression model to explain accuracy on tracking task.

This regression is using predictors for oxytocin/placebo (drug), alcohol intake (Units per week), and their interaction, with heart rate (HR), anxiety (STAI), depression (BDI), alexithymia (TAS-20), drug sequence and oxytocin plasmatic level as control variables, restricted to subjects with oxytocin plasmatic data. Formula: Accuracy ~ Drug * Units per week + HR + STAI + BDI + TAS-20+ Sequence + Plasma + (1 + Drug|ID); signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

In order to check the effect of plasma OT level on accuracy, we compared a model restricted to the participants with plasma OT information and a second one similar to the first one but including plasma OT levels as predictor. Adding plasma OT levels did not significantly improve the model fit ($\chi^2_{(14)} = 2.33$; $p = 0.13$). Moreover, the main effect of plasma was not significant ($\beta = 0.05$, SE = 0.04, $p = 0.16$; see

Table 5-4 and

Table 5-5).

5.5.3 Discrimination Task

	β	Std. Error	z value	*p* value	
(Intercept)	0.097	0.107	0.904	0.366	
Drug	0.236	0.140	1.686	0.092	.
Units per week	-0.002	0.005	-0.346	0.730	
HR	-0.013	0.006	-2.043	0.041	*
STAI	-0.010	0.007	-1.511	0.131	
BDI	-0.004	0.008	-0.435	0.664	
TAS-20	0.001	0.008	0.170	0.865	
Sequence	0.032	0.137	0.236	0.813	
Drug*Unit	0.018	0.008	2.239	0.025	*

Table 5-6 Mixed-effects regression model to explain accuracy on discrimination task.

This regression using predictors for oxytocin/placebo (drug), alcohol intake (units per week), and their interaction, with heart rate (HR), anxiety (STAI), depression (BDI), alexithymia (TAS-20) and drug sequence as control variables, including all participants. Formula: Accuracy ~ Drug * Units per week + HR + STAI + BDI + TAS-20 + Sequence + (-1 + Drug|ID); signif. codes: 0.01 '*' 0.05 '.' 0.1

Results of the mixed-effects regression model, for the (interoceptive/exteroceptive, cross-modal) discrimination task, are presented in

Table 5-6. Crucially, we found a significant interaction between drug and units of alcohol (β = 0.02, SE = 0.01, *p* = 0.025): The more alcohol drank, the more OT increases interoceptive accuracy compared to placebo (Figure 5.1).

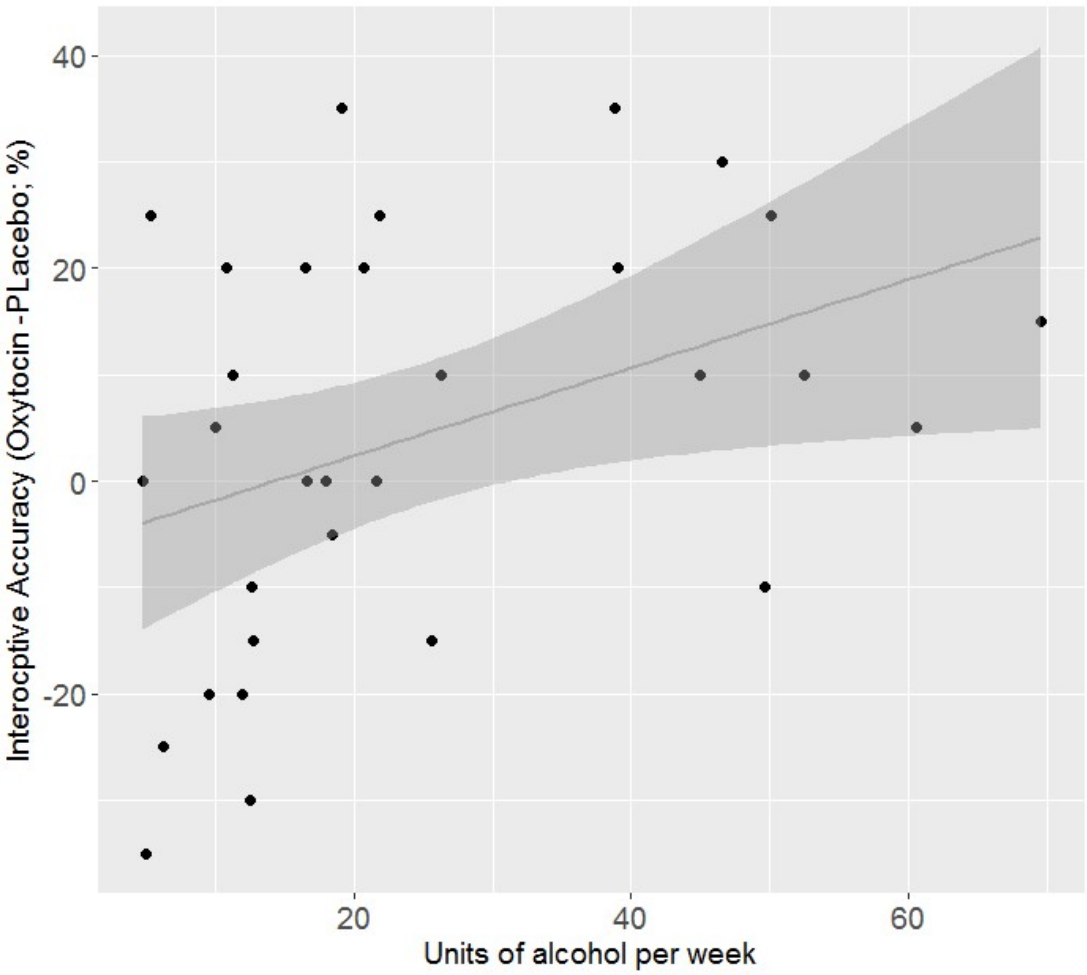


Figure 5.1Scatterplot illustrating the interaction between Drug (Oxytocin/Placebo) and alcohol units per week on interoceptive accuracy during the heartbeat discrimination task.

The y-axis is displaying the difference between interoceptive accuracy under oxytocin and under placebo, in percentage. The shaded area represents the standard error.

We also found a trend of main effect of drug on accuracy, with greater accuracy under OT than under placebo (β = 0.23, SE = 0.14, *p* = 0.092). A significant main effect of heart rate was also observed: participants with lower heart rate were more accurate than participants with higher heart rate (β = -0.01, SE = 0.01, *p* = 0.041).

No main effect of anxiety, depression, and alexithymia or OT/placebo sequence was observed.

	β	Std. Error	z value	*p* value	
(Intercept)	0.075	0.111	0.670	0.503	
Drug	0.255	0.147	1.732	0.083	.
Unit	-0.002	0.005	-0.493	0.622	
HR	-0.013	0.007	-2.009	0.045	*
STAI	-0.011	0.007	-1.524	0.128	
BDI	-0.005	0.009	-0.537	0.591	
TAS	0.003	0.008	0.321	0.748	
Sequence	0.065	0.142	0.460	0.646	
Drug*Unit	0.022	0.009	2.483	0.013	*

	β	Std. Error	z value	*p* value	
(Intercept)	0.082	0.111	0.738	0.460	
Oxytocin/placebo (drug)	0.245	0.144	1.698	0.089	.
Units per week	-0.001	0.005	-0.292	0.771	
HR	-0.010	0.007	-1.470	0.141	
STAI	-0.011	0.007	-1.545	0.122	
BDI	-0.007	0.009	-0.866	0.387	
TAS-20	0.001	0.008	0.141	0.888	
Sequence	0.057	0.139	0.412	0.680	
Plasma	-0.217	0.130	-1.666	0.096	.
Drug*Unit	0.021	0.008	2.525	0.012	*

Table 5-7 Mixed-effects regression model to explain accuracy on discrimination task.

This regression is using predictors for oxytocin/placebo (drug), alcohol intake (units per week), and their interaction, with heart rate (HR), anxiety (STAI), depression (BDI), alexithymia (TAS-20), drug sequence and plasma OT level as control variables, restricted to participants with plasma OT data. Formula: Accuracy ~ Drug * Units per week + HR + STAI + BDI + TAS-20 + Sequence + Plasma + (-1 + Drug|ID); signif. codes: 0.01 '*' 0.05 '.' 0.1

In order to check the effect of plasma OT level on accuracy, we compared one model, restricted to the participants with plasma OT information, to a second model, similar to the first one but including plasma OT levels as predictor. Adding plasma OT levels did not significantly improve the model fit ($\chi^2_{(11)} = 2.74$; $p = 0.10$). Moreover, the main effect of plasma was not significant ($\beta = -0.21$, SE = 0.13, $p = 0.10$; **Error! Reference source not found.** &Table 5-7).

5.6 Discussion

In the present study, we examined interoceptive processing in alcohol users and characterised the impact of intranasal OT on these processes.

5.6.1 Main findings

Our main findings were that OT administration was associated with a reduction of interoceptive accuracy on the tracking task. However, it tended to be associated with an increase of interoceptive accuracy on the discrimination task. Moreover, we found a significant interaction between OT and alcohol intake on the discrimination task: Compared to placebo, OT administration was associated with improved interoceptive accuracy in heavy drinkers, but not in mild social drinkers. Interestingly, we did not find any main effect of units of alcohol on either interoceptive tasks; indicating that (non-clinical) alcohol consumption alone did not seem to be a strong predictor of interoceptive accuracy. Unlike alcohol-dependent patients, these alcohol users do not seem to be markedly impaired in interoceptive accuracy (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). However, interaction between alcohol intake and OT was observed independently of heart rate, alexithymia, anxiety or depression (as we controlled for these potential confounds). Moreover, the fact that this interaction was found only in the discrimination task (and not in the tracking task) suggests that OT does not have a general or fundamental impact on interoceptive processing.

Indeed, while these two tasks are often used interchangeably in the objective measurement of interoception, heartbeat discrimination and heartbeat tracking tasks involve different cognitive mechanisms (Garfinkel *et al.*, 2015, Garfinkel *et al.*, 2016b). Heartbeat tracking requires the participant to focus only on his/her internal cardiac sensations, whereas performance of the heartbeat discrimination task requires the participant to attend flexibly to, switch perception between, and integrate external (e.g. sound) and internal (e.g. actual heartbeat) cues. Therefore, in the discrimination task, this integration of both internal and external sensorial information is crucial for making accurate judgments of synchronicity. While specific studies of the integration of internal and external cues have yet to be undertaken in alcohol use disorders, there is evidence to suggest that the multimodal integration of emotional information is impaired in alcohol-dependent individuals (Maurage *et al.*, 2009, Brion *et al.*, 2017). For example, electroencephalography reveals a reduced amplitude and increased

latency of event-related potentials during cross-modal emotional processing in alcohol-dependent patients. However, this deficit is not observed in binge drinking suggesting that sensory integration processes are more vulnerable to chronic alcohol use compared to more acute alcohol intoxication or other drinking patterns (Maurage *et al.*, 2008, Brion *et al.*, 2017).

The ability to switch between internally and externally focused attention is evoked by the discrimination task. This capacity is impaired in specific psychiatric disorders, including obsessive and compulsive disorders (Stern *et al.*, 2017). A cognitive-physiological theory of 'alcohol myopia' suggests that alcohol allows drinkers to narrow their perceptual abilities in order to focus in more immediate and salient aspects of experience (e.g. externally-oriented thoughts) (Steele and Josephs, 1990, Fairbairn and Sayette, 2013). This is supported by the recent demonstration that alcohol disrupts key nodes within the salience network, suggesting compromised internal monitoring (Padula *et al.*, 2011, Gorka *et al.*, 2017, Grodin *et al.*, 2017). Interestingly, intranasal OT is proposed to direct attentional resources from internal to external cues, through concurrent modulation of functional connectivity within the ventral attention network and salience network (Abu-Akel *et al.*, 2015, Shamay-Tsoory and Abu-Akel, 2016, Brodmann *et al.*, 2017, Yao *et al.*, 2017). These elements, plausibly account for the different directions of OT effects on the two interoceptive tasks, and inform understanding of the potential mechanism through which OT enhances interoceptive discrimination accuracy in heavy drinkers compared to mild social drinkers.

The alcohol myopia theory suggests that heavy drinkers are biased toward the processing of interoceptive cues and consequently experience difficulty in switching attention to exteroceptive cues Heavy drinkers, before marked alcohol-induced neurodegeneration, may not be overtly impaired and might employ compensatory mechanisms to manage deficits in attentional switching. However, we expect deficits become exacerbated and pathological with prolonged heavy drinking. The expression of such deficits may be mitigated by OT: As already mentioned, intranasal OT increases attentional resources toward exteroceptive stimuli. Correspondingly, we showed OT-induced reduction of accuracy during interoceptive tracking. Indeed, since the tracking task mainly involves internally focused attention (Garfinkel *et al.*, 2015), OT may directly weaken attentional resource allocation toward bodily sensations, compromising interoceptive performance, and perhaps boost the capacity to switch

from internal to external focused attention. Alternatively, by increasing externally focused attention, OT may potentiate the integration of internal-external inputs. These complementary mechanisms can also explain why OT tended to increase interoceptive accuracy on the discrimination task and why heavy drinkers, potentially less able to switch between internal and external cues, show a greater benefit from OT administration than mild social drinkers.

5.6.2 Limitations

The results of this study should be considered in light of several limitations. First, low heart rate is associated with increased accuracy on cardiac interoceptive tasks, an effect that was also observed on both heartbeat tracking and discrimination tasks. Relatedly, 'beliefs' about heart rate may further influence heartbeat counting performance (Ring and Brener, 1996, Ring *et al.*, 2015). Here, this issue was managed by including heart rate as a co-variable in analyses to account for its bias on cardioception. A second limitation of the study is the generalisation of our findings to other dimensions of interoception. Even if it has been shown that cardioception aligns with sensitivity to gastric functions (Herbert *et al.*, 2012), it would be interesting to investigate this relationship in alcohol use disorders. The final limitation was the study sample was composed only of males (to avoid menstrual cycle variability within our within-subject design). Further studies are needed to verify if our findings regarding OT modulation of attentional resources are generalizable to females. Finally, we measured basal plasma OT level to account for inter-individual differences, however knowledge of the relationship between central and peripheral endogenous oxytocin, and pharmacokinetic aspects of intranasal oxytocin action remain rudimentary (Quintana *et al.*, 2015, Valstad *et al.*, 2016, Valstad *et al.*, 2017).

5.6.3 Conclusion

In conclusion, this study is, to date, the first study to examine the impact of OT on interoceptive performance accuracy in relation to non-clinical alcohol use. Our results do not suggest pure interoceptive impairment in alcohol users, but instead highlight a potential reduced flexibility of attentional resource allocation between internal and external cues; importantly, this impairment might be mitigated by acute intranasal oxytocin intake.

In the next chapter, we will explore if intranasal oxytocin administration modulates functional brain activity in function of visceral input.

Chapter 6 Oxytocin, interoception and empathy-for-pain: A neuroimaging study[4]

[4] As first author I designed the study and undertook all stages of data collection analyses and interpretation with the guidance of my senior colleagues and the assistance of Dr Pfeifer. In the text that follows, as in the manuscript in preparation, this group contribution is acknowledged in my use of plural personal pronouns.

6.1 Abstract

The emotional feeling associated with empathy for other people observed to be in pain states is likely to be underpinned by interoception. Interoception is classically defined as the central representation of internal states. The processing of emotional stimuli and one's own pain is modulated by interoceptive cardiac signals that occur phasically; Arterial baroreceptor discharges at systole communicate the strength and timing of individual heartbeats. Here, we tested whether these phasic interoceptive signals modulate empathy-for-pain. Moreover, the hormone oxytocin (OT) is proposed to enhance empathy and to modulate the processing (precision-weighting) of interoceptive, so we further tested whether OT administration impacts empathy-for-pain via interoceptive mechanisms.

Male subjects (N=32) attended three sessions to perform psychometric tests and an fMRI empathy-for-pain task, after intranasal administration of OT or placebo (40 units (IU)). Pictures of hands in painful or non-painful context were presented at systole or diastole. Effects of drug, emotion and cardiac timing on behaviour and brain activity was tested using general and mixed-effects linear models.

Across conditions, activation was observed within regions implicated in pain and empathy-for-pain, with greater signal in the right compared to the left insula. OT administration, compared to placebo, attenuated the neural reactivity within some regions, including anterior cingulate cortex. However, the attenuating effect of OT was blocked by stimulus presentation at cardiac systole i.e. during interoceptive baroreceptor signalling.

Our data suggest that OT alters the processing of salient social cues, interacting with interoceptive signals. Our findings may inform targeted use of OT in psychiatric conditions linked to aberrant interoceptive processing.

Keywords Affective neuroscience, Emotion, Empathy for pain, fMRI, Insula, Interoception Oxytocin

6.2 Introduction

Empathy is crucial to social cognition and is expressed for (higher-order) thoughts and emotional feeling states and for lower-level physical sensations such as pain (Decety and Jackson, 2004, Singer *et al.*, 2004, Kanske *et al.*, 2017). Within the brain the empathic processing of another's pain engages a network of regions called the empathy-for-pain matrix, which encompasses the dorsal anterior/middle cingulate cortex (ACC, MCC) and bilateral insulae (for review Lamm *et al.*, 2011).

Interoception is hypothesised to underpin empathy: people with poor interoceptive accuracy (Garfinkel *et al.*, 2015) have difficulty in labelling their own emotions, in recognizing emotional facial expressions, and in inferring the mental state of others (Terasawa *et al.*, 2014, Betka *et al.*, 2017, Bornemann and Singer, 2017, Shah *et al.*, 2017). Moreover, people with better interoceptive accuracy tend to express an enhanced capacity for imitating others, and show an increased sensitivity/decreased tolerance to pain (Pollatos *et al.*, 2012, Ainley *et al.*, 2014). These findings are consistent with the postulation that empathic understanding arises from a simulation (interoceptive prediction) of likely internal bodily state, requiring the integration of subsequent interoceptive afferent signals into emotional representations of both self and other. The insular cortex is proposed to be the neural substrate in charge of such integration (Singer *et al.*, 2009).

Heartbeats are a very important source of interoceptive sensations which influence our behaviour (Łukowska *et al.*, 2018). The processing of brief stimuli can be modulated by presentation on and off the heartbeat. Though the literature remains mixed, two trends seem to occur. Some studies find that the timing of a visual or nociceptive stimulus in synch with, or at the same frequency as, the heartbeat will supress its processing (Lacey and Lacey, 1970, Walker and Sandman, 1979, Walker and Sandman, 1982, Eckberg and Sleight, 1992, Mini *et al.*, 1995, Angrilli *et al.*, 1997, Gray *et al.*, 2009, Gray *et al.*, 2010). Within the brain, a deactivation of the insular cortex is observed when visual stimulation is delivered at the cardiac frequency (Salomon *et al.*, 2016). In contrast, the perceived emotional intensity of facial expressions of disgust and fear is enhanced by presentation at systole (e.g. during natural baroreceptor activation), with corresponding changes in reactivity of affective brain circuitry (Gray *et al.*, 2012, Garfinkel *et al.*, 2014).

Oxytocin (OT) is a neuropeptide hormone with a major role in mother-child bonding. OT also contributes more generally to prosocial behaviours across mammalian species (Kendrick *et al.*, 1987, Carter, 2003, Young and Wang, 2004, Parker *et al.*, 2005). In humans, intranasal administration of OT can increase trust and attention toward social stimuli including faces (Guastella *et al.*, 2008, Keri and Kiss, 2011). Moreover, OT increases sensitivity (recognition and detection) of facial emotions (Schulze *et al.*, 2011, Leknes *et al.*, 2013). Intranasal OT administration improves inferences about others' social emotions, i.e. the cognitive component of empathy (Domes *et al.*, 2007b, Aoki *et al.*, 2014) and, across individuals, endogenous (salivary) OT levels correlate positively with both objective and subjective measures of emotional competence and interpersonal skills (Koven and Max, 2014).

Behaviourally, OT can also enhance empathy-for-pain (e.g. when adopting the other but not self perspective; Abu-Akel *et al.*, 2015) and extend empathy beyond its usual in-group bias (Shamay-Tsoory *et al.*, 2013). Within the brain, only two studies have explored the impact of intranasal OT on vicarious pain processing: the first study did not observe an OT-induced effect on neural correlates of empathy-for-pain, whereas the second study observed OT-induced deactivation within secondary somatosensory cortices, insula, and MCC (Singer *et al.*, 2008, Bos *et al.*, 2015). Given the postulate that empathy-for-pain arises from interoceptive afferent signalling, one possible explanation is that (intranasal) OT reduces the salience of interoceptive signals (Louvel *et al.*, 1996, Quattrocki and Friston, 2014, Yao *et al.*, 2017, Betka *et al.*, 2018).

6.3 Aims and hypotheses

In this study, we sought to characterise the impact of phasic visceral feedback on empathy-for-pain processing. Within the empathy-for-pain matrix, we expected an attenuation of neural responses at ventricular systole (during baroreceptor activity) relative to diastole (baroreceptor inactivity) when viewing painful pictures compared to non-painful pictures.

The investigation of how interoceptive signals modulate the neural correlates of empathy further provides a novel opportunity to investigate the impact of intranasal OT on viscerosensory processes. Specifically, we predicted that OT administration will modulate the impact of cardiac timing on empathy for pain, compared to placebo.

Based on earlier literature, we hypothesized that OT will decrease the activation of the empathy-for-pain matrix. However, taking into account the hypothesis that OT attenuates interoceptive precision, we predicted that intranasal OT would attenuate the degree of cortical inhibition induced by baroreceptor activity at systole. Thus, activity within the empathy-for-pain matrix will be reduced by OT, but these effects will be primarily visible at diastole and attenuated by systolic baroreceptor effects.

6.4 Methods summary

Full methodological details are described in Chapter 2, section 2.2.

6.4.1 Procedure and participants

To test our hypotheses, thirty-two male volunteers (mean age 25.1 years; range 18–36 years) took part in the experiment.

Each participant came three times to the laboratory. The first session was a baseline session during which psychometric/demographic information and a blood sample were collected. During the second and the third sessions, participants performed an empathy-for-pain paradigm within the scanner during fMRI, after intranasal spray administration of 40 units (IU) of oxytocin or after placebo. The drug/placebo sequence was randomized for each participant, the experimenter was double-blind.

The empathy task consisted of pictures illustrating a hand, either in a painful or in a non-painful context (Jackson *et al.*, 2005). Each picture was presented twice, once at diastole and once at systole. Correspondingly, the design was a 2x2x2 repeated measures design with 2 levels of emotion (pain, no pain), 2 levels of cardiac cycle (systole/diastole), and 2 levels of drug (oxytocin/placebo).

The empathy-for-pain task involved the presentation of (1) a fixation cross (3000ms), (2) a picture (150ms) presented at either systolic or diastolic phase of cardiac cycle, followed by a jitter of 1, 3 or 5s, and (3) a visual analogue scale (VAS; 3000ms). During the time of presenting the VAS, participants were asked to rate “How painful was the picture?”, using a scale of 0 (not painful at all) to 100 (very painful). See Figure 6.1A-B for a histogram detailing the precision of stimulus presentation in relation to cardiac cycle in the experiment conducted within the MRI scanner and Figure 6.1C for a visual depiction of the paradigm.

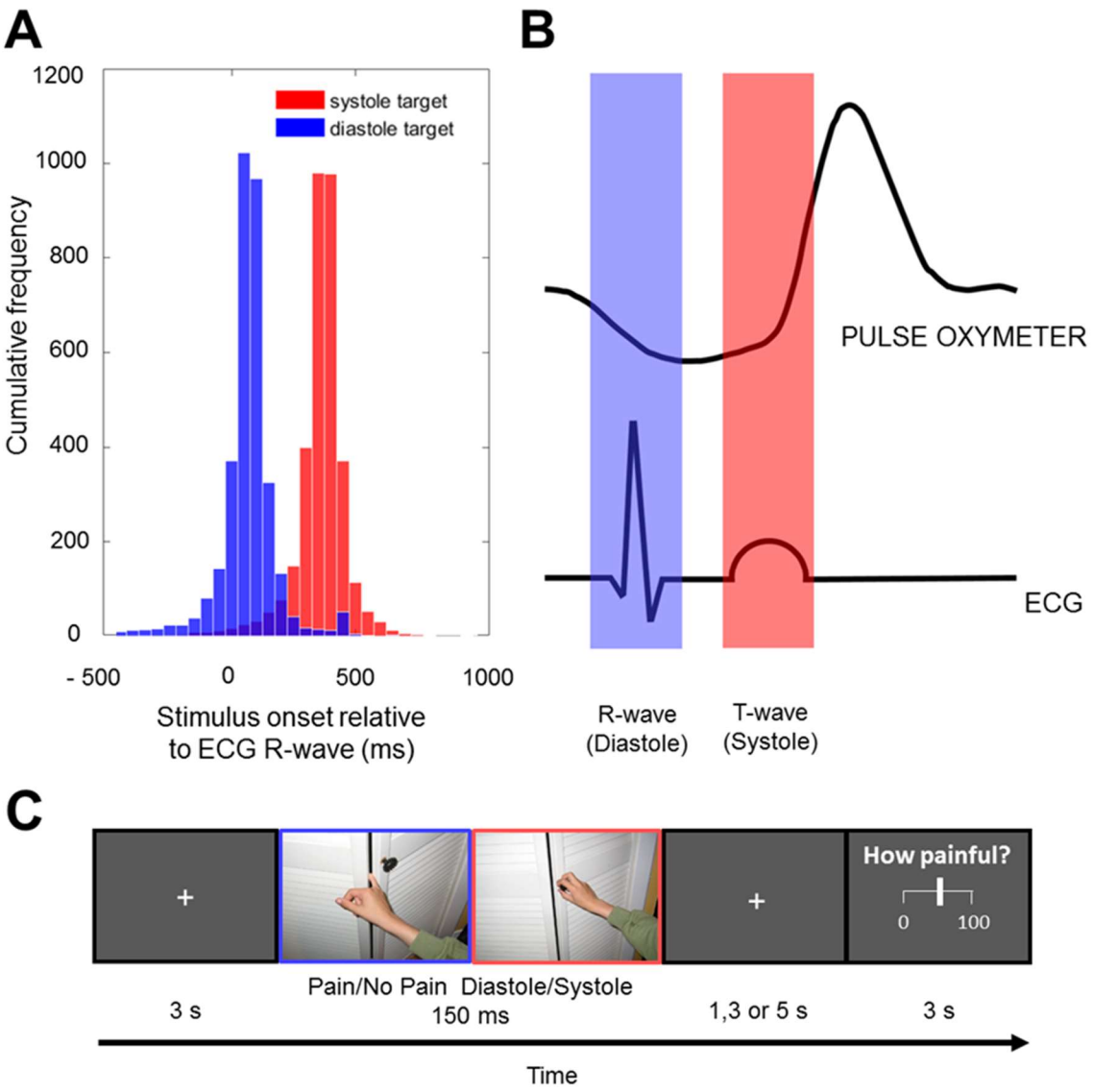


Figure 6.1 Empathy-for-pain paradigm and cardiac timing manipulations.

(A) Histogram detailing pictures presentation timing in relation to cardiac cycle within the MRI scanner. (B) Stimulus presentation was time-locked to coincide with two distinct points of the cardiac cycle: cardiac diastole in blue (ECG R-wave) and cardiac systole in red (ECG R-wave + 300ms, equivalent to ECG T-wave). (C) For the empathy-for-pain task, pictures of hand (painful context or non-painful context) were time-locked to diastole or systole. Participants made subsequent trial-by-trial pain intensity rating using a VAS.

6.4.2 Statistical Analyses

6.4.2.1 Behavioural data

At the behavioural level, the analysis of pain ratings used a linear mixed-effects model, since the outcome was continuous, and was conducted using the lme4 package (Bates *et al.*, 2015) in the R environment (version 3.4.2; RCoreTeam, 2013). P values were computed using lmerTest package (Kuznetsova *et al.*, 2014).

Ratings were analysed with drug (2 levels: Placebo=0; OT=1), emotion (2 levels: No Pain=0; Pain=1), Cardiac Timing (2 levels: Diastole=0; Systole=1) and their interactions, as fixed factors. The intercept reflected the average pain rating in the placebo no pain diastole condition. Participants were treated within the analysis as a random factor.

This basic model was then compared to a similar model that also included anxiety, depression, alexithymia, alcohol intake, sequence, interbeat interval, jitter and plasma OT levels to test confounding effects of these variables. We contrasted the goodness of fit of these models using likelihood ratio tests.

6.4.2.2 Functional data

At the neuroimaging level, functional brain images were pre-processed (including realignment and projecting into standard space). At the first level, task events were analysed in a general linear model composed of two sessions (OT and Placebo). These were entered into a full factorial second-level analysis, with drug, emotion and cardiac timing as non-independent (repeated measures) factors.

Contrasts were generated to test for overarching experimental effects (F contrast: [identity matrix]), for drug effects for both emotion and cardiac timing (Oxytocin Pain Systole; Oxytocin Pain Systole; Oxytocin No Pain Systole; Oxytocin No Pain Systole; Placebo Pain Systole; Placebo Pain Systole; Placebo No Pain Systole; Placebo No Pain Systole), drug effect (Placebo > Oxytocin), emotion effect (Pain > No Pain), and cardiac effect (Diastole > Systole ; T contrasts). Some contrasts were generated to test for interactions (PL(PvsNP) > OT(PvsNP) ; PL(PDvsNPD) > PL(PSvsNPS); OT(PSvsNPS) > OT(PDvsNPD)).

Statistic images were thresholded at an initial cluster-forming threshold of $p<0.001$ for cluster-wise False Discovery Rate (FDR) correction for multiple comparisons at $p<0.05$ (Chumbley and Friston, 2009). Significant clusters were localised according to the Anatomy toolbox (v 2.2b, Eickhoff *et al.*, 2005). Contrast estimate effect size plots were generated in SPM for each condition, at the peak coordinate of significant (empathy-for-pain related) regions according to the F test for all effects.

6.5 Results

6.5.1 Psychometric, plasma and physiological measures

	Minimum	Maximum	Mean	Std. Deviation
TAS-20	36.00	74.00	55.34	9.61
Units of alcohol per week	4.50	69.50	24.18	18.16
BDI	0.00	48.00	12.72	9.84
STAI	22.00	66.00	47.88	12.15
Baseline plasma OT levels (pg/mL)	0.95	3.80	1.72	0.72
Mean Interbeat Interval* (ms; Placebo session)	715.36	1279.86	940.58	152.76
Mean Interbeat Interval* (ms; Oxytocin session)	644.64	1273.09	895.93	153.18

Table 6-1 Psychometric measures; *During the task

Means, standard deviations, and ranges of psychometric measures, basal plasma OT level as well as mean interbeat interval were computed for both tasks (

Table 6-1).

6.5.2 Drug impact on cardiovascular activity during the task

	β	SE	df	t	*p*	
Intercept	940.589	27.005	31.003	34.831	0	***
Oxytocin	-44.656	19.037	31.013	-2.346	0.026	*

Table 6-2 Mixed-effects linear model assessing predicting the effect of oxytocin on interbeat interval (IBI) duration.

The model includes IBI as outcome, drug as fixed factors, and participants as random factor, with random correlated intercepts and slopes, in function of the drug. The intercept reflects the average IBI in the placebo condition.

A main effect of drug on the IBI was observed: OT reduced the IBI compared to placebo (i.e. increased heartrate; β = -44.66; SE = 19.04; t = -2.346, *p* = 0.0256,

Table 6-2).

6.5.3 Behavioural performance

	β	SE	df	t	*p*	
Intercept	19.363	2.475	34.392	7.823	0.000	***
Drug	-0.267	1.371	67.468	-0.195	0.846	
Emotion	43.582	0.905	8046.889	48.169	0.000	***
Cardiac	0.89	0.903	8047.142	0.985	0.324	
Emotion*Cardiac	1.661	1.273	8046.869	1.305	0.192	
Drug*Cardiac	-0.443	1.272	8046.997	-0.348	0.728	
Emotion*Cardiac	-1.853	1.278	8047.023	-1.449	0.147	
Drug*Emotion* Cardiac	0.629	1.8	8046.936	0.35	0.727	

Table 6-3 Mixed-effects linear model predicting changes in pain rating.

The model includes drug (2 levels: Placebo=0; OT=1), emotion (2 levels: No Pain =0; Pain=1), Cardiac Timing (2 levels: Diastole=0; Systole = 1) and their interactions, as fixed factors; participants, as random factor The intercept reflects the average pain rating in the placebo no pain diastole condition.

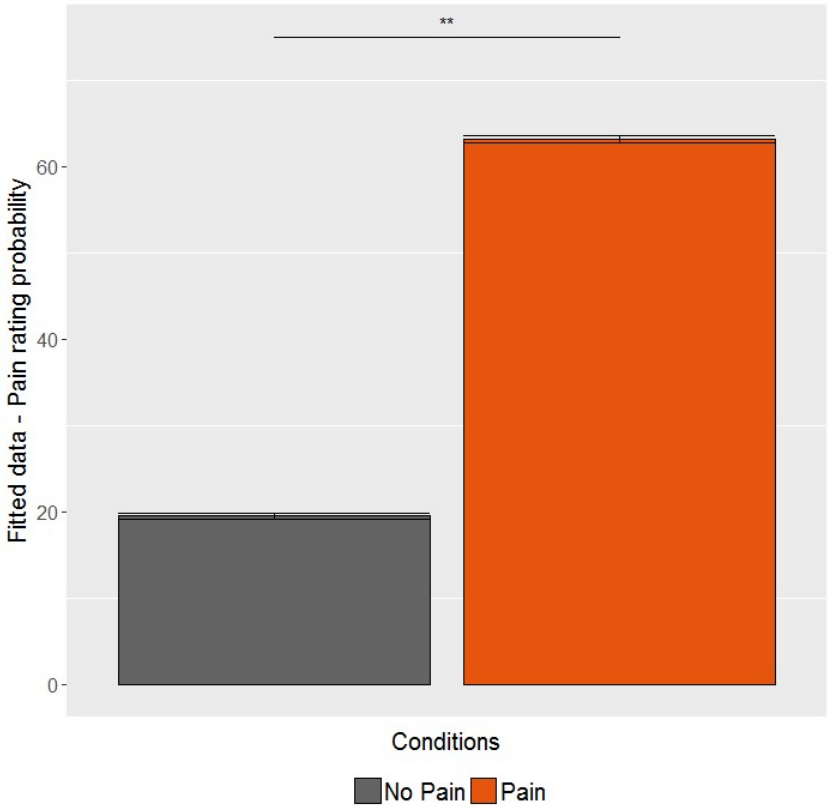


Figure 6.2 Bar plot with error bars demonstrating the pain effect of emotion on pain rating: pain stimuli are rated more highly than no pain.

($p < 0.01$).

A significant main effect of emotion was observed. Painful pictures were rated as more painful than non-painful pictures (see Figure 6.2; $p < 0.001$,

Table 6-3). No effect of drug or cardiac timing was observed. The addition of control variables (anxiety, depression, alcohol units per week, alexithymia, sequence, interbeat interval, endogenous plasmatic OT or jitter) to the model did not significantly improve the goodness of fit (Basic model: AIC =72 103; Model with covariates: AIC = 72 106; comparison: χ^2 (8) = 13.402; $p = 0.098$).

6.5.4 Neuroimaging

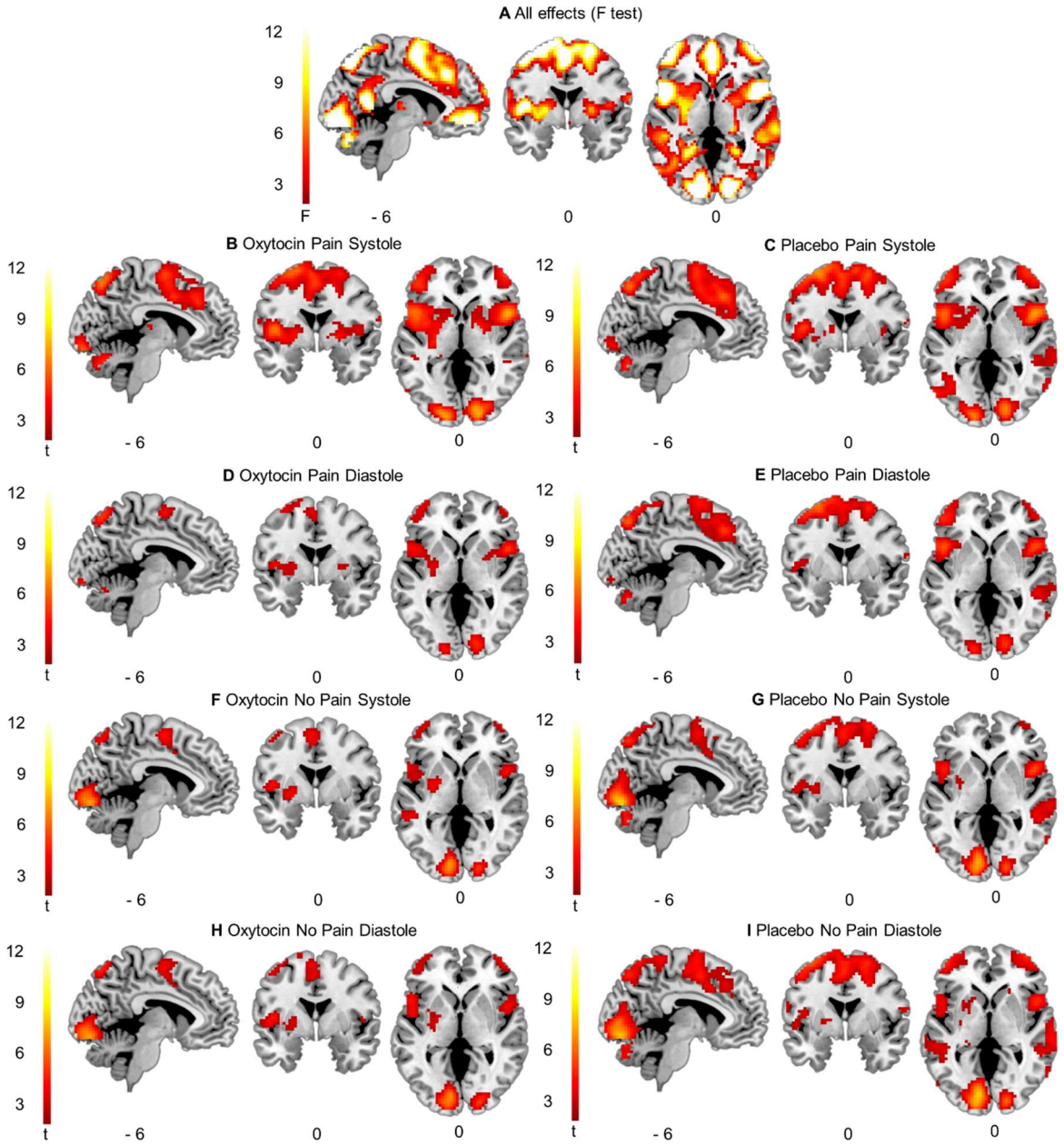


Figure 6.3 Activity during viewing pictures across experimental conditions.

Oxytocin globally reduces the cerebral activation compared to placebo, except in the pain systole condition. (A) F test of all experimental effects, (B) Oxytocin Pain Systole; (C) Placebo Pain Systole; (D) Oxytocin Pain Diastole; (E) Placebo Pain Diastole; (F) Oxytocin No Pain Systole; (G) Placebo No Pain Systole; (H) Oxytocin No Pain Diastole; (I) Placebo No Pain Diastole. All images thresholded at cluster-wise FDR $p < 0.05$ (cluster-forming threshold $p < 0.001$).

Activity associated with viewing of pain and no pain stimuli, presented at systole and diastole in both drug conditions, was examined using an F contrast testing for all experimental effects. Activations were observed across a canonical set of pain processing regions, including inferior, superior and middle frontal gyri, precentral and postcentral gyri, superior parietal lobule, cerebellum and precuneus. This network encompassed activation of the empathy-for-pain matrix, engaging anterior insulae (AI; extending to inferior frontal gyri); ACC and MCC. Also, brain areas were activated implicated in the representation of information concerning body parts e.g. fusiform gyrus, (Figure 6.3A, Supplementary Table 6-4).

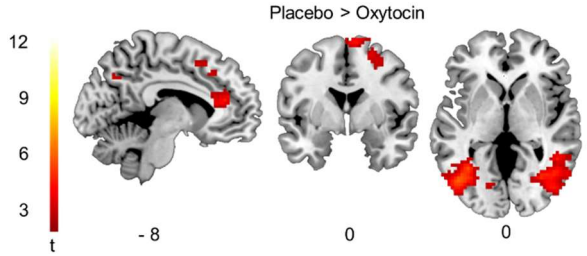


Figure 6.4 Activity during all experimental conditions under placebo minus all experimental conditions under oxytocin.

All images were thresholded at cluster-wise FDR p<0.05 (cluster-forming threshold p<0.001).

Examining the experimental effects for emotion and cardiac timing for OT and placebo (Oxytocin Pain Systole; Oxytocin Pain Diastole; Oxytocin No Pain Systole; Oxytocin No Pain Diastole; Placebo Pain Systole ;Placebo Pain Diastole; Placebo No Pain Systole; Placebo No Pain Diastole; Figure 2B, D, F, H, C, E, G, and I respectively; Supplementary Table 6-4) revealed activation in both drug conditions across precuneus, superior parietal lobule, superior frontal gyrus, lingual gyrus, occipital and frontal poles, cerebellum, as well as putamen. While bilateral AI were activated for all experimental conditions, these regions were especially activated by the pain systole condition. In addition, activation of the ACC was generally attenuated by OT, except during the pain systole condition. This effect was not observed under placebo. Direct testing confirmed that ACC was deactivated by OT, relative to placebo. Similarly, OT also deactivated adjacent paracingulate cortex, supplementary motor area, and the

superior frontal gyrus and lateral occipital cortex (Figure 6.4; Supplementary Table 6-4.J). All other whole-brain contrasts testing for drug, emotion, cardiac differences or interactions did not attain significance at corrected thresholds.

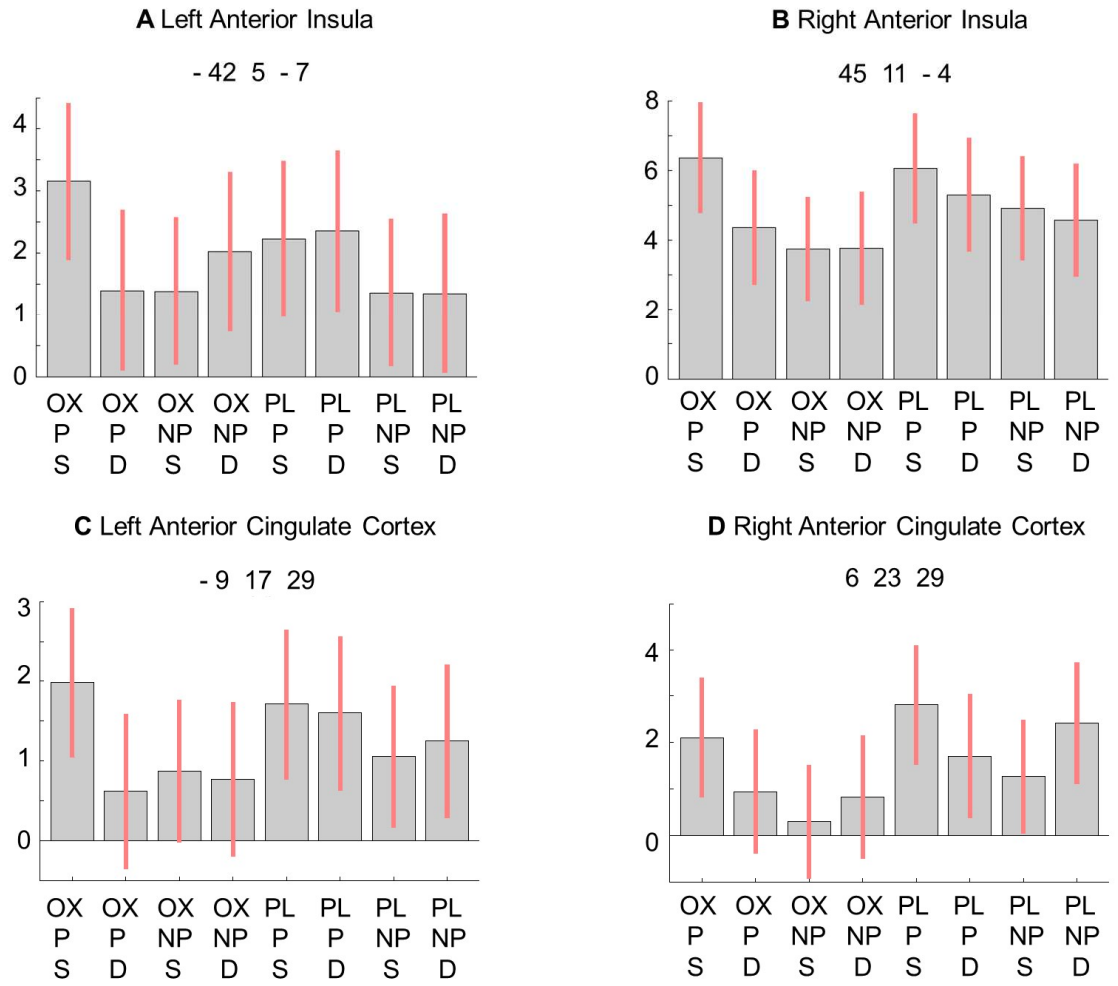


Figure 6.5 Contrast estimate effect size plots.

For the bilateral Anterior Insulae (A-B), and bilateral Anterior Cingulate Cortices (C-D), respectively, for (left-to-right) Oxytocin Pain Systole (OXPS); Oxytocin Pain Diastole (OXPD); Oxytocin No Pain Systole (OXNPS); Oxytocin No Pain Diastole (OXNPD); Placebo Pain Systole (PLPS); Placebo Pain Diastole (PLPD); Placebo No Pain Systole (PLNPS), and Placebo No Pain Diastole(PLNPD).

Contrast estimate effect size plots, representing activity at the peak coordinates of bilateral AI and bilateral ACC clusters, identified according to the F test for all experimental effects, are presented in Figure 6.5. These reveal generally greater activity within right AI when compared to the left AI across all experimental conditions (Figure 6.5A &B). Under placebo, left AI showed increased activation for pain versus no pain condition, irrespective of cardiac timing. However, within right AI, this

differential response was less evident, suggesting a more linear relationship wherein pain induced more activation than the non-pain conditions, and systole induced more activation than the diastole conditions. Strikingly, under OT, the general activation in bilateral AI was reduced, except in the pain systole condition, where the activation was preserved or even increased. The pattern of activation within anterior cingulate cortex was less clear. However, in line with the previous results, bilateral ACC appeared more active in placebo conditions compared to OT conditions (Figure 6.5C &D). Indeed, OT reduces activation across the ACC, except within the left ACC during the pain systole. Nevertheless, these regional trends did not survive stringent significance threshold testing as formal interactions in whole-brain contrasts.

6.6 Discussion

In the present study, we first sought to characterise the impact of (phasic) interoceptive cardiac signals on empathy-for-pain processing. In addition, we investigated a novel question related to the impact of intranasal OT on the relationship between visceral feedback and empathic brain activations.

6.6.1 Main findings

We found that momentary states of cardiovascular arousal, occurring at systole when baroreceptors are active, are associated with decreases in activity within two key pain-matrix regions, the right anterior insula, and anterior cingulate cortex, when participants received intranasal OT. Although we observed predicted effects, many results did not attain threshold significance when corrected for multiple comparisons in whole brain imaging analysis when corrected for multiple comparisons, potentially due to a power issue. Nevertheless, three important observations can be emphasized. The AI appeared to be specifically engaged in the integration of emotive information with interoceptive signals (associated with cardiac timing). Intranasal OT was observed to generally reduce brain activations across experimental conditions. However, the activation associated with processing painful pictures at cardiac systole was unaffected by OT administration.

We observed a main effect of viewing the task stimuli, irrespective of cardiac timing or drug condition, wherein activity was elicited across cerebral areas previously implicated in processing and perception of pain. The empathy-for-pain matrix, composed of the bilateral AI and ACC/MCC, was thus activated. However, these areas also play a crucial role in salience attribution (Seeley *et al.*, 2007, Menon and Uddin, 2010), another less-specific account for the activation of these brain regions during our task. Indeed, participants were required to attend to the brief presentation of picture stimuli. As part of the “salience network” (Seeley *et al.*, 2007), the AI is hypothesized to detect the most relevant bottom-up events in both internal and external environments, and to temporarily initiate attentional control inputs, which are subsequently sustained by ACC (Menon and Uddin, 2010). Interestingly, activation of right AI was generally greater compared to the left AI, across all conditions. These data are also in line with the salience hypothesis, as the switching between (externally-

oriented) central-executive and (self-referential) default-mode networks involves specifically the right AI (Sridharan *et al.*, 2008).

In addition, the engagement of right AI in our experiment is likely to support the monitoring and appraisal of predicted visceral inputs which constitute a potential basis for selfhood (Craig, 2002, Critchley, 2004, Critchley *et al.*, 2004, Critchley and Seth, 2012, Babo-Rebelo *et al.*, 2016). It is proposed that posterior insular cortices bilaterally receive an initial representation of interoceptive information or physical pain, thereafter the second-order interoceptive representation is constructed within right AI, before projection onto prefrontal regions (Craig *et al.*, 2000, Brooks *et al.*, 2002, Craig, 2002). However, to allow for necessary switching between main cerebral networks, internal physiological inputs received by the (right) AI need to be integrated with the representation of external sensory information (Singer *et al.*, 2009). Recently, a growing body of evidence supports the crucial role of right AI in these integrative processes (Garfinkel *et al.*, 2014, Salomon *et al.*, 2016, Salomon *et al.*, 2018). Consequently, *via* our manipulation of stimulus presentation on and off the heartbeats, we engaged mechanisms enabling both interoceptive/exteroceptive integration and associated switching of cognitive resources, processes in which the right AI is specifically implicated.

Our second key finding was the OT-induced attenuation of cerebral activation: across all experimental conditions, intranasal OT, relative to placebo, was associated with decreased activation over cerebral areas including paracingulate cortex, supplementary motor area, superior frontal gyrus and lateral occipital division. The reduction in the reactivity of the ACC by OT was particularly noteworthy. This result extends evidence showing that intranasal OT suppresses anterior cingulate activity when processing emotional faces (Labuschagne *et al.*, 2012, Kanat *et al.*, 2015, Luo *et al.*, 2017) and reduces MCC activation during empathy-for-pain (Bos *et al.*, 2015). Interestingly, both ACC and MCC (regions of dorsal ACC) are characterized by the presence of Von Economo neurons; large projection neurons from cingulate, AI and specific frontal regions, that occur only in specific social mammals (Allman *et al.*, 2011). Based on their particularly concentrated presence in humans and great apes, it is postulated that these specialised pyramidal neurons play an important role in salience attribution, social cognition and even consciousness (Critchley and Seth, 2012). The dorsal cingulate, including MCC, is connected to cognitive and motor-

related areas, as well as thalamic nuclei and spinal cord. Rostrally, the ACC is increasingly also connected to 'limbic' structures involved in motivation behaviour and emotional responses (Stevens *et al.*, 2011). However, both these regions react to unpleasant/noxious stimulation (Vogt, 2005, Shackman *et al.*, 2011). Indeed, ACC encodes the motivational and affective dimension of pain, preparing behavioural responses to pain and other negatively-valenced stimuli (Morrison *et al.*, 2004). Consequently, reaction times are speeded when faced with a potentially noxious challenge (Morrison *et al.*, 2007). It is possible that the OT-induced attenuation of activation in the ACC might compromise the preparation and production of defensive actions. This hypothesis is consistent with observed analgesic effects of OT, modulating pain threshold and discomfort (Ohlsson *et al.*, 2005, Rash *et al.*, 2014), and corresponding autonomic reactions coordinated through ACC activation (Critchley *et al.*, 2003). Therefore, the observed ACC deactivation might be a signature of reduced sympathetic activity after OT administration (Norman *et al.*, 2011).

Our third finding is that, while OT reduced activation within the empathy-for-pain matrix, this effect was blocked when viewing pain stimuli at systole. At ventricular systole, arterial baroreceptors are activated by phasic ejection of blood from the heart (Bronk and Stella, 1932). A suppression of cortical excitability specific to baroreceptor activation is widely acknowledged within the literature (Lacey and Lacey, 1970, Droste *et al.*, 1994, Angrilli *et al.*, 1997, Edwards *et al.*, 2001). Indeed, presentation of stimuli time-locked with baroreceptor activation typically decreases activation of the insula, via the interoceptive pathway (Gray *et al.*, 2009, Makovac *et al.*, 2015). Stimuli occurring at the same frequency as the heartbeat are also inhibited (Salomon *et al.*, 2016). Interestingly, there is evidence to suggest that OT elicits a reduction in interoceptive processing (Louvel *et al.*, 1996, Ohlsson *et al.*, 2005, Black *et al.*, 2009, Engle *et al.*, 2012). Indeed, intranasal OT administration can decrease visceral symptoms in patients with irritable bowel syndrome (Louvel *et al.*, 1996) and reduce objective performance accuracy on a heartbeat tracking task, via modulation of right AI activation (Yao *et al.*, 2017, Betka *et al.*, 2018). These observations are corroborated by work in animals showing that OT can reduce sensitivity to bladder distension (Black *et al.*, 2009, Engle *et al.*, 2012). One model proposes that OT modulates interoceptive precision, facilitating attention deployment toward external cues and enhancing associative learning between internal and external stimuli

(Quattrocki and Friston, 2014). By extension, OT, through amplification of interoceptive signal-to-noise, may enhance the processing of baroreceptor inputs, in the context of its usual suppression of cortical excitability. This effect would benefit the processing of emotional and salient external cues. Correspondingly, a clear pattern of preserved insular and cingulate activation during pain stimulation at systole was observed after OT administration. Further studies involving pharmacological manipulation and connectivity analyses might usefully explore this potential mechanism.

6.6.2 Limitations

The present study should be considered in light of several constraints. First, our empathy-for-pain paradigm used pictures of hands in painful and non-painful contexts. A more efficient way to tap into empathic cerebral responses of vicarious pain might have used more explicit cues informing about another's affective state. Indeed, this kind of paradigm elicits greater activation in cerebral structures associated with inferring and mentalizing other's mental states (Lamm *et al.*, 2011). A second limitation was that we did not screen our sample for atypical/variant pain processing, notably mirror-sensory or mirror-pain synaesthesia, (Grice-Jackson *et al.*, 2017a). Moreover, different subpopulations of 'pain responders' show distinct patterns of functional neural connectivity between AI and the temporoparietal junction (Grice-Jackson *et al.*, 2017a, Grice-Jackson *et al.*, 2017b). Finally, there are methodological considerations: Given the time constraint inherent to the combination of pharmacological manipulation and neuroimaging, we did not measure change in PTT after the drug administration. It is possible that OT modulated PTT, which may have impacted cardiac-contingent stimulus presentation. However, PTT correlates with systolic blood pressure, which is not modulated by acute intranasal OT (Rash and Campbell, 2014). Moreover, multiband scanning at a magnetic field of 1.5T constrained the statistical power of our study. Nevertheless, a clear pattern of preserved activation during pain stimulation under OT, at systole, emerged for AI and ACC. Higher MRI field strength and better statistical power may add further detail regarding wider neural correlates of this effect.

6.6.3 Conclusion

Our findings support a mediating role of right AI in interoceptive monitoring and salience attribution. Even so, OT seems to be a facilitator of empathy, yet, our data extends previous empirical evidence for an OT-induced neural deactivation and a disengagement of the empathy-for-pain matrix. Finally, a clear pattern of preserved activation within the insular and cingulate cortices emerged under OT, during pain stimulation at systole. Taken together, our data suggests that OT alters the relationship between afferent interoceptive signals and the processing of relevant external cues.

In the next chapter, we will explore more in depth how emotional stimulation might impact behaviour through changes in autonomic and visceral input.

6.7 Supplement section

Table 6-4 Local maxima of significant clusters per contrast.

Maxima are localised according to the Anatomy toolbox (V2.2b; 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, *F* / *t* = *F* / *t* stat at this voxel. (A-G) Peaks are listed at p<0.05 FDR cluster corrected (cluster-forming threshold: p<0.001). *No label in Anatomy toolbox.

Cluster	Region	Hemisphere	MNI coordinates			
			x	y	z	*F* / *t*
	A All effects (F test)					
1	Superior Parietal Lobule	R	15	-70	59	36.19
	Precuneus	L	-12	-70	56	34.89
	Cerebellum (Crus 1)	L	-15	-88	-13	32.44
	Lingual Gyrus	L	-15	-91	-4	31.07
	Fusiform Gyrus	L	-27	-49	-7	30.68
	SupraMarginal Gyrus	R	51	-34	50	28.27
	Middle Frontal Gyrus	R	39	41	26	27.51
	Precentral Gyrus	L	-42	-13	59	26.72
	Lingual Gyrus	R	18	-91	-1	26.63
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	17	26.18
	Occipital Pole*	R	48	14	-1	25.58
2	Middle Orbital Gyrus	L	0	56	-4	20.70
	Anterior Cingulate Cortex	L	0	38	2	15.43
	Middle Orbital Gyrus	L	-27	35	-10	11.30
	Superior Medial Frontal Gyrus	L	0	56	44	10.17
	Superior Medial Frontal Gyrus	L	0	59	38	9.69
	Superior Medial Frontal Gyrus	L	-3	62	35	8.97
	Frontal Pole*	L	-6	68	23	8.74
	Frontal Pole*	R	3	71	14	8.47
	Subcallosal Cortex*	L	0	11	-10	8.29
	Middle Orbital Gyrus	R	0	23	-4	7.51
	Superior Frontal Gyrus	L	-18	44	50	5.07
3	Precentral Gyrus	R	36	-16	68	13.12
	Precentral Gyrus	R	48	-16	59	6.56
	Postcentral Gyrus	R	30	-34	74	5.50
	Superior Frontal Gyrus	R	21	-16	77	5.19
	Precentral Gyrus	R	51	-13	56	4.94
	Postcentral Gyrus	R	27	-40	74	4.22
4	Middle Orbital Gyrus	R	30	35	-10	11.73
	B Oxytocin Pain Systole					
1	Precuneus	L	-12	-70	56	8.49
	SupraMarginal Gyrus	R	51	-34	50	8.20
	Lingual Gyrus	R	18	-88	2	7.64
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	17	7.62
	Frontal Operculum Cortex*	R	48	14	-1	7.58

	Precuneus	R	12	-67	59	7.55
	Superior Parietal Lobule	R	18	-73	56	7.49
	Lingual Gyrus	L	-15	-91	-13	7.42
	Middle Occipital Gyrus	L	-15	-94	-1	7.37
	Middle Frontal Gyrus	R	42	41	23	7.28
	Superior Parietal Lobule	R	27	-67	56	6.97
	C Oxytocin Pain Diastole					
1	Precuneus	L	-9	-70	59	6.13
	SupraMarginal Gyrus	R	51	-34	50	6.05
	Precuneus	R	12	-70	59	5.57
	Postcentral Gyrus	L	-48	-37	56	5.45
	Precentral Gyrus	L	-42	-22	62	5.11
	Inferior Parietal Lobule	L	-39	-55	50	4.77
	Inferior Parietal Lobule	L	-39	-43	41	4.11
	Inferior Parietal Lobule	L	-45	-40	41	3.87
	Angular Gyrus	R	36	-61	44	3.72
2	Lingual Gyrus	R	18	-88	2	5.46
	Cerebellum (VI)	R	15	-88	-10	5.01
	Cerebellum (Crus 1)	R	36	-58	-31	4.54
	Cerebellum (VI)	R	30	-61	-31	4.45
	Cerebellar Vermis (6)	R	6	-73	-16	4.06
	Cerebellar Vermis (7)	R	3	-73	-22	3.91
	Cerebellum*	R	27	-49	-28	3.88
	Cerebellum (IV-V)	R	9	-61	-16	3.81
	Cerebellum*	L	-6	-67	-22	3.65
3	Inferior Frontal Gyrus (p. Orbitalis)	R	51	14	-1	5.44
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	14	5.13
	Putamen	R	30	2	2	3.75
	Insular Cortex*	R	33	11	2	3.60
	Pallidum	R	24	-10	2	3.42
4	Frontal Operculum Cortex*	L	-48	11	-1	4.59
	Insula Cortex	L	-45	2	5	4.24
	Insula Cortex	L	-39	8	2	4.14
	Pallidum*	L	-27	-7	-4	4.07
	Putamen*	L	-33	-1	-1	3.77
	Inferior Frontal Gyrus (p. Opercularis)	L	-57	11	14	3.68
	Inferior Frontal Gyrus (p. Opercularis)	L	-60	14	20	3.55
5	Frontal Pole*	R	48	44	-13	4.60
	Middle Frontal Gyrus	R	45	47	20	4.15
	Middle Frontal Gyrus	R	39	41	26	4.03
	Inferior Frontal Gyrus (p. Triangularis)	R	51	35	29	3.81
	Middle Frontal Gyrus	R	45	53	8	3.73
6	Lingual Gyrus	L	-12	-91	-13	5.03
	Middle Occipital Gyrus	L	-18	-94	2	4.77
7	Cerebellum (Crus 1)	L	-42	-58	-31	5.10
	Cerebellum (Crus 1)	L	-33	-58	-31	4.97
8	Frontal Pole*	L	-39	59	2	4.19

	Middle Orbital Gyrus	L	-42	53	-4	4.04
	Middle Orbital Gyrus	L	-45	44	-4	4.04
9	Middle Frontal Gyrus	L	-33	50	23	4.42
	Middle Frontal Gyrus	L	-42	38	32	3.37
10	Precentral Gyrus	L	-33	-1	65	4.06
	Posterior-Medial Frontal	L	-12	5	74	3.75
	Superior Frontal Gyrus	L	-18	2	74	3.66
11	Posterior-Medial Frontal	L	-6	-4	62	4.44
	Medial Cingulate Cortex	L	-3	5	47	3.24
	D Oxytocin No Pain Systole					
1	Precuneus	L	-12	-73	56	5.48
	Postcentral Gyrus	L	-45	-13	59	5.44
	Superior Parietal Lobule	L	-24	-67	59	4.77
	Postcentral Gyrus	L	-48	-37	56	4.17
	Inferior Parietal Lobule	L	-48	-40	44	4.16
	Inferior Parietal Lobule	L	-36	-58	56	4.15
	Postcentral Gyrus	L	-48	-28	59	4.00
	Precentral Gyrus	L	-45	5	53	3.58
2	Cerebellum (VI)	L	-12	-85	-13	7.55
	Lingual Gyrus	L	-12	-88	-1	7.13
	Cerebellum (Crus 1)	L	-36	-55	-31	5.03
	Cerebellum (Crus 1)	L	-27	-70	-25	3.60
3	Superior Parietal Lobule	R	15	-67	62	6.92
	Superior Parietal Lobule	R	15	-73	59	6.91
	SupraMarginal Gyrus	R	51	-34	47	5.59
4	Middle Frontal Gyrus	R	39	41	26	4.98
	Frontal Pole*	R	42	59	2	4.14
	Middle Frontal Gyrus	R	42	29	35	3.74
	Middle Orbital Gyrus	R	45	53	-4	3.68
	Frontal Pole*	R	45	47	-13	3.68
	Middle Frontal Gyrus	R	39	56	17	3.41
5	Inferior Frontal Gyrus (p. Opercularis)	R	60	11	17	4.69
	Insula Cortex	R	48	11	-1	4.65
	Inferior Frontal Gyrus (p. Opercularis)	R	57	14	5	3.90
	Insular Cortex	R	45	2	11	3.26
6	Heschls Gyrus	L	-42	-25	17	5.16
	Middle Temporal Gyrus	L	-57	-37	2	4.31
	Middle Temporal Gyrus*	L	-48	-34	-1	4.20
7	Lingual Gyrus	R	21	-88	2	5.50
	Cuneus	R	21	-94	14	4.26
8	Middle Frontal Gyrus	L	-36	50	23	4.63
	Frontal Pole*	L	-45	50	-7	4.02
	Frontal Pole*	L	-39	59	2	3.91
	Frontal Pole*	L	-45	50	14	3.32
9	Insular Cortex	L	-45	-1	8	4.72
	Temporal Pole	L	-51	14	-4	3.89

10	Posterior-Medial Frontal	L	-6	-4	59	4.69
	MCC	L	-6	8	41	3.33
11	Insular Cortex*	L	-30	-4	-4	4.44
	Putamen	L	-24	-1	-1	4.24
12	Cerebellum (VI)	R	33	-52	-31	4.43
	E Oxytocin No Pain Diastole					
1	Cerebellum (Crus 1)	L	-12	-85	-16	7.68
	Lingual Gyrus	L	-9	-85	-1	6.89
	Lingual Gyrus	L	-12	-82	-4	6.88
	Lingual Gyrus	L	-12	-91	-4	6.84
	Superior Occipital Gyrus	L	-18	-91	11	6.00
	Cerebellum (Crus 1)	L	-39	-58	-28	5.11
	Cerebellum (Crus 1)	L	-45	-61	-28	4.97
	Cerebellum*	L	-18	-67	-31	4.12
2	Precentral Gyrus	L	-42	-13	56	5.74
	Precentral Gyrus	L	-30	-22	68	4.89
	Postcentral Gyrus	L	-48	-31	56	4.57
	Postcentral Gyrus	L	-45	-40	59	4.41
	Precentral Gyrus	L	-30	-34	68	4.25
	Middle Frontal Gyrus	L	-39	11	53	3.85
	Middle Frontal Gyrus	L	-39	14	44	3.82
	Middle Frontal Gyrus	L	-45	17	47	3.77
	Superior Frontal Gyrus	L	-15	2	74	3.74
	Superior Frontal Gyrus	L	-15	-7	77	3.68
	Middle Frontal Gyrus	L	-36	8	59	3.64
3	Precuneus	R	12	-70	59	6.98
	Superior Parietal Lobule	R	15	-76	56	6.96
	Precuneus	L	-9	-70	59	5.58
	Precuneus	L	-12	-73	56	5.48
	Superior Parietal Lobule	L	-24	-67	59	4.62
	Inferior Parietal Lobule	L	-39	-58	50	3.61
	Angular Gyrus	L	-36	-61	41	3.58
	Angular Gyrus	R	42	-61	44	3.14
4	Middle Frontal Gyrus	R	39	41	23	5.76
	Middle Orbital Gyrus	R	45	50	-7	4.90
	Frontal Pole*	R	48	47	-10	4.88
	Middle Frontal Gyrus	R	42	56	11	4.32
	Middle Frontal Gyrus	R	33	32	32	4.30
	Middle Frontal Gyrus	R	30	53	29	4.29
	Middle Frontal Gyrus	R	39	59	8	4.23
	Middle Orbital Gyrus	R	45	56	2	4.20
5	Middle Frontal Gyrus	L	-30	50	23	4.65
	Frontal Pole*	L	-42	44	-16	4.24
	Frontal Pole*	L	-45	47	-10	4.21
	Middle Orbital Gyrus	L	-42	50	-7	4.21
	Middle Frontal Gyrus	L	-36	56	14	4.02
	Frontal Pole*	L	-33	62	-1	3.64

	Middle Frontal Gyrus	L	-33	38	32	3.49
6	Temporal Pole	L	-51	14	-4	4.85
	Insular Cortex	L	-45	-1	8	4.84
	Superior Temporal Gyrus	L	-48	2	2	4.70
	Putamen*	L	-30	-7	-4	4.01
	Putamen	L	-24	-1	2	3.87
	Hippocampus	L	-30	-16	-10	3.44
7	Lingual Gyrus	R	21	-88	2	5.73
8	Insular Cortex	R	48	8	-4	4.60
	Inferior Frontal Gyrus (p. Opercularis)	R	60	11	14	4.51
	Insular Cortex	R	48	5	8	4.11
9	SupraMarginal Gyrus	R	51	-34	47	4.91
	SupraMarginal Gyrus	R	48	-46	50	4.54
10	Cerebellum (VI)	R	9	-64	-16	4.63
	Cerebellum*	R	27	-49	-28	4.49
	Cerebellum (Crus 1)	R	39	-61	-28	4.28
	Cerebellum*	R	39	-55	-37	4.07
	Cerebellar Vermis (7)	C	3	-70	-22	3.90
	Cerebellum (VI)	R	30	-61	-31	3.72
	Cerebellum*	R	18	-55	-22	3.62
11	Superior Temporal Gyrus	L	-45	-28	17	4.81
	Insular Cortex*	L	-30	-25	20	4.35
	Heschls Gyrus	L	-33	-28	17	4.34
12	Posterior-Medial Frontal	L	-6	-4	62	4.38
	MCC	L	-6	8	41	3.37
	F Placebo Pain Systole					
1	Superior Parietal Lobule	L	-15	-70	56	8.37
	Inferior Frontal Gyrus (p. Opercularis)	R	54	11	17	8.05
	Middle Frontal Gyrus	R	42	41	23	7.35
	Frontal Operculum Cortex*	R	48	14	-1	7.26
	SupraMarginal Gyrus	R	51	-37	50	7.24
	Precentral Gyrus	L	-30	-4	68	7.18
	Insular Cortex	R	45	11	2	7.16
	Middle Frontal Gyrus	L	-33	53	23	7.08
	Middle Frontal Gyrus	L	-27	-1	65	7.06
	Cerebellum (VI)	R	21	-85	-13	7.05
	Inferior Frontal Gyrus (p. Orbitalis)	R	51	17	-7	7.04
	G Placebo Pain Diastole					
1	Superior Parietal Lobule	L	-15	-70	56	7.30
	Superior Parietal Lobule	R	15	-73	59	6.66
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	17	6.43
	Middle Frontal Gyrus	L	-30	-1	68	6.41
	Inferior Frontal Gyrus (p. Orbitalis)	R	51	17	-7	6.40
	Middle Frontal Gyrus	L	-33	53	23	6.25
	Middle Frontal Gyrus	R	39	41	26	6.22
	Middle Frontal Gyrus	R	30	8	62	6.19
	Inferior Frontal Gyrus (p. Orbitalis)	L	-48	14	-4	6.05

	Precentral Gyrus	L	-36	-19	62	5.87
	Inferior Parietal Lobule	L	-39	-49	44	5.87
2	Cerebellum (Crus 1)	R	33	-64	-28	6.13
	Cerebellum (Crus 1)	R	21	-85	-16	5.88
	Cerebellum (Crus 1)	L	-42	-61	-28	5.59
	Cerebellum (VI)	R	12	-79	-13	5.53
	Cerebellum*	R	33	-52	-34	5.39
	Inferior Temporal Gyrus	R	54	-58	-19	5.30
	Cerebellum (VIII)	L	-3	-73	-28	5.20
	Cerebellum*	R	36	-55	-37	5.18
	Inferior Occipital Gyrus	L	-54	-67	-10	5.15
	Middle Occipital Gyrus	L	-15	-94	-1	5.01
	Cerebellum (Crus 1)	L	-27	-70	-25	4.94
	H Placebo No Pain Systole					
1	Superior Parietal Lobule	R	15	-73	59	7.32
	Postcentral Gyrus	L	-45	-13	59	6.88
	Precuneus	L	-12	-73	56	6.37
	Middle Frontal Gyrus	R	30	5	65	5.85
	Superior Parietal Lobule	L	-24	-64	56	5.84
	Precentral Gyrus	L	-30	-4	68	5.49
	SupraMarginal Gyrus	R	48	-37	50	5.45
	Postcentral Gyrus	L	-54	-19	50	5.35
	Precentral Gyrus	L	-39	-4	62	5.26
	Inferior Parietal Lobule	L	-42	-40	41	5.17
	Posterior-Medial Frontal	L	0	2	59	5.13
2	Cerebellum (VI)	L	-6	-82	-10	8.34
	Lingual Gyrus	L	-12	-91	-4	7.52
	Cerebellum (Crus 1)	L	-42	-61	-28	6.35
	Lingual Gyrus	R	18	-91	-1	5.89
	Cerebellum (Crus 1)	R	39	-64	-25	5.78
	Superior Occipital Gyrus	R	24	-91	14	5.71
	Cerebellum (VI)	R	33	-52	-31	5.65
	Cerebellum*	R	27	-52	-28	5.50
	Cerebellum (VIII)	L	-3	-73	-28	5.41
	Cerebellum*	R	36	-55	-34	5.31
3	Middle Frontal Gyrus	R	39	41	26	5.90
	Middle Frontal Gyrus	R	33	50	26	5.14
	Middle Frontal Gyrus	R	51	17	47	4.65
	Middle Frontal Gyrus	R	42	53	14	4.50
	Frontal Pole*	R	48	44	-10	4.26
	Middle Orbital Gyrus	R	27	56	-7	3.60
	Middle Orbital Gyrus	R	42	56	-4	3.47
	Frontal Pole*	R	36	59	-4	3.39
4	Inferior Frontal Gyrus (p. Orbitalis)	R	48	14	-4	6.01
	Inferior Frontal Gyrus (p. Orbitalis)	R	51	17	-7	5.93
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	17	5.26
	Inferior Frontal Gyrus (p. Orbitalis)	R	39	23	-13	4.68

	Insular Cortex	R	33	20	8	4.37
5	Inferior Frontal Gyrus (p. Orbitalis)	L	-45	14	-4	5.16
	Inferior Frontal Gyrus (p. Opercularis)	L	-60	8	20	3.92
	Insular Cortex	L	-42	2	8	3.88
	Putamen*	L	-33	-1	5	3.82
	Inferior Frontal Gyrus (p. Triangularis)	L	-57	14	17	3.82
	Inferior Frontal Gyrus (p. Triangularis)	L	-57	23	23	3.57
	Temporal Pole	L	-54	11	-13	3.48
6	Middle Frontal Gyrus	L	-33	50	20	5.15
7	Superior Temporal Gyrus	L	-45	-31	17	4.40
	Planum Temporale*	L	-39	-31	8	3.25
	I Placebo No Pain Diastole					
1	Superior Parietal Lobule	R	15	-70	62	7.67
	Postcentral Gyrus	L	-45	-13	56	7.47
	Middle Frontal Gyrus	R	42	41	26	6.99
	Middle Frontal Gyrus	R	33	53	29	6.55
	Superior Frontal Gyrus	R	27	8	65	6.43
	Superior Parietal Lobule	L	-15	-70	56	6.34
	Precentral Gyrus	L	-33	-4	65	6.25
	Middle Frontal Gyrus	L	-33	50	23	6.24
	Superior Parietal Lobule	L	-24	-67	59	6.10
	Precentral Gyrus	L	-39	-4	62	6.04
	SupraMarginal Gyrus	R	48	-49	47	6.02
2	Cerebellum (VI)	L	-9	-85	-10	8.19
	Lingual Gyrus	L	-12	-82	-1	8.13
	Lingual Gyrus	R	18	-91	-1	6.28
	Middle Occipital Gyrus	L	-21	-91	14	6.17
	Superior Occipital Gyrus	R	24	-91	14	6.09
	Cerebellum (VI)	R	18	-88	-10	5.95
	Cerebellum (Crus 1)	L	-42	-64	-22	5.68
	Cerebellum (Crus 1)	L	-42	-58	-28	5.68
	Inferior Temporal Gyrus	R	60	-52	-13	5.46
	Cerebellum (Crus 1)	L	-27	-70	-25	5.42
	Cerebellum (VI)	R	27	-58	-28	5.36
3	Inferior Frontal Gyrus (p. Orbitalis)	R	48	14	-4	5.63
	Inferior Frontal Gyrus (p. Opercularis)	R	60	11	11	4.76
	Inferior Frontal Gyrus (p. Opercularis)	R	57	11	17	4.65
	Inferior Frontal Gyrus (p. Orbitalis)	R	39	23	-13	4.29
4	Rolandic Operculum	L	-42	-28	17	4.88
	Middle Temporal Gyrus	L	-66	-31	5	4.12
	Middle Temporal Gyrus*	L	-45	-34	-4	4.11
	Middle Temporal Gyrus	L	-60	-37	5	4.05
	Middle Temporal Gyrus	L	-54	-40	2	4.02
	Superior Temporal Gyrus	L	-48	-37	11	3.89
	Middle Temporal Gyrus*	L	-48	-46	-1	3.52
5	Putamen*	L	-24	14	8	4.58
	Caudate*	L	-21	14	-7	3.80

	Pallidum	L	-18	2	2	3.74
	Putamen	L	-30	-10	2	3.49
	Putamen*	L	-27	8	-4	3.44
6	Inferior Frontal Gyrus*	L	-54	14	-1	4.40
	Inferior Frontal Gyrus (p. Orbitalis)	L	-51	17	-4	4.32
	Frontal Operculum Cortex*	L	-48	11	-1	4.20
	Inferior Frontal Gyrus*	L	-57	14	5	4.17
	Temporal Pole	L	-48	5	-1	4.16
	Insular Cortex	L	-42	-4	11	4.03
	Inferior Frontal Gyrus*	L	-60	14	17	3.95
	Inferior Frontal Gyrus (p. Opercularis)	L	-60	2	17	3.93
7	Thalamus	L	-18	-22	11	5.09
8	Caudate Nucleus	R	18	20	8	4.68
	Insular Cortex*	R	30	20	8	3.54
9	Frontal Orbital Cortex*	R	21	20	-7	3.28
	J Placebo > Oxytocin					
1	Middle Temporal Gyrus	R	48	-64	5	5.64
	Fusiform Gyrus	R	42	-76	-13	5.60
	Inferior Temporal Gyrus*	R	48	-46	-4	5.48
	Lateral Occipital Cortex*	R	36	-64	2	5.16
	Lateral Occipital Cortex*	R	30	-64	29	4.61
	Inferior Temporal Gyrus	R	54	-34	-10	4.56
	Precuneus	R	21	-64	32	4.53
	Middle Temporal Gyrus	R	42	-67	14	4.36
	Calcarine Gyrus	R	24	-64	14	4.36
	Cuneus	R	18	-82	41	4.19
	Cerebellum (VII)	R	33	-73	-40	4.05
2	Lateral Occipital Cortex*	L	-39	-61	2	6.60
	Inferior Temporal Gyrus	L	-45	-70	-1	6.21
	Fusiform Gyrus	L	-36	-49	-7	5.40
	Middle Occipital Gyrus	L	-36	-79	26	4.79
	Cerebral White Matter*	L	-30	-52	20	4.69
	Cerebellum (IV-V)	L	-18	-46	-13	4.65
	Middle Occipital Gyrus	L	-45	-85	8	4.58
	Middle Occipital Gyrus	L	-39	-82	14	4.37
	Precuneus Cortex*	L	-27	-58	14	4.26
3	Posterior-Medial Frontal	R	12	5	71	5.23
	Middle Frontal Gyrus	R	30	8	62	4.10
	Middle Frontal Gyrus*	R	30	5	47	4.09
	Middle Frontal Gyrus	R	30	8	53	4.02
	Superior Frontal Gyrus	R	24	-1	59	4.02
	Precentral Gyrus	R	33	-4	53	3.57
4	Posterior-Medial Frontal	L	-3	17	50	4.38
	Posterior-Medial Frontal	L	-6	14	53	4.34
	Superior Medial Gyrus	L	-3	23	47	4.26
	Superior Medial Gyrus	R	9	29	56	4.12
	MCC	R	15	17	38	4.08

	Posterior-Medial Frontal	R	9	17	50	3.90
	MCC	L	-12	23	38	3.66
	Superior Medial Gyrus	L	-9	29	41	3.55
	Superior Frontal Gyrus	R	18	23	56	3.50
	Superior Medial Gyrus	R	9	23	65	3.45
5	Paracingulate Gyrus*	L	-15	38	14	4.96
	ACC	L	-9	38	17	4.84

Chapter 7 Relationship between systolic blood pressure, as a baroreflex index, and decision-making during emotional processing: a correlational study[5]

[5] As first author I designed the study and undertook all stages of data collection analyses and interpretation with the guidance of my senior colleagues and the assistance of Dr Pfeifer. In the text that follows, as in the manuscript in preparation, this group contribution is acknowledged in my use of plural personal pronouns.
This chapter led to a scientific publication submission in an international journal:
Betka, S., Watson, D., Garfinkel, S., Pfeifer, G., Sequeira, H., Duka, T., Critchley, H. (submitted) Relationship between systolic blood pressure, as a baroreflex index, and decision-making during emotional processing in male alexithymic subjects: a correlational study. *Psychosomatic Medicine*

7.1 Abstract

Emotional feelings are expressed in body and mind through the subjective experience of physiological changes. In previous work, the subliminal priming of anger was shown to increase systolic blood pressure (SBP). This increase predicted the slowing of response times (RT) when making lexical decisions, suggesting that baroreflex-related autonomic changes and their interoceptive (feedback) representations influence cognitive processes. Alexithymia is a subclinical affective dysfunction characterized by difficulty in identifying emotions. Atypical autonomic and interoceptive profiles are observed in alexithymia. Therefore, we sought to identify mechanisms through which SBP fluctuations during emotional processing influence decision-making, and test whether alexithymia contributes to this relationship.

Thirty-two male participants performed an affect priming paradigm and completed the Toronto Alexithymia Scale. Emotional faces were briefly presented (20ms) prior a short-memory task. RT accuracy and SBP were recorded on a trial-by-trial basis. Generalized mixed-effects linear models were used to evaluate the impact of emotion, physiological changes, alexithymia score, and their interactions on behavioural outcomes.

A main effect of emotion was observed on performance accuracy. Participants were more accurate on trials with anger primes, compared to neutral primes. Greater accuracy was related to an increased SBP. An interaction between SBP and emotion was observed on RT: increased SBP was associated with RT prolongation in the anger priming condition, yet this relationship was absent in the sadness priming. Alexithymia did not significantly moderate any of the above relationships.

In conclusion, our data indicate how peripheral autonomic responses to affective challenges guide cognitive processes. We discuss how these findings fit within an established theoretical framework proposed by Lacey and Lacey (1970).

Keywords: Systolic blood pressure (SBP), Baroreflex, Alexithymia, Affective priming, Emotion, EFE

7.2 Introduction

Emotional feeling states originate in part through the subjective experience and cerebral representation of peripheral physiological reactions to affective stimuli (James, 1884, Lange, 1912). Internal changes also inform and influence perceptual experience, allocation of attentional resources, emotional processing and decision-making (Bechara *et al.*, 1996, Gray *et al.*, 2010, Park *et al.*, 2014, Makovac *et al.*, 2015, Garfinkel *et al.*, 2016c, Makovac *et al.*, 2017, Łukowska *et al.*, 2018).

One established way of testing how autonomic changes influence cognitive processes is the use of an affective priming paradigm. Here, the emotional valence of a briefly presented stimulus (i.e. the prime) affects the processing of subsequent stimuli. For example, in a recent study, the subliminal presentation of the word “ANGER” (vs “RELAX”) as a prime, just prior to rapid judgments of letter-strings, increases systolic blood pressure in healthy individuals(Garfinkel *et al.*, 2016c). Interestingly, the magnitude of this increase predicts RT prolongation on the task (Garfinkel *et al.*, 2016c). Increased systolic blood pressure is also found in studies using emotional faces as primes (Gendolla and Silvestrini, 2011, Silvestrini and Gendolla, 2011b, Silvestrini and Gendolla, 2011c, Lasauskaite *et al.*, 2013; for review see van der Ploeg et al., 2017).

However, alexithymia is characterized by an impairment in the representation, and integration of interoceptive bodily responses (Apfel and Sifneos, 1979). Indeed, alexithymic subjects show abnormal emotional, interoceptive and physiological profiles (Neumann *et al.*, 2004, Grynberg *et al.*, 2012, Grynberg and Pollatos, 2015, Lane *et al.*, 2015, Murphy *et al.*, 2017). For example, alexithymic individuals show greater systolic blood pressure reactivity and rate themselves as more anxious during the stress of blood donation (Byrne and Ditto, 2005). During anger recall, cardiac responses are attenuated in alexithymic subjects (Neumann *et al.*, 2004) but then the recovery (of diastolic blood pressure recovery and cardiac preejection period) is slower, compared to the recovery of non-alexithymic individuals (Neumann *et al.*, 2004). In addition, alexithymia is more prevalent among hypertensive patients than in the general population (Gage and Egan, 1984, Todarello *et al.*, 1995, Jula *et al.*, 1999).

7.3 Aims and hypotheses

We sought to identify how specific physiological changes (indexed by systolic blood pressure) evoked by emotional primes influenced cognition, specifically the accuracy and speed of decision-making.

A second aim was to test whether alexithymia contributed to this relationship.

We predicted that affective priming with brief anger stimuli would be associated with increases in systolic blood pressure that influence accuracy and response times during a short-memory task. We hypothesized that alexithymia may partly mediate the relationship between physiological changes and decision-making through its association with increased systolic blood pressure at rest.

Given the well-documented co-occurrence of depression, anxiety and alcohol use disorders in alexithymia (Finn *et al.*, 1987, Hendryx *et al.*, 1991), we additionally assessed these variables in order to control for potential cofounding effects. Moreover, we did not have any strong hypothesis regarding the impact of alcohol use on systolic blood pressure, given the young age of our participants.

7.4 Methods summary

Full methodological details are described in Chapter 2 section 2.2.

7.4.1 Procedure and participants

To test our hypotheses, thirty-two male volunteers (mean age 25.1 years; range 18–36 years) took part in the experiment.

The experiment happened during the first baseline session of a series of tests (no drug administration). During that session, demographic and psychometric information were collected. Then, participants performed the priming task, while their beat-to-beat systolic blood pressure, accuracy and reaction time were recorded, for each trial.

Each trial began with a 1000 ms fixation cross, followed by the Emotional Facial Expression (EFE; 20 ms) and a backward mask (125 ms). This rapid series of events was immediately followed by a string of seven letters that remained on screen for 750ms, followed by a backward mask of seven “X” letters (750 ms). Then a target letter appeared at the centre of the screen until participants made a decision (max 2000ms), denoting whether or not the target letter was present in the string by pressing the right or the left arrow key, respectively. Next, a visual analogue scale allowed participants to rate their confidence for each trial, from “zero” to “extreme” (3000ms).

7.4.2 Statistical Analyses

One participant was excluded due to a depression score above three standard deviations of the mean.

We used mixed-effects modeling to test effects of the variables (accuracy, RT and SBP), measured on trial by trial basis, (Barr *et al.*, 2013).

Accuracy was analyzed using a generalized linear mixed model as the outcome was binary (binomial family; Inaccurate =0; Accurate =1). To satisfy normality assumption, reaction times were also analyzed using a generalized linear mixed-effects model (Lo and Andrews, 2015). After fitting different density to the observed reaction times distribution, the relative quality of the models were estimated using Akaike information criterion (AIC). The lower AIC (e.g. best fit) was observed when a Gamma distribution was fitted to the observed reaction time distribution.

The same basic model was tested for each of the two outcomes (i.e. accuracy and reaction time). The basic model included systolic blood pressure, emotion (3 levels: Neutrality=0; Sadness=1; Anger=2), TAS score and the interactions terms as predictors. Given the established influence of age on blood pressure reactivity, age was included in the basic model as a control variable. Finally, participants were specified as a random (subject) factor, allowing for random intercepts.

The basic model was then compared to a similar model that also included anxiety, depression and alcohol intake to test confounding effects of these variables, contrasting the goodness of fit of the models using likelihood ratio tests.

As post-hoc analyses, we wanted to explore if accuracy, reaction times and systolic blood pressure were related to a deceleration at the heart rate level. To do so, we analysed the heart rate variability in the frequency domain.

7.5 Results

Table 7-1 Mean, standard deviations, range as well as uncorrected Kendall's tau correlation coefficients (τ) for psychometric, physiological and behavioural measures

		Correct Answer (%)	Reaction Times (ms)	Sytolic Blood Pressure (SBP; mmHg)	Alexithymia (TAS-20)	Unit of alcohol per week	Depression score (BDI)	Mean	Std. Dev	Minimum	Maximum
Correct Answer (%)	τ	-						83.47	8.80	54.17	97.92
	p	.									
Reaction Times (s)	τ	-0.090	-					1020.30	139.67	730	1380
	p	0.484	.								
Sytolic Blood Pressure (SBP; mmHg)	τ	-0.108	0.11	-				125	19	78	161
	p	0.403	0.386	.							
Alexithymia (TAS-20)	τ	-0.173	0.128	-0.08	-			55.13	9.69	36	74
	p	0.183	0.315	0.529	.						
Unit of alcohol per week	τ	0.037	0.131	0.153	0.063	-		24.57	18.32	4.5	69.5
	p	0.772	0.300	0.228	0.622	.					
Depression score (BDI)	τ	-0.114	0.039	-0.079	0.265	-0.039	-	11.58	7.57	0	29
	p	0.383	0.759	0.540	0.041	0.759	.				
Trait Anxiety (STAI)	τ	0.110	0.064	-0.125	0.162	-0.086	0.450	47.71	12.31	22	66
	p	0.401	0.621	0.331	0.213	0.506	0.001				

7.5.1 Mean, standard deviations, correlation and sample characterisation

A total of thirty-one participants was included in the analyses. Means, standard deviations, and ranges, as well as uncorrected correlation coefficients between psychometric, physiological and behavioral measures, are presented in Table 7-1. Interestingly, we did not observe significant correlations across psychometric data, behavioral and physiological measures. For example, we did not find a significant relationship between systolic blood pressure and accuracy and reaction times. Moreover, in this sample, there was also no significant positive correlation between alexithymia and resting systolic blood pressure. Concerning alexithymia scores, 10 subjects were characterized as non alexithymic (32.26%), 10 subjects were characterized as intermediate (32.26%), 11 subjects were characterized as alexithymic (35.48%), based on TAS-20 cut off (Bagby *et al.*, 2006).

7.5.2 Accuracy

	β	SE	z	p	
Intercept	1.56	0.13	12.25	0.00	***
Sadness	-0.01	0.12	-0.09	0.93	
Anger	0.49	0.13	3.80	0.00	***
Systolic Blood pressure (SBP)	1.56	0.60	2.59	0.01	*
TAS-20	0.01	0.01	0.51	0.61	
Age	-0.06	0.03	-2.28	0.02	*
Sadness:SBP	-0.59	0.59	-1.01	0.31	
Anger:SBP	-1.14	0.64	-1.79	0.07	.
Sadness:TAS-20	-0.01	0.01	-1.03	0.30	
Anger:TAS-20	-0.01	0.01	-0.77	0.44	
SBP:TAS-20	-0.10	0.05	-1.90	0.06	.
Sadness:SBP:TAS-20	0.04	0.05	0.92	0.36	
Anger:SBP:TAS-20	-0.02	0.06	-0.39	0.70	

Table 7-2 Mixed-effects regression model to explain accuracy.

This regression is using Emotion (Neutrality, Sadness, Anger), systolic blood pressure (SBP) alexithymia (TAS-20) and their interactions as predictors, and, age as control variable. signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Accuracy was analyzed with emotion (3 levels: Neutrality=0; Sadness=1; Anger=2), systolic blood pressure, TAS and their interactions as fixed factors. The participant (subject) variable was defined as a random factor (see methods section). The model controlled for age. The distribution was set as binomial (see

Table 7-2).

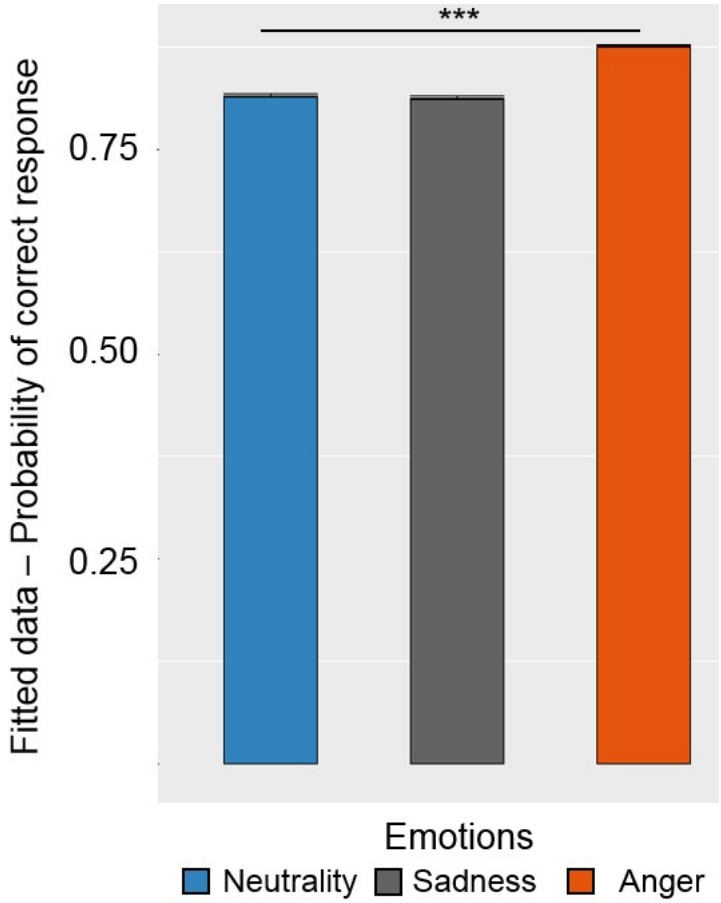


Figure 7.1 Main effect of emotion on probability of being accurate (*** $p < 0.001$)

There was a main effect of emotion; anger primes elicited increased accuracy compared to sadness and neutrality conditions (β = -0.50, SE = 0.13, $p < 0.001$; see Figure 7.1). A main effect of systolic blood pressure was also observed: greater systolic blood pressure was related to better accuracy (β = 1.56, SE = 0.6, $p < 0.05$).

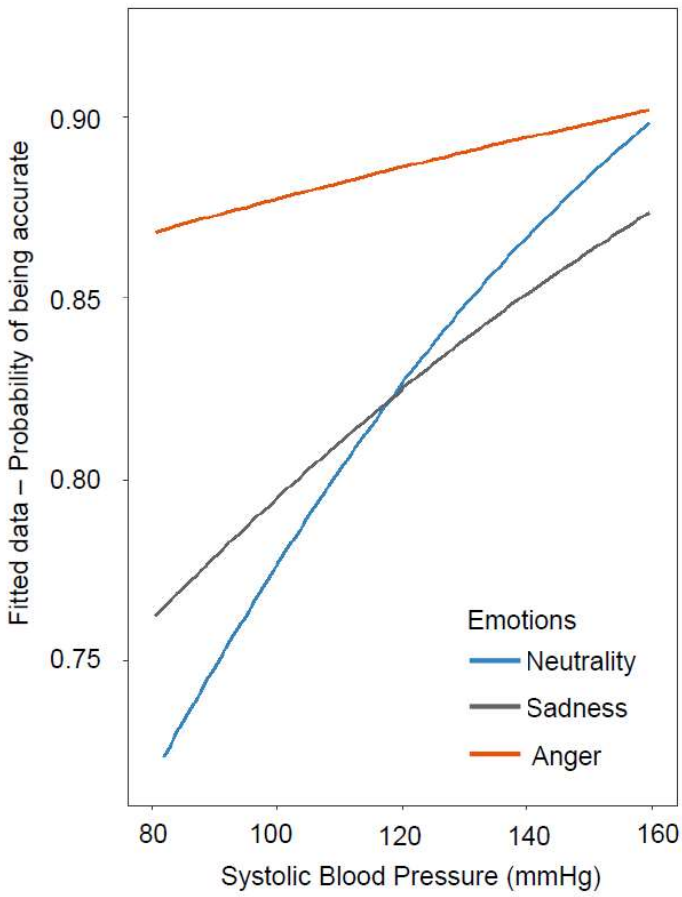


Figure 7.2 Trend of interaction between systolic blood pressure and emotion on probability of being accurate (p = 0.07)

A trend of interaction between systolic blood pressure and emotion (anger vs neutrality) was observed: low systolic blood pressure seemed to be associated with better accuracy in the anger condition compared to the neutral condition. However, increased blood pressure seemed to lead to similar accuracy in both conditions (β = -1.14, SE = 0.64, p = 0.07; see

Figure 7.2).

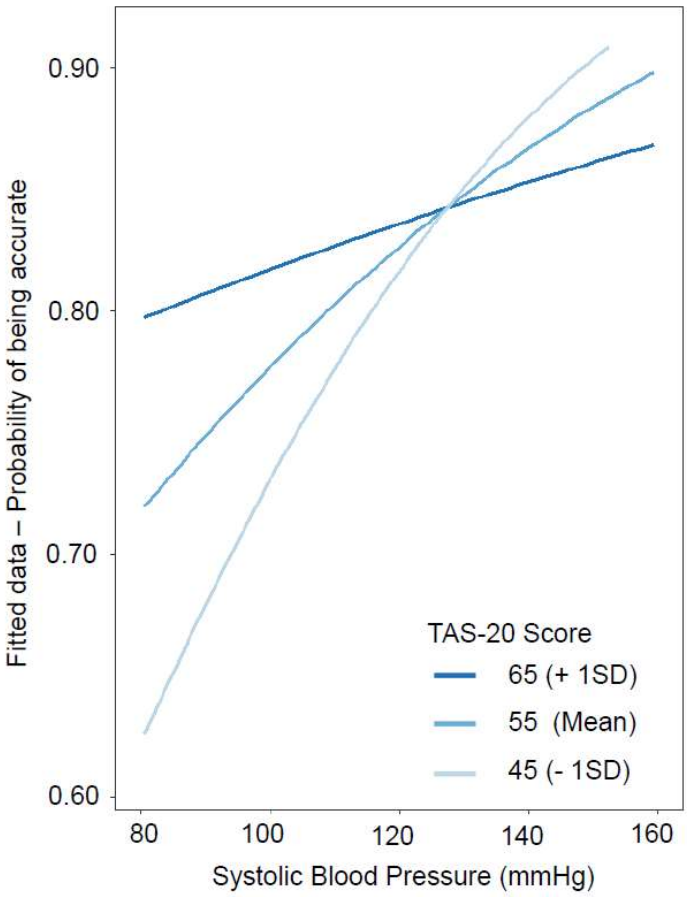


Figure 7.3 Trend of interaction between systolic blood pressure and Alexithymia (TAS-20 scores) on probability of being accurate.

To illustrate this interaction, high alexithymic (Mean TAS-20 score + 1SD = 65), intermediate (Mean TAS-20 score = 55) and non alexithymic (Mean TAS-20 score -1SD = 45) were plotted. (p = 0.058).

Interaction between TAS-20 and systolic blood pressure was also observed as a trend: While in non-alexithymic individuals, performance accuracy correlated positively with systolic blood pressure, alexithymic individuals showed increased accuracy at lower systolic blood pressures and their gain in accuracy with increasing systolic blood pressure was reduced compared to non-alexithymic individuals (β = -0.1, SE = 0.05, p = 0.058; see

Figure 7.3). Furthermore, there was a main effect of age (β = -0.06, SE = 0.03, p < 0.05). Addition of control variables (anxiety, depression, alcohol intake) to the model did not significantly improve the goodness of fit (Basic model: AIC =2586.9; Model with covariates: AIC = 2590.4; comparison: χ^2 (3) = 2.53; p = 0.47).

7.5.3 Reaction Time

	β	SE	z	p	
Intercept	1.01	0.03	30.08	0	
Sadness	-0.01	0.01	-1.03	0.3	
Anger	0	0.01	-0.07	0.95	
Systolic Blood pressure (SBP)	-0.14	0.08	-1.69	0.09	.
TAS-20	0	0	-0.51	0.61	
Age	0	0.01	0.26	0.79	
Sadness:SBP	0.2	0.06	3.36	0	***
Anger:SBP	-0.01	0.06	-0.19	0.85	
Sadness:TAS-20	0	0	0.94	0.35	
Anger:TAS-20	0	0	-1.01	0.31	
SBP:TAS-20	-0.01	0.01	-1.23	0.22	
Sadness:SBP:TAS-20	-0.01	0	-1.58	0.11	
Anger:SBP:TAS-20	0	0	0.27	0.79	

Table 7-3 Mixed-effects regression model to explain reaction times.

This regression is using Emotion (Neutrality, Sadness, Anger), systolic blood pressure (SBP) alexithymia (TAS-20) and their interactions as predictors, and, age as control variable. signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

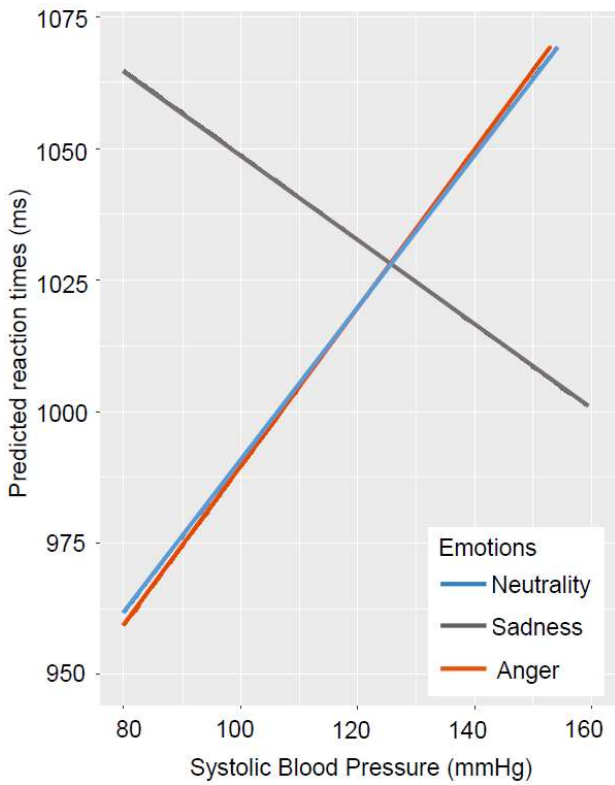
Reaction times were analyzed with emotion (3 levels: Neutrality=0; Sadness=1; Anger=2), systolic blood pressure, TAS and their interactions as fixed factors. The participant variable was defined as a random factor (see methods section above). The model controlled for age. The distribution was set as gamma (see

Table 7-3).

There was an interaction between systolic blood pressure and emotion (sadness vs. neutrality conditions; β = -0.20, SE = 0.06, $p < 0.001$; see Figure 7.4): For neutral (and anger) primes, increases in systolic blood pressure were associated with longer reaction times. However, increases in blood pressure during the sadness prime condition were associated with faster reaction times. A trend of main effect of systolic blood pressure on reaction time was observed (β = -0.14, SE = 0.08, $p = 0.09$). Addition of control variables (anxiety, depression, alcohol intake) to the model did not significantly improve the goodness of fit (Basic model: AIC =736.17; Model with

Figure 7.4 Interaction between systolic blood pressure and emotion on reaction times.

($p < 0.001$)



covariates: AIC = 741.50; comparison: χ^2 (3) = 0.67; $p = 0.88$).

7.5.4 Post-hoc analyses

In order to explore if accuracy, reaction times and systolic blood pressure were related to a deceleration at the heart rate level, we analysed the heart rate variability in the frequency domain.

		Correct Answer	Reaction Times	Systolic Blood Pressure (SBP)	Mean	Std. Dev	Minimum	Maximum
Mean Interbeat Interval (ms)	Kendall's tau	-.323	0.084	0.08	842.69	106.00	606.3	1086.3
	Sig. (2-tailed)	0.012	0.507	0.529				
Low cardiac frequencies (%)	Kendall's tau	0.097	-.254	0.215	48.83	11.59	21.9	73.5
	Sig. (2-tailed)	0.453	0.045	0.089				
High cardiac frequencies (%)	Kendall's tau	-0.18	0.099	0.017	25.25	12.14	8.3	64
	Sig. (2-tailed)	0.162	0.434	0.892				
Ratio Low/high cardiac frequencies	Kendall's tau	0.158	-0.133	0.074	2.50	1.60	0.34	7.07
	Sig. (2-tailed)	0.23	0.301	0.568				

Table 7-4 Mean, standard deviations, range as well as Kendall's tau correlation coefficients for heart rate variability-related and behavioural measures.

Means, standard deviations, and ranges, as well as uncorrected correlation coefficients between physiological and behavioral measures, are presented in Table 7-4. Interestingly, a negative correlation was observed between mean interbeat interval and the percentage of correct responses: greater accuracy was associated with increased heart rate (τ= -.323, p = 0.012). Reaction times were negatively correlated with low frequencies percentage suggesting an association between shorter reaction times and increased sympathetic (or mixed) activity (τ= -.254; p = 0.045). We did not observe suprathreshold correlations across HRV-related measures and systolic blood pressure.

7.6 Discussion

In this present study, we tested how systolic blood pressure changes influence cognition during the processing of emotional primes, and explored how alexithymia might contribute to this relationship.

7.6.1 Main findings

We found an effect of emotional valence on the accuracy of letter-string judgments: individuals were more accurate in their judgments when primed by angry faces compared to neutral faces. Moreover, better accuracy was associated with increased systolic blood pressure, regardless of the emotion. However, systolic blood pressure interacted also with the valence of affective priming on reaction time responses, where increasing blood pressure slowed reaction times following anger and neutrality primes, but did not impact responses following sadness primes. We saw no significant direct moderating effect of alexithymia on accuracy or reaction times, in our participants. Perhaps, in this context, we were underpowered to test for this effect. However, our data indicated relative primacy of physiological effects and early emotional processing over higher-order alexithymic differences.

Alexithymia did not seem to correlate with blood pressure level in our sample. Nevertheless, a trend of interaction between systolic blood pressure and alexithymia on performance accuracy was observed. Non-alexithymic individuals seemed to be more accurate in conditions associated with increased systolic blood pressure. Alexithymic individuals seemed to be more accurate under low blood pressure compared to non-alexithymic individuals, whereas the inverse was observed under high blood pressure. Overall, alexithymic participants did not seem to benefit cognitively from blood pressure increases as much as non-alexithymic participants. However, future studies with greater statistical power should clarify the relationship between behaviour, autonomic reactivity and alexithymia.

Our first main finding was an effect of valence of the affective primes on decision-making accuracy. Here, participants were more accurate on the judgment task after being primed by angry faces compared to neutral faces. These results are in line with existing literature: briefly flashed visual anger

stimuli influenced behavior, even without stimulating explicit affective responses (Murphy and Zajonc, 1993, Winkielman *et al.*, 2005, Gendolla, 2012). Anger is a negative-valenced and particularly salient emotion which preferentially captures attentional resources (Pinkham *et al.*, 2010, Feldmann-Wustefeld *et al.*, 2011, Hodsoll *et al.*, 2011, Shasteen *et al.*, 2014, Burra *et al.*, 2016, Burra *et al.*, 2017). Presentation of angry face stimuli can increase visual short-term memory and working memory via modulation of basal ganglia activation (Jackson *et al.*, 2008, Jackson *et al.*, 2009). One potential explanation for increased accuracy after anger priming is the triggering of a hyper-vigilant state by the emotional anger prime, enhancing attentional deployment to improve task performance. Alternatively, when primed by anger, the participants experienced a subjective reduction in task demand and a consequent increased ease in performance, when compared to the neutral and sadness priming conditions (Gendolla and Silvestrini, 2011, Chatelain *et al.*, 2016). The latter is consistent with the coupling of anger to appetitive and approach motivational systems (Russell, 2003, Carver and Harmon-Jones, 2009). Angry facial expressions facilitate the generation of approach rather than avoidance motor responses (Wilkowski and Meier, 2010) and dynamic angry faces increase motor corticospinal excitability, mediating implicit and automatic responses to threat (Hortensius *et al.*, 2016). However, we did not find a significant main effect of emotion on reaction times.

Our second main finding, nevertheless, was a significant interaction between priming condition and systolic blood pressure, on reaction times. In both anger and neutral priming conditions, increases in systolic blood pressure evoked increased reaction times. However, this linear relationship was absent in the sadness priming condition: reaction times did not seem to be modulated by systolic blood pressure changes. Moreover, in the sadness condition, lower systolic pressure was associated with longer reaction times. Blood pressure increases following verbal anger primes have been previously observed to predict a prolongation of reaction time on a lexical task (Garfinkel *et al.*, 2016c). The difference between these effects of anger and sadness primes parallels earlier findings: sadness primes during an easy task can increase cardiovascular responses compared to anger primes, yet in a difficult task, the

reverse pattern is found (Freydefont *et al.*, 2012). Moreover, even masked sadness stimuli are associated with greater perceived difficulty and less ease when performing tasks (increased reactions times) and therefore, increase the likelihood of disengagement when task demand becomes excessive.(Silvestrini and Gendolla, 2011c, Silvestrini and Gendolla, 2011a). However, our data did not show any reaction time increase during the sadness condition, as might be predicted by the “implicit affect primes effort” (IAPE) model of Gendolla as a sign of disengagement.

Our third main finding was that, on a trial-by-trial basis, task performance accuracy was related to increased systolic blood pressure. These data extend the existent literature. As shown by intra-arterial recordings, a sympathetic mechanism is implicated in engendering the blood pressure increases that accompany simple reaction-time tasks, (Obrist *et al.*, 1974, Paller and Shapiro, 1983). Lower blood pressure correlates with poorer performance on a visuospatial attentional task in young hypotensive women (Wharton *et al.*, 2006, Cellini *et al.*, 2013). Moreover, pharmacological elevation of blood pressure improves cognitive performance in hypotensive patients (Duschek *et al.*, 2007). Typically a rise in blood pressure activates arterial baroreceptors which ultimately inhibit both cardiac and cortical activity, impacting cognitive processes (Rau *et al.*, 1993, Kimmerly, 2017). Natural or artificial baroreceptor stimulation can inhibit somatosensory afferent information flow (including pain; Angrilli *et al.*, 1997, Gray *et al.*, 2010). This cardiac afferent mechanism is postulated to reduce input from the external environment and, thereby reduce inattention and distractibility. Correspondingly, our data (i.e. higher systolic blood pressure, higher accuracy), accompanied by a potential heartrate deceleration (reducing afferent cardiac feedback to the brain and thereby limiting interference with cognitive processes) might have been broadly in line with the notion of an increase in attentive observation of the environment (Lacey and Lacey, 1970). Speculatively, our observed increased blood pressure could have regulated cardiac output - via vagal influences. This control might have thus facilitated sensorimotor performance (Cellini *et al.*, 2013, Park *et al.*, 2013, Park and Thayer, 2014). Indeed, cardiac deceleration is itself modulated by arousal (e.g. threat) supporting the hypothesis of an

evolutionary survival strategy (Hare *et al.*, 1970, Libby *et al.*, 1973). However, we are cautious as we only observed a statistical trend in the interaction between blood pressure and emotion on performance accuracy. For the neutral condition, increased systolic blood pressure was associated with greater accuracy. First, we recognised the relevance of this observation to Lacey and Lacey's (1970) hypothesis. This model dating back to the 1970s suggests that baroreceptor activation has a lower-level inhibitory influence on sensory processing and cortical excitability. To test our findings in relation to this hypothesis, we conducted post-hoc analyses. These showed that greater accuracy and shorter reaction times were associated with faster heart rate and an increase in power of low frequency heart rate variability, respectively. Given the absence of an association between heartbeat deceleration or increased parasympathetic activity index (e.g. high frequency heart rate variability) and accuracy, our data does not seem to support Lacey and Lacey's hypothesis. Instead, the rise in systolic blood pressure and the greater accuracy seem to be both driven by increased arousal induced by affective priming. This interpretation is congruent with the observed increased accuracy, in the absence of a modulation bodily state, under anger priming condition. Moreover, this discrepancy between different emotional conditions suggests the involvement of emotion-specific pathways (Lacey and Lacey, 1970, Brooks *et al.*, 2012).

A secondary aim of the study was to characterise the impact of alexithymia on these relationships between bodily changes and behaviour. We found a trend of an interaction between alexithymia and systolic blood pressure. Compared to non-alexithymic, alexithymic individuals benefitted least from systolic blood pressure fluctuations. Alexithymia is typically characterized by increased blood pressure and reduced interoceptive abilities (Gage and Egan, 1984, Todarello *et al.*, 1995, Jula *et al.*, 1999, Brewer *et al.*, 2016, Betka *et al.*, 2017, Bornemann and Singer, 2017, Murphy *et al.*, 2017). Given their atypical autonomic profiles, one could postulate that alexithymic people may also attribute less salience to bodily changes and show impaired integration of autonomic information when compared to non-alexithymic. Compensatory strategies developed by alexithymic individuals may explain, in part, why they

are not impaired on the task. For example, an alexithymic individual might use information related to bodily actions (e.g. increased somatosensory and motor areas activation), rather than affective states, to label emotional faces correctly (Ihme *et al.*, 2014). Alexithymic individuals may have particular difficulty in processing and using automatically high-arousal emotional information in the context of cognitive challenges (Vermeulen *et al.*, 2006). In that way, our data also suggest reduced integration of highly relevant emotional information in alexithymia. Further studies should clarify this relationship.

7.6.2 Limitations

We recognize some limitations of this study. A larger sample would increased statistical power and sensitivity sufficiently to explore the impact of inter-individual characteristics on relationships between body and behaviour. For example, it would have been interesting to measure trait anger and hostility, which is a factor known to modulate the effects of subliminal anger primes (Wilkowski and Robinson, 2008, Garfinkel *et al.*, 2016c). Another limitation is the absence of an awareness check to establish the degree to which the brief (20ms) primes were not processed consciously. We, therefore, cannot guarantee that our affective prime stimuli were rendered fully subliminal (van der Ploeg *et al.*, 2017). However, this would be unusual for our rapid presentation of the primes. In addition, recent studies highlight the importance of taking into account both affective and cognitive dimensions of alexithymia as their autonomic signatures seem to differ (Cecchetto *et al.*, 2017, Martínez-Velázquez *et al.*, 2017). Unfortunately, we did not have this degree of granularity within the present dataset. Finally, we did not have any strong hypothesis regarding the impact of alcohol use on systolic blood pressure, given the young age of our participants. To add alcohol use in the statistical models did not significantly improve the fit. Future studies involving bigger sample of participants or groups comparison should explore the impact of alcohol use on how specific physiological changes (indexed by systolic blood pressure) evoked by emotional primes influence cognition, specifically the accuracy and speed of decision-making.

7.6.3 Conclusion

In conclusion, our data demonstrates the interacting effects of peripheral autonomic changes and affective states in guiding mental processes.

Chapter 8 General discussion

"Et pourtant, il faut bien tenter de dessiner le schéma qui permet de mieux comprendre le monde même si nous savons qu'il n'est pas l'image de l'exacte vérité."

[And yet, we must try to draw the diagram that allows to better understand the world even if we know that it is not the image of the exact truth]

Claude Béata*, Au risque d'aimer* (2013), p110

8.1 Key findings and contributions of the present work

My thesis attempted to describe the relationship between the capacity for emotional regulation, as indexed by alexithymia, interoceptive processes, and alcohol use, using multimodal techniques.

In Chapter 3, I sought to characterise relationships between subjective measures of alexithymia, sensitivity to bodily sensations and alcohol consumption, using mediation analyses to infer likely causality. I found that self-assessment measures of alexithymia and, more specifically, difficulties to identify feelings, mediate the relationship between self-assessment measures of sensitivity to bodily sensations and of alcohol use.

In Chapter 4, my aim was to describe the mechanism, within the brain, that can explain the interoceptive impairments observed in alcohol use disorders. Combining Magnetic Resonance Spectroscopy (MRS) with psychometric ratings, I explored the relationship between alcohol-related measures (i.e. severity of alcohol use, craving, and compulsion) and middle insular cortical neurochemistry (glutamate plus glutamine (Glx) and N-acetylaspartate plus N-acetylaspartylglutamate (TNAA) metabolite concentrations) in alcohol users. I was also interested in associations between alcohol-related measures and insular morphology (volume and surface gyrification) and used voxel and surface-based morphometry. I found that alcohol use severity was predictive of reduced insular gyrification. Moreover, insular glutamate-plus-glutamine concentration was negatively correlated with alcohol use severity, but also with a more behavioural facet of alcohol use; alcohol compulsion. Interestingly, this reduction in insular metabolites mirrored reduced insular gyrification, allowing the possibility to discuss general alcohol-related insular grey matter atrophy.

In Chapter 5, my aim was to explore the interaction between interoception, oxytocin and alcohol use. Therefore, I sought to characterize the impact of intranasal oxytocin (OT) on interoceptive processing in alcohol users, using classical behavioural interoceptive tasks. I showed that intranasal OT evoked a decrease in interoceptive accuracy on the heartbeat tracking task, but also tended to reduce interoceptive abilities on the heartbeat discrimination task. However, importantly, the more participant drank alcohol, the more OT

increased interoceptive accuracy when compared to placebo, potentially driven by an attentional mechanism. Alexithymia did not significantly contribute to this relationship.

In Chapter 6, my aim was to characterise the functional cerebral activity underlying empathy-for-pain (using multiband fMRI), and address underlying interoceptive contributions using physiological (cardiac timing presentation) but also pharmacological (through OT administration) manipulations of visceral input. I found that the right anterior insula (AI) was involved in the integration of emotional exteroceptive stimulations and interoceptive signals. Also, while OT generally decreased the activation of the empathy-for-pain matrix across experimental conditions, the processing of painful pictures at cardiac systole seemed less (or not) affected by OT administration. This highlights the interacting contributions of putative top-down oxytocin-mediated, and bottom-up cardiac afferent aspects of interoception in this non-clinical panel of alcohol users. Neither overall alcohol use nor alexithymia score influenced pain ratings on this task.

In Chapter 7, my aim was to understand better the relationship between bodily sensations and decision-making during emotional processing, and how this relates to alexithymia. I sought to identify how physiological changes (indexed by beat-to-beat systolic blood pressure) evoked by emotional primes (using an affective priming paradigm) influence cognition, specifically the accuracy and speed of decision-making. A second aim was to test whether alexithymia contributed to this relationship. I found that participants were more accurate in their judgments when primed by anger compared to neutral stimuli. Also, better accuracy was associated with increased systolic blood pressure, which seemed to be driven by the increased arousal induced by the affective priming. Finally, neither alexithymia (nor alcohol use) significantly modulated these relationships.

Taken together, these results suggest that conscious access to emotional states partly arises from the processing of internal bodily sensations, which also guide and influence behavioural response. This specific integration seems to involve specifically the insular cortex and can be modulated by the

administration of psychoactive substance such as the oxytocin. Moreover, in this work, we accumulated evidence in favour of a disruption of the insular cortex integrity associated with heavy alcohol use, which might explain deficits in integrating and processing visceral inputs. In the following sections, conclusions from all the experiments are discussed in a broader context.

8.2 Implications

The work contained within this thesis has great potential impact both at theoretical and clinical levels. First, it seems important to discuss the nature of alexithymia.

8.2.1 Alexithymia, no more than an interoceptive failure?

Alexithymia, etymologically, means “without word for emotions” (Apfel and Sifneos, 1979) and, for a long time, has been considered as a coping mechanism (Bogutyn *et al.*, 1999, Panayiotou *et al.*, 2015), due to its tight relationship with abuse and abnormal attachment profile (Fukunishi *et al.*, 1997, Fukunishi *et al.*, 1999, De Rick and Vanheule, 2006, Thorberg *et al.*, 2011, Besharat and Khajavi, 2013, Koelen *et al.*, 2016). I disagree with the argument that alexithymia is a coping mechanism; this implies that alexithymic individuals make an (unconscious) choice. Alexithymia does not seem to be a simple labelling problem, the result of Chapter 3 strengthens the hypothesis that alexithymia is, in fact, the outcome of a failure of interoception. As already mentioned, alexithymic individuals show impairments in emotional recognition, automatic imitation, empathy, self-awareness, theory of mind, and body representation malleability (Moriguchi *et al.*, 2006, Moriguchi *et al.*, 2007, Grynberg *et al.*, 2012, Grynberg and Pollatos, 2015, Lane *et al.*, 2015, Scarpazza *et al.*, 2015, Georgiou *et al.*, 2016, Lyvers *et al.*, 2017, Scarpazza *et al.*, 2017). Interestingly, alexithymia does not seem to be a purely verbal (labelling) problem (Lemche *et al.*, 2004), but instead appears to be rooted within the individual’s own body. Indeed, growing literature supports the notion that much of what is described as alexithymia seems to be the expression of a general interoceptive failure: alexithymic individuals show reduced cardiac and respiratory interoceptive accuracy (Brewer *et al.*, 2016, Shah *et al.*, 2016b, Murphy *et al.*, 2017), yet show a hypersensitivity to bodily sensations (e.g.

pain, physical symptoms, palpation/touch) at the subjective level (Sivik, 1993, Lumley *et al.*, 1996, Nyklicek and Vingerhoets, 2000, Nakao *et al.*, 2002, Lumley *et al.*, 2005, Kano *et al.*, 2007, Kojima, 2012, Kojima *et al.*, 2014).

These observations are in line with the results in Chapter 3. I found that alexithymia, and, more precisely difficulties in identifying feelings, mediated the relationship between sensitivity to bodily sensations and alcohol use. It is important to be cautious when discussing “statistical causality”; nevertheless, this finding suggests that impaired sensitivity to bodily sensations impacts the ability to identify feelings which, itself, modulates alcohol use. All relationships were positively associated. In that sense, higher self-report of bodily sensations sensitivity was related to increasing alexithymic features. This finding is in line with an overestimation of subjective bodily sensations in alexithymia. At the objective level, alexithymia is linked to reduced interoceptive accuracy (Brewer *et al.*, 2016, Shah *et al.*, 2016b, Murphy *et al.*, 2017). I did not replicate these findings in Chapter 5: alexithymia did not seem to impact interoceptive accuracy on the heartbeat tracking or discrimination task. However, in Chapter 7, I explored the relationship between physiological changes during emotional processing and behaviour, I found a trend of interaction between alexithymia and systolic blood pressure. In non-alexithymic subjects, a positive relationship was observed between accuracy on the task and increases in systolic blood pressure. This relationship was less clear in alexithymic individuals, suggesting they benefitted least from systolic blood pressure fluctuations. Taken together, it seems that a bigger sample and greater statistical power are needed to confirm a significant relationship between interoceptive and autonomic impairments. Nevertheless, one potential explanation for this trend is that alexithymic subjects might attribute less salience to bodily changes, and show impaired integration of autonomic information when compared to non-alexithymic. For example, cardiac changes induced by a behavioural error may serve as an interoceptive source of information when making metacognitive judgments about previous performance (Łukowska *et al.*, 2018). Error monitoring can be apprehended by looking at event-related potentials (ERP), e.g. the error-related negativity (ERN; Falkenstein *et al.*, 1991, Wessel, 2012), a frontocentral ERP occurring

shortly after an error, or the error positivity (Pe), a more parietal positive ERP which seems to be modulated by error awareness (Murphy *et al.*, 2012). Interestingly, during an emotional face categorisation task, alexithymia is unrelated to the number of errors but instead modulates ERN amplitude (Maier *et al.*, 2016). High alexithymic subjects show reduced error monitoring, as indexed by the ERN, when compared to low alexithymic participants. Moreover, this reduction correlates with difficulty in identifying feelings (Maier *et al.*, 2016). Altogether, these observations are in line with abnormal processing of interoceptive afferent signals, potentially leading to aberrant error monitoring and impaired emotional processing.

Importantly, as component hubs within the salience network (SN), both the anterior cingulate cortex (ACC) and the insular cortex are hypothesised to play a crucial role in salience attribution to stimuli from internal and external milieus (Seeley *et al.*, 2007, Sridharan *et al.*, 2008, Menon and Uddin, 2010). Moreover, the right anterior insula (AI) is implicated in supporting a representation of visceral responses that is accessible to awareness, correspondingly providing a substrate for subjective feeling states (Critchley *et al.*, 2004). In addition, both the SN and right AI cortex show robust activation during error processing and awareness (Orr and Hester, 2012, Ham *et al.*, 2013, Klein *et al.*, 2013). As alexithymia is characterized by subjective hypersensitivity to bodily sensations, but difficulty in identifying feelings, one can postulate that alexithymia is characterized by a general *metacognitive blindness*. In other words, alexithymic individuals may struggle to integrate low-level bodily sensations into high-level subjective feelings, with conscious access. This postulation is corroborated by a study showing that anterior insular cortex lesions can lead to acquired alexithymia (Hogeveen *et al.*, 2016). However, as attractive as this postulate is, the Pe (e.g. marker of error awareness) does not seem to be influenced by features of alexithymia (Maier *et al.*, 2016). Likewise, alexithymic individuals show reduced cardiac and respiratory interoceptive accuracy (Murphy *et al.*, 2017). This suggests that not only declarative access to subjective feelings but also the apprehension of less integrated internal events (e.g. heartbeat sensation) is disrupted in alexithymia. It would therefore be interesting to measure the magnitude of the

heartbeat-evoked potential (HEP), which is hypothesised to reflect cortical processing of cardiac afferent inputs (Montoya *et al.*, 1993) alongside the measurement of the temporal perception of heartbeat sensation, using a multi-interval discrimination task (Clemens, 1984, Brener and Kluvitse, 1988, Ring and Brener, 1992, Brener and Ring, 2016), in alexithymia. I would predict that alexithymia will be characterized by a reduced and delayed HEP. At the behavioural level, I further expect alexithymia to be associated with less precision in the temporal perception of heartbeat sensation. Also, it is possible that the abnormal amplitude or latency of the HEP might predict the temporal uncertainty of heartbeat sensation.

One of the limitations of studying alexithymia is caused by the way the concept is measured. Indeed, while a growing body of evidence suggests that alexithymia is characterized by an inability to feel and report accurately one's bodily sensations, in addition to having difficulty identifying and describing subjective feelings, researchers still label an individual as alexithymic based on self-assessment questionnaires. In other words, the 'sub-clinical diagnosis' of alexithymia is made on the basis of one's ability to recognize this lack of insight. I feel this is a major limitation, which may bias the selection of the alexithymic sample by potentially not capturing individuals with the worst alexithymia level (e.g. not able to recognize their impairments). Therefore, the development of objective ways to measure alexithymia is crucially needed. In this thesis, I found evidence showing that interoception and autonomic reactivity are both impaired in (and might, in fact, be the cause of) alexithymia. Therefore, it would be very interesting to measure multi-domain interoceptive abilities as well as autonomic reactivity (HRV, electrodermal activity, blood pressure, electroencephalography, electrogastrography) in a very large sample of people with and without alexithymia. By using classification tools such as machine learning, one could try to optimise the prediction of alexithymia scores based on all these objective measures. This way, it would also be possible to isolate and characterise sub-types of alexithymia, as has already been hypothesised (Vorst and Bermond, 2001).

Finally, these findings have important implications for the current definitions of alexithymia: alexithymia is defined conventionally as a personality construct,

whereby characteristic difficulties in emotion labelling are a possible outcome of interoceptive failure. The relationship between interoception and alexithymia might reflect a conceptual overlap. An extended definition of alexithymia, however, might thus describe the disorder on a broader spectrum of interoceptive dysfunction. This latter definition presents alexithymia on a continuum. Nevertheless, focused studies are still needed to understand better the mechanisms through which interoception contributes to alexithymia. I believe that alexithymia can be reduced to an interoceptive failure, which in turn might favour the development of alcohol use disorders.

8.2.2 Alcohol use disorder and interoception

A second main question of this thesis was the relationship between interoception and alcohol use disorders. In Chapter 4, I found that alcohol use severity and alcohol compulsion were associated with reduced insular glutamate-glutamine concentration and, that this metabolite concentration was predicted by reduced insular gyrification. Moreover, gyrification reduction was predicted by alcohol use severity.

These findings are in line with the insular atrophy observed in patients suffering from alcohol use disorders (Trick *et al.*, 2014, Xiao *et al.*, 2015, Thayer *et al.*, 2016, Yang *et al.*, 2016). Also, it is recognized that chronic alcohol intake leads to increase glutamatergic neurotransmission, which, in the case of acute cessation of drinking (withdrawal), leads to glutamatergic neurotoxicity, neuronal death and potential reduced grey matter volume (Lovinger *et al.*, 1989, Lovinger, 1993, Trujillo and Akil, 1995, Tsai *et al.*, 1995). Therefore, we postulated that the alcohol use of our participants led to excitotoxicity and atrophy which itself caused the reduction in insular metabolite concentrations. The insular cortex is implicated in supporting a wide range of behaviours from homeostatic regulation to consciousness (Tsakiris and Critchley, 2016). Also, the insula contributes to attribute salience and motivational dimensions to relevant stimulations from the environment (Menon and Uddin, 2010). In the context of addiction, it is proposed that alcohol or drug craving (i.e. the interoceptive component of drug seeking) involves the insular cortex. Indeed, patients with insular lesions show disruption of smoking

addiction (Naqvi et al., 2007). In addition, treatment combining high frequency deep repetitive transcranial magnetic stimulation (rTMS) targeted to the lateral PFC and bilateral insulae significantly reduced tobacco consumption and nicotine dependence (Dinur-Klein *et al.*, 2014). In Chapter 4, I found that reduced glutamate plus glutamine concentration was negatively related to alcohol craving indexed by alcohol compulsion. This is in line with a MRS study showing decreased glutamate concentration in the right thalamus of patients suffering from obsessive-compulsive disorder (OCD). Here, glutamate concentrations and patients' compulsion scores negatively correlate (Zhu *et al.*, 2015). This study did not measure glutamate in insular cortex, yet, insular cortices are reciprocally connected to thalami (Mufson and Mesulam, 1984, Craig, 2002). In a second study of the same research group, compulsion was associated with greater right insular cortex intrinsic activity in OCD patients (Zhu *et al.*, 2016). Also, increased insular activation (to alcohol-relevant pictures) predicts subsequent transition to heavy drinking in students (Dager *et al.*, 2014). Interestingly, evidence suggests that rTMS increases glutamatergic neurotransmission (Michael *et al.*, 2003, Yue *et al.*, 2009, Croarkin *et al.*, 2016) and can enhance neurogenesis in animals (Ueyama *et al.*, 2011, Zhang *et al.*, 2014), also cortical thickness in depressed patients (Boes *et al.*, 2018). One could postulate that deep rTMS targeting insular cortices might decrease alcohol compulsion, *via* regulation of insular glutamatergic neurotransmission and insular grey matter volume recovery. This notion adds to converging evidence for insular cortex dysfunction in drug abuse and addiction (Naqvi and Bechara, 2010, May *et al.*, 2013, Migliorini *et al.*, 2013, Berk *et al.*, 2015, Senatorov *et al.*, 2015).

These findings build upon a growing neuroscientific understanding of brain mechanisms implicated in substance and alcohol use disorders. For example, neuroimaging studies of alcohol-dependent adolescents relate the structural integrity of white matter around right insula to obsessions and craving for alcohol (Chung and Clark, 2014). Since the right insular cortex is particularly implicated as a key interoceptive hub within the brain (Critchley *et al.*, 2004), that has a preeminent role in the representation of internal bodily state (Medford and Critchley, 2010), these findings can be regarded as an indirect

demonstration of a relationship between interoception and alcohol-related behaviours. Sensitivity to bodily sensations appears impaired across different populations of substance misusers, from methamphetamine users (May *et al.*, 2013), to adolescent cannabis users (Migliorini *et al.*, 2013, Berk *et al.*, 2015). Related patient groups with compulsive 'addictive' behaviours, including anorexia nervosa (Kerr *et al.*, 2016) and internet gaming disorder show similar patterns (Zhang *et al.*, 2016).

In alcohol-dependent patients, interoceptive accuracy is impaired and this impairment correlates with alcohol craving (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). Contrary to the literature, in Chapter 5, I did not find that alcohol use was related to poorer interoceptive accuracy on the heartbeat tracking task nor on the heartbeat discrimination task. This suggests that interoceptive accuracy is not impaired in non-clinical alcohol users. In fact, I found a significant interaction between OT and alcohol intake on performance of the discrimination task: compared to placebo, OT administration was associated with improved interoceptive accuracy in heavy drinkers, but not in mild social drinkers. As this effect was not observed in the tracking task, I tried to explain the discrepancy in terms of key differences between the two tasks: while the tracking task requires attention to internal bodily signals (e.g. counting heartbeats), the discrimination task requires simultaneous attention to, and integration of, internal (e.g. heartbeat) and external (e.g. auditory) information (Garfinkel *et al.*, 2015, Garfinkel *et al.*, 2016b). Importantly, multisensory integration of emotional stimulation is impaired in alcohol use disorders (Maurage *et al.*, 2008, Maurage *et al.*, 2009, Maurage and Campanella, 2013). Given the potential modulatory role of OT in salience attribution (Yao *et al.*, 2017), I postulated that OT might favour integration between internal and external stimuli; and that heavy drinkers (i.e. more impaired) might thus benefit from OT more than mild social drinkers. Even if interoceptive accuracy was not impaired *per se* in this sample of alcohol users, it would be interesting to gain follow up longitudinal data. Indeed, in line with findings in clinical alcohol-dependent patients, I predict the aggravation of interoceptive abilities as a consequence of prolonged alcohol misuse. Moreover, this worsening would be predicted by insular neurochemical and structural changes.

However, in Chapter 4, I acknowledge that I considered the reduction of insular cortical folding as an alcohol-related atrophy, since insular gyrification index was inversely predicted by alcohol use severity. Gyrification index is usually used to quantify differences in cortical folding reflecting structural abnormalities of neurodevelopmental origin. For example, there is extensive literature exploring the relationship between gyrification and prenatal alcohol exposure in children and adolescents (De Guio *et al.*, 2014, Kuhn *et al.*, 2016, Hendrickson *et al.*, 2017, Hendrickson *et al.*, 2018). To my knowledge, the gyrification index has not been used to evaluate alcohol-related structural changes in adults, despite evidence suggesting surface-based morphometry (SBM) is able to capture subtler grey matter changes than voxel-based morphometry (VBM; Hutton *et al.*, 2009, Kelly *et al.*, 2013). Nevertheless, it is important to notice that the reduced gyrification/metabolites concentration may be considered as a predisposition toward alcohol use. For example, reduced left insula grey matter volume is observed in children at familial risk of developing alcohol use disorders (Sharma and Hill, 2017). Longitudinal studies would help clarify the relationship between alcohol use disorders and insular cortex integrity. Nonetheless, the potential hypothesis of a predisposition aligns with findings in Chapter 3.

In Chapter 3, I found that the relationship between subjective measures of sensitivity to bodily sensations and alcohol use was mediated by difficulties in describing feelings. In other words, when taking in account alexithymia, sensitivity to bodily sensations was not significantly predicting alcohol use anymore. This also explains why sensitivity to bodily sensations was not significantly correlated to alcohol use, suggesting the absence of a direct relationship between subjective report of body sensations and alcohol intake. Instead, the results of my mediation analysis show that sensitivity to bodily sensations impacted the expression of alexithymia which modulated alcohol use. These findings are supported by the literature showing that improvement in interoceptive accuracy was associated with a reduction in alexithymia scores (Bornemann and Singer, 2017). Also, alexithymia is not modulated by abstinence in alcohol-dependent individuals (de Timary *et al.*, 2008), but is a predictor of relapse (Loas *et al.*, 1997, Berking *et al.*, 2011); suggesting that

alexithymia modulates alcohol use, rather than the other way around. In that sense, it is possible that some interoceptive abilities predispose to alcohol use disorders. Strikingly, the majority of the studies looking at sensitivity to bodily sensations in adolescent drug users might be usefully re-interpreted (Migliorini *et al.*, 2013, Strigo *et al.*, 2013, Berk *et al.*, 2015). Therefore, we are in need of longitudinal studies looking at the evolution of the relationships between interoceptive abilities, emotional skills, cerebral structures and alcohol/drug use, from childhood to adulthood. Recently, a promising technique was developed to assess interoceptive abilities in infants, using electroencephalogram (Maister *et al.*, 2017). Another important implication of the results of my mediation analyses is that by modulating an individual's sensitivity to bodily sensations, we might reduce her/his deficits in describing emotional feelings and, potentially, reduce her/his alcohol consumption. As shown by Bornemann and Singer, in the context of a 9-month body scan training, interoceptive accuracy improvement at 3 months predicts the reduction in alexithymia at the end of the training (Bornemann and Singer, 2017). It would be very interesting to implement this kind of training in patients suffering from alcohol use disorders, while monitoring alcohol use before, during and after the training. One study already documented the benefit to her/his well-being of engaging in "mood self-assessment" by the alcohol-dependent patient (i.e. to pay attention to the emotional state the patient was in) (Krentzman *et al.*, 2015). Future therapeutic training should focus on interoceptive abilities in alcohol use disorders.

Finally, findings within this thesis can be interpreted in the framework of specific conceptual models, which might give mechanistic insights into the development of social cognition.

8.2.3 Emotion, interoception, oxytocin and predictive coding.

In Chapter 3, one main finding was that difficulties in identifying feelings, rather than difficulties describing feelings or externally oriented thinking, mediated the relationship between subjective bodily sensations and alcohol intake. These results are coherent with other studies of alcohol and substance users indicating a specific relationship between interoceptive accuracy and difficulties in identifying emotions (Sönmez *et al.*, 2016). Again, given the interpretation of the mediation analyses, it seems that bodily sensations inform abilities to identify feelings, as suggested by a previous study (Bornemann and Singer, 2017). Interestingly, good interoceptive abilities are also associated with stable body representations, greater emotional Theory-of-Mind processing, better ability to describe one's own emotion and increased recognition of emotional facial expressions (Tsakiris *et al.*, 2011, Terasawa *et al.*, 2014, Shah *et al.*, 2017).

Altogether, these observations are coherent with the hypothesis of an 'interoceptive simulation mechanism', within a predictive coding framework. Predictive coding is a framework used to simulate potential neural mechanisms underlying cognitive process; predictive coding models constantly generate and update hypotheses via feedback loops (Friston *et al.*, 2006). It is postulated that the understanding of one's own affective states arises from top-down simulation (interoceptive prediction) of likely bodily state and the integration of subsequent interoceptive afferent signals into the affective representation of self (Seth, 2013). The fact that sensitivity to bodily sensations predicted the ability to identify feelings, fits into an interoceptive predictive coding model.

However, the association between patterns of bodily sensations and "labelled feelings" must be learned. Fotopoulou and Tsakiris claim that, given the immaturity of the motor and sensory systems of the infant, this learning process is fundamentally social (Fotopoulou and Tsakiris, 2017). By interacting with the infant, the caregiver modifies interoceptive states of the offspring and allows the offspring to generate interoceptive predictions. Caregiver-infant interactions also favour the infant's "mentalization" of its

physiological states, a fundamental process for social cognition development. In this thesis, I would like to make the link between the two models proposed by Fotopoulou and Tsakiris' and by Quattrocki and Friston: both, I think, propose that OT plays a crucial role in the infant's "mentalization" of its physiological states. Indeed, OT may modulate the salience or prediction of interoceptive signals in order to favour learning (e.g. acquisition, construction or update of a generative model) (Quattrocki and Friston, 2014).

In the attachment theory proposed by Bowlby, the infant is genetically predisposed to need emotional support (e.g. dependence toward caregiver) and becomes "attached" to it, in order to favour survival (Bowlby, 1969). As its name gives it away, OT (from Ancient Greek ὀξύς,oxús, "swift" and τόκος - tókos, "childbirth") plays an essential role in labour and childbirth (Blanks and Thornton, 2003). However, the role of OT goes beyond labour and is important for attachment development, mother-infant pair-bonding and, more generally for motherhood (Gimpl and Fahrenholz, 2001, Carter, 2003, Young and Wang, 2004, Feldman, 2007, Swain *et al.*, 2007, Buchheim *et al.*, 2009, Bell *et al.*, 2014, Bosch and Young, 2017, Carter, 2017, Chambers, 2017). OT is also implicated in other forms of social behaviour: OT is shown to improve capacity for "mind-reading", empathy, self-other distinction and mimicry of emotional faces (Guastella *et al.*, 2010, Hurlemann *et al.*, 2010, Colonnello *et al.*, 2013, Panksepp and Panksepp, 2013). Also, OT administration can increase trust, enhance attention toward socially relevant stimuli and increase the detection of emotional signals of others (Domes *et al.*, 2007a, Guastella *et al.*, 2008, Keri and Kiss, 2011, Schulze *et al.*, 2011, Ellenbogen *et al.*, 2012, Lischke *et al.*, 2012, Van and Bakermans-Kranenburg, 2012, Leknes *et al.*, 2013, Perry *et al.*, 2013, Shamay-Tsoory *et al.*, 2013, Tollenaar *et al.*, 2013, Clark-Elford *et al.*, 2014, Kanat *et al.*, 2015). Recently, neuroimaging studies found that, under OT, participants chose to be closer to other people, and this effect was mediated by dorsal striatum activation (Cohen *et al.*, 2018). In patients with irritable bowel syndrome, intranasal OT administration can decrease visceral perception (Louvel *et al.*, 1996). In animals, OT can reduce sensitivity to bladder distension (Black *et al.*, 2009, Engle *et al.*, 2012). Intranasal OT reduces objective measures of interoception on a heartbeat tracking task.

(Yao *et al.*, 2017). These observations are consistent with Quattrocki and Friston's postulate in which OT is proposed to narrow the attribution of salience to internal bodily signals. This OT neuromodulation might then facilitate attentional deployment toward relevant external cues, which in turn favours associative learning between internal and external cues, promoting social cognition (Quattrocki and Friston, 2014). In the view of Fotopoulou and Tsakiris, OT might also favour infant's "mentalization" of its own physiological states (Fotopoulou and Tsakiris, 2017).

Importantly, some of the results from Chapter 5 are in line with this postulate. First, I found that OT administration reduced interoceptive accuracy on the tracking task. Contrary to the discrimination task, the tracking task requires participants to pay attention to their internal bodily sensations. This finding is in line with the OT-related (interoceptive) sensory attenuation. Moreover, OT selectively improves the interoceptive accuracy of heavy drinkers on the discrimination task. The discrimination task requires participants to pay attention to internal and external stimulations and to integrate them. Also, multimodal integration is impaired in alcohol-dependent subjects (Maurage *et al.*, 2008, Maurage *et al.*, 2009). In addition, given the cognitive-physiological theory of 'alcohol myopia', alcohol allows drinkers to narrow their perceptual abilities and to focus in more immediate and salient aspects of experience (e.g. externally-oriented thoughts) (Steele and Josephs, 1990, Fairbairn and Sayette, 2013). Indeed, acute alcohol use is shown to disrupt the key nodes of the salience network, suggesting compromised internal monitoring (Padula *et al.*, 2011, Gorka *et al.*, 2017, Grodin *et al.*, 2017). Thus, it is possible that alcohol users have difficulties disengaging from their internal milieu/inner thoughts, suggesting potential impairments in switching between internal to external stimulations. For example, this kind of deficit has been found in subjects suffering from OCD (Stern *et al.*, 2017). The fact that OT is beneficial for heavy drinkers in the discrimination task, but not in the tracking task, is, therefore, in line with the role of OT in attentional deployment toward the external milieu.

It is very likely that the insular cortex is involved in interoceptive attenuation and attentional deployment. As part of the “salience network” (Seeley *et al.*, 2007), the AI is hypothesized to detect the most relevant bottom-up events among internal and external environments, and to temporarily initiate attentional control inputs, which, then, are sustained by ACC (Menon and Uddin, 2010). The right AI is involved in switching between central-executive and default-mode networks (Sridharan et al., 2008). Moreover, during the heartbeat tracking task combined with presentation of external stimulation (emotional faces), both OT-induced increased right AI activation and OT-induced increased functional connectivity between the anterior and posterior insula correlate negatively with interoceptive accuracy scores (Yao *et al.*, 2017). Similarly, in Chapter 6, we found that while OT reduced activation within the AI and ACC, this effect was blocked when viewing pain stimuli at systole. At ventricular systole, arterial baroreceptors are activated by phasic ejection of blood from the heart, triggering interoceptive signals that are sent to the brain (Bronk and Stella, 1932). These observations are consistent with the hypothesis of an attenuation of interoceptive prediction errors by OT, indexed by an activation of the anterior insular cortex, in the context of competing interest between interoceptive signals and relevant external stimulations. This increased activation might also be the results of an attentional switch from internal toward external milieu (Sridharan et al., 2008). By extension, this effect would benefit the processing of emotional and salient external cues. Nevertheless, further studies should directly test for the role of oxytocin in interoceptive attenuation, in the switch between internal and external attention and in associative learning. Recently, evidence of dopamine-mediated precision weighted unsigned prediction error signals (i.e. the degree to which an outcome is unexpected) has been found in the dorsal ACC, using computational reinforcement-learning modelling of behaviour on functional MRI data (Haarsma *et al.*, submitted). It would be interesting to apply similar computational modelling techniques to explore the role of OT in social cognition development.

I have integrated data from the literature with my key findings to propose a schematic model of the role of the insular cortex in salience attribution (see **Error! Reference source not found.**). Salience attribution seemed to be modulated by alcohol misuse (Figure 8.2) and by intransal oxytocin adminstration (Figure 8.3). These thesis findings should be considered in the light of several constraints.

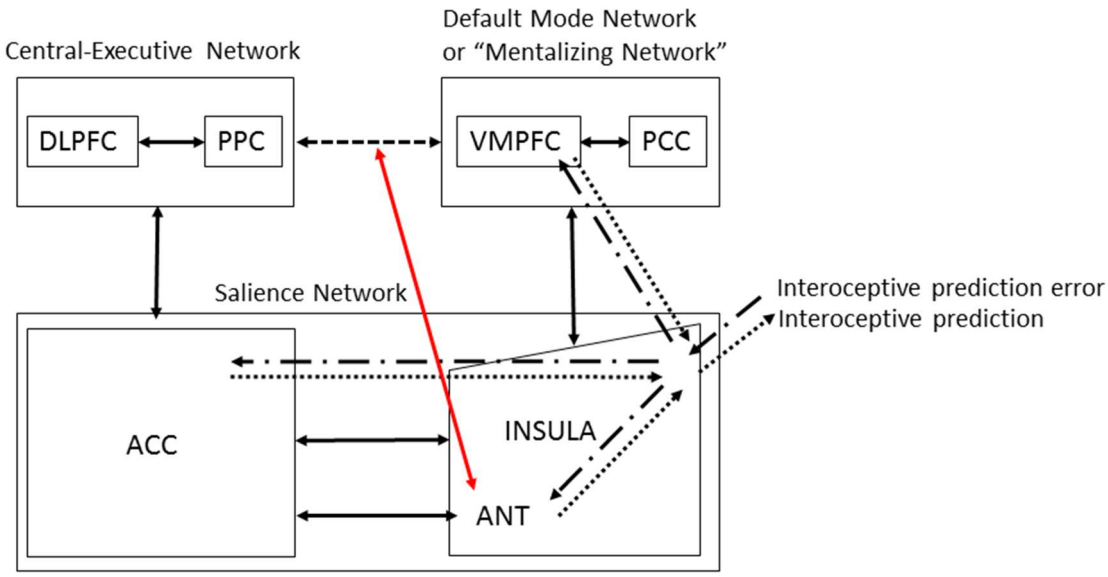


Figure 8.1 Schematic model about the role of the insular cortex in salience attribution

The Central-Executive Network (CEN) is composed of the dorsolateral prefrontal cortex (DLPFC) and the posterior parietal cortex. The Default Mode Network (DNM), also known as the mentalizing network, is composed of the ventromedial prefrontal cortex (VMPFC) and the posterior cingulate cortex (PCC). The Salience Network is composed of the anterior insula (ANT) and of the anterior cingulate cortex (ACC). CEN and DNM are anti-correlated (black dashed arrow) and the anterior insula is able to coordinate the switch between these two networks (e.g. external versus internal attention; red arrow). Agranular cortices such as anterior insula, ACC and VMPFC are hypothesised to send interoceptive changes predictions (interoceptive prediction; black dotted line) while granular parts compute and send interoceptive predictions errors (black dotted/dashed line) back to visceromotor cortices in order to modify predictions

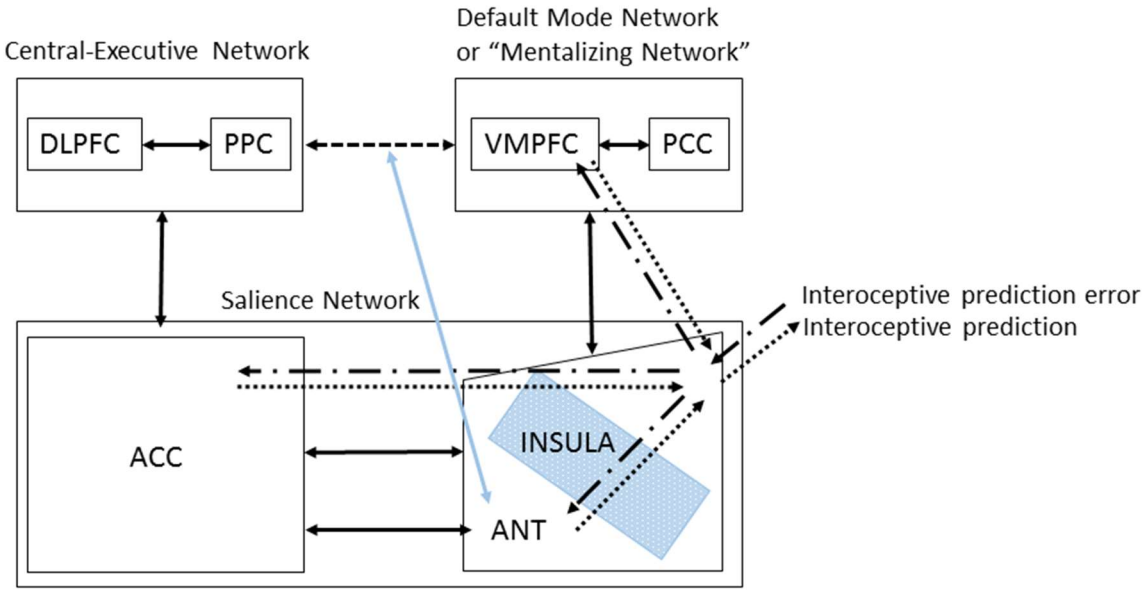


Figure 8.2 Schematic model about the role of the insular cortex in salience attribution, in alcohol users.

In alcohol misuse, the switch between CEN and DMN seems to be impaired (dashed blue arrow). Also a part of mid-insula (potentially dysgranular cortex) show reduced grey matter and reduced glutamate-plus-glutamine concentration due to heavy alcohol use (blue square); this might have an impact on the progression of interoceptive prediction and prediction error signals.

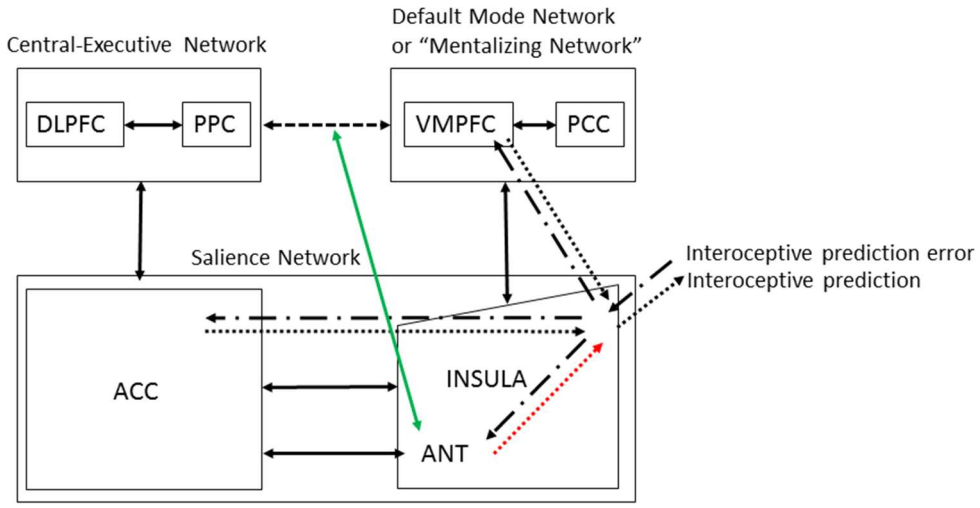


Figure 8.3 Schematic model about the role of the insular cortex in salience attribution, after inhalation of oxytocin.

Intranasal oxytocin seems to switch attention toward the external environment by reinforcing CEN-DMN interaction (green arrow). Oxytocin also leads to a concomitant attenuation of interoceptive signal (modulation of prediction error weight; red dotted arrow). This is mediated by an increased activation of the anterior insula (updating predictions signals and switching between DMN/CEN) and an increased intrinsic insular functional connectivity (potentially between agranular and granular insular cortices). The simultaneous interoceptive attenuation and attentional deployment toward the external environment induced by oxytocin might favour associative learning. Moreover, the involvement of the DMN could explain the mentalization of interoceptive signals crucial for social cognition development.

8.3 Limitations of the general project

This PhD thesis was composed of two different projects, divided into five experiments.

The main limitation of study 1 was our (pragmatic) use of self-report questionnaires to assess alexithymia, alcohol consumption, and especially sensitivity to bodily sensations. The measurement of alexithymia using self-report was not optimal either, given that alexithymic subjects, by definition, show biased insights into their bodily and emotional states. Future studies should lead to the development of an objective measure of alexithymia (e.g. inferred from multi-dimensional interoceptive accuracy). A second limitation was the use of cross-sectional design, which restricted our interpretations in term of causation. Prospective cohort studies could clarify the nature of relationships between interoception, alexithymia and risk-taking behaviours such as alcohol use disorders.

The main constraint of study 2 lies in its methodological complexity. My PhD project was ambitious and involved many inter-related factors of interest (i.e. interoception, alexithymia, alcohol use and oxytocin). This complexity made it harder for me to answer fully all my research questions as definitive conclusions. Arguably a simpler design may have helped. For example, instead of correlational approaches to the dataset (also a limitation of this work), if a meaningful control group can be identified then future studies would benefit from looking at differences between groups. The recruitment of thirty-two participants was satisfactory for experiments involving a within-subject design (Chapters 5 and 6). However, this sample was low for experiments involving one sample analyses included in Chapters 4 and 7. Fortunately, this work will provide important empirical data to inform the computation of future power calculations for relevant projects. In Chapter 4 and 6, my neuroimaging scanning was conducted at a low magnetic field homogeneity (1.5 Telsa), and this, despite the multiband acquisition, resulted in low statistical power to detect weaker effects. If possible, it would be useful for both experiments to be replicated at higher magnetic field (giving enhanced sensitivity through greater signal-to-noise). Concerning OT administration, the use of nasal spray

was convenient and widely used at the time when I designed the project (Guastella *et al.*, 2013). However, even after training, some participants showed difficulties in self-administrating the nasal spray. One potential alternative would have been the use of a nebulizer, which has been shown to be as efficient as nasal spray (Dal Monte *et al.*, 2014b). Also, it would have been interesting to collect blood before and after each drug administration, in order to add basal endogenous and exogenous plasmatic OT levels as covariates in the analyses. For example, it is possible that heavy drinkers damaged their blood-brain barrier, which could have modulated the amount of exogenous OT going into their brain (Haorah *et al.*, 2005, Haorah *et al.*, 2007).

Finally, I would like to add a more theoretical limitation to my work, which also extends to the current field of interoception. The limitation lies in the definition of interoception itself. Interoception used to be restricted conceptually to signals coming from the internal environment (e.g. information coming from the viscera and glands) as defined by Sherrington (1907). Recently, the importance of interoceptive processes is widely recognised, which has led to an increasing amount of research on the topic. However, the term interoception is sometimes used loosely and, broadened, for example, to include sensorial dimensions such as taste, touch, muscular effort and even subjective measurement of efferent autonomic activity; domains in which a pure interoceptive element is highly debatable (May *et al.*, 2013, Migliorini *et al.*, 2013, Berk *et al.*, 2015, Murphy *et al.*, 2017). For example, in Chapter 3, I deliberately used the term *sensitivity to bodily sensation* instead of *interoception* as I was using the Body Perception Questionnaire (BPQ) (Porges, 1993). The BPQ measures awareness of bodily events, which, for the majority, are not interoceptive (i.e. sweaty palms *versus* urge to urinate). Also, I think it is important to stress that interoception is part of, but is not a synonym for, body-brain interactions. Indeed, interoception is restricted to afferent signals from the body to the brain. Therefore, I think it is important to be careful and precise in what we consider to be interoceptive. I also think that it could be interesting to refer to the Sherrington's exclusive definition of interoception, by using the term *visceroception* in a new and stricter nomenclature.

8.4 Future directions for alcohol research

People suffering from drug and alcohol use disorders (AUD) show impaired emotional abilities. Difficulties in recognizing emotional facial expressions, prosody or postures are observed in alcohol-dependent individuals. (Maurage *et al.*, 2008, Maurage *et al.*, 2009, D'Hondt *et al.*, 2014). In addition, reduced emotional awareness, reduced empathy and interpersonal problems are commonly reported among drug and alcohol users (Kornreich *et al.*, 2002, Verdejo-Garcia, 2014). Furthermore, the presence of poor emotional skills predicts relapse, even after apparently effective cognitive-behavioural therapy (Berking *et al.*, 2011).

Recently, visceroception has acquired a resurgence of interest. Indeed, sensitivity to bodily signals coming from the viscera plays a crucial role in the generation of conscious affective states, and correspondingly, seems to underlie social cognition skills (Singer *et al.*, 2009, Ainley *et al.*, 2014, Terasawa *et al.*, 2014). I recently reported that difficulties in identifying, and differentiating between emotional feelings (a possible outcome of aberrant processing of bodily sensations) play a role in social drinking (Betka *et al.*, 2017). In addition, I found that alcohol use might be associated with an impairment in the ability to switch attention between interoceptive and exteroceptive cues (Betka *et al.*, 2018). Two other studies describe reduced objective measures of interoception in AUD; the authors also report a positive correlation between interoceptive impairment and subjective alcohol craving (Ates Çöl *et al.*, 2016, Sönmez *et al.*, 2016). Interestingly, interoception and drug craving share common cerebral substrates, notably the insular cortex (Gray and Critchley, 2007, Naqvi *et al.*, 2007), which shows reduced structural, functional and neurochemical integrity in AUD (O'Daly *et al.*, 2012, Senatorov *et al.*, 2015, Grodin *et al.*, 2017, Kohno *et al.*, 2017, Betka *et al.*, submitted). It is therefore likely that the insular cortex plays a crucial role in drug-seeking via interoceptive processes.

To date, the relationship between interoception, emotional regulation, and subjective craving in AUD, remains rather unexplored. Therefore, as part of my postdoctoral research, I would like to acquire high-resolution structural (DTI

NODDI), functional (Resting State) and spectroscopic data to quantify the integrity of the component nodes of the interoceptive network in AUD. In parallel, I hope to define causality in interactions between interoceptive neural centres relevant to AUD, by applying advanced neuroimaging analyses methods (Dynamic Causal Modelling; Multivariate Pattern Analysis) to the combination of electrophysiological and neuroimaging datasets. I would like to measure alcohol craving, interoceptive sensitivity as well as the integration of exteroceptive and interoceptive signals in AUD, by combining psychophysics and visceral physiological approaches (e.g. multi-interval task). In addition, I would like to identify the impact of anti-craving medication (e.g. Acamprosate, Nalmefene) on interoceptive processes at the subjective, behavioural and cerebral levels in AUD.

Finally, I think it is important to use computational neuroscience in future research to model processes relevant to AUD (e.g. update of generative models, weight of prediction errors, contribution of different cortical layers using layer fMRI techniques), and apply these models to test hypotheses arising from an interoceptive predictive coding model of AUD.

8.5 Conclusion

In humans, emotional states can arise from the body *via* interoceptive signals integrated within the insular cortex. These signals carry high motivational weights and guide both adaptive and maladaptive behaviours, including substance misuse. In this PhD thesis, my results extend the literature beyond alcohol addiction, by showing that non-clinical alcohol drinkers also display abnormal bodily sensitivity. Correspondingly, this work supports the hypothesized contribution of interoceptive processes to the development and, potentially, the maintenance of alcohol use disorders. My observations motivate the need to take equally into account interoceptive processes in the understanding and treatment of compulsive and addictive behaviour. More broadly, further research is needed to investigate the role of interoception in addiction. Nevertheless, therapeutic intervention targeting interoception could potentially decrease the likelihood of alcohol use disorders.

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Appendices

Ethics Approval Studies 1 & 2

BSMS Research Governance & Ethics Committee (RGEC)
Chair: Professor Kevin Davies
Deputy Chair: Professor Bobbie Farsides
Secretary: Miss Caroline Brooks
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16/06/2015

Professor Hugo D Critchley
Brighton & Sussex Medical School
Clinical Imaging Sciences Centre (CISC)
Brighton and Sussex Medical School
University of Sussex
Falmer, Brighton
BN1 9RR

Dear Professor Critchley

Full Study Title: **Impact of oxytocin on emotional regulation in binge drinkers: Behavioural and fMRI investigations**

R&D Ref No. : **15/093/CRI**

I am writing to inform you that the Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) has now assessed your application and granted **Research Governance Approval** to proceed with the above named project.

This letter acknowledges that you have the necessary internal regulatory approvals. The sites covered by this approval include:

- Clinical Imaging Sciences Centre (CISC) University of Sussex

Conditions of Approval
The approval covers the period stated in the Research Governance & Ethics Committee (RGEC) application and will be extended in line with any amendments agreed by the RGEC. Research must commence within 12 months of the issue date of this letter. Any delay beyond this may require a new review of the project resources.

Amendments
Project amendment details dated after the issue of this approval letter should be submitted to RGEC for review and formal approval. Please submit your application for an amendment to the Committee (via rgec@bsms.ac.uk) using the 'Request for an Amendment Form'.

Monitoring
The Medical School has a duty to ensure that all research is conducted in accordance with the University's Research Governance Code of Practice. In order to ensure compliance the department undertakes random audits. If your project is selected for audit you will be given 4 weeks notice to prepare all documentation for inspection.

It is your responsibility to inform me in the event of early termination of the project or if you fail to complete the work.

I wish you luck with your project.

Yours sincerely

Professor Kevin Davies
Chair of the BSMS Research Governance and Ethics Committee

Online Survey – Study 1

Q1 Thank you for taking the time to look at our questionnaire!

Social alcohol consumption is common in countries such as Britain, and elsewhere in Europe. To understand this habit, it is necessary to explore the potential relationship between social drinking and emotional regulation. This study aims to examine whether emotional awareness influences social alcohol consumption. It would appear that there are few studies on this topic and very little data is currently available; for this reason, it is necessary to conduct an initial online screening study.

This questionnaire is the first step of a larger project. If you express an interest (case to tick at the end of the questionnaire), you may be invited to take part in further linked studies involving neuroscientific investigations.Please note, participation at this stage does not mean that you will be obliged to participate in future. Some question will be asked about your use of alcohol and other drugs.There may be some repetition. Please take a few minutes (~20 min) to fill it out.This questionnaire has been designed as part of a project run by the Brighton and Sussex Medical School. It has been approved by the BSMS Research Governance and Ethics Committee (BSMS RGEC).Your participation in this experiment is completely voluntary and you have the right to withdraw from this experiment at any time, without giving an explanation, including after your participation and the data collection of this experiment has been completed. Furthermore, withdrawing from this experiment will have no impact on your marks, assessments or future studies.

If you would like to be entered into a prize draw for £25 completing this study please enter your email address in the final question: this is not compulsory. Your email address will not be stored with any of the response data and any information we receive will be kept strictly confidential and handled in accordance with the Data Protection Act 1998.Your email address will be kept in a password protected excel spread sheet in case you should you wish to withdraw your data from the study (which you may do at any stage) and in order to hold the prize draw when data collection is complete. By clicking on

the “I agree” option below you are consenting to take part in this study and indicating that you understand that any information offered from this investigation will be treated as strictly confidential and handled in accordance with the data protection act 1998.

- ❍ I agree (1)
- ❍ I disagree (2)

If I disagree Is Selected, Then Skip To End of Survey

Q2 What year were you born?

- ❍ 2000 (1)
- ❍ ….
- ❍ 1900 (101)

Q3 What month are you born?

- ❍ January (1)
- ❍ February (2)
- ❍ March (3)
- ❍ April (4)
- ❍ May (5)
- ❍ June (6)
- ❍ July (7)
- ❍ August (8)
- ❍ September (9)
- ❍ October (10)
- ❍ November (11)
- ❍ December (12)

Q4 What date are you born?

- ❍ 1 (1)
- ❍ 2 (2)
- ❍ 3 (3)
- ❍ 4 (4)
- ❍ 5 (5)
- ❍ 6 (6)
- ❍ 7 (7)
- ❍ 8 (8)
- ❍ 9 (9)
- ❍ 10 (10)
- ❍ 11 (11)
- ❍ 12 (12)
- ❍ 13 (13)
- ❍ 14 (14)
- ❍ 15 (15)
- ❍ 16 (16)
- ❍ 17 (17)
- ❍ 18 (18)
- ❍ 19 (19)
- ❍ 20 (20)
- ❍ 21 (21)
- ❍ 22 (22)
- ❍ 23 (23)
- ❍ 24 (24)
- ❍ 25 (25)
- ❍ 26 (26)
- ❍ 27 (27)
- ❍ 28 (28)
- ❍ 29 (29)
- ❍ 30 (30)
- ❍ 31 (31)

Q5 Gender

- ❍ Male (1)
- ❍ Female (2)
- ❍ Other (3)

Answer If Gender Female Is Selected

Q18 Are you currently pregnant or breastfeeding?

- ❑ Yes (1)
- ❑ No (2)

If Yes Is Selected, Then Skip To End of Survey

Q29 Do you have any children?

- ❍ Yes (1)
- ❍ No (2)

Answer If Do you have any children? Yes Is Selected

Q30 If yes, how many?

- ❍ 1 (1)
- ❍ 2 (2)
- ❍ 3 (3)
- ❍ 4 (4)
- ❍ 5 (5)
- ❍ 6 (6)
- ❍ 7 (7)
- ❍ 8 (8)
- ❍ 9 (9)
- ❍ more than 10 (10)

Q6 Is English your native language?

- ❍ Yes (1)
- ❍ No (2)

Answer If Is English your native language? No Is Selected

Q7 Are you fluent in writing and reading English?

- ❍ Yes (1)
- ❍ No (2)

If No Is Selected, Then Skip To End of Survey

Q8 What is your dominant hand? For example, which hand to you use to write with?

- ❍ Right (1)
- ❍ Left (2)

Q9 What is the highest level of education you have completed?

- ❍ Less than High School (1)
- ❍ High School / GED (2)
- ❍ Some College (3)
- ❍ 2-year College Degree (4)
- ❍ 4-year College Degree (5)
- ❍ Masters Degree (6)
- ❍ Doctoral Degree (7)
- ❍ Professional Degree (JD, MD) (8)

Q17 Do you have a history of high blood pressure or any other cardiovascular condition ?

- ❍ Yes (1)
- ❍ No (2)

Q19 Do you have a history of psychological or psychiatric problems ?

- ❍ Yes (1)
- ❍ No (2)

Answer If Do you have a history of psychological or psychiatric problems ? Yes Is Selected

Q20 If yes, please state which disease you are suffer from below:

Q21 Are you taking any medication (apart from oral contraceptive pill)?

- ❍ Yes (1)
- ❍ No (2)

Answer If Are you taking any medication (apart from oral contraceptive pill)? Yes Is Selected

Q22 If yes, please state which medications you are taking below:

Q23 Do you smoke?

❍ Yes (1)
❍ No (2)

Answer If Do you smoke? Yes Is Selected

Q24 Please estimate, on average, how many cigarettes you smoke a day?

_____ Number of cigarettes by day (1)

Q25 Do you use cannabis?

❍ Yes (1)
❍ No (2)

Answer If Do you use cannabis? Yes Is Selected

Q26 Please estimate, on average, how many grams you use a week?

_____ Number of grams of cannabis by week (1)

Q28 Do you use other drugs (expect alcohol, tobacco and cannabis)?

❍ Yes (1)
❍ No (2)

Answer If Do you use other drugs (expect alcohol, tobacco and cannabis)? Yes Is Selected

Q27 If yes, please, can you state the frequency of drug use below:

	Never (1)	Rarely (2)	Sometimes (3)	Most of the Time (4)	Always (5)
Cocaine (1)	❍	❍	❍	❍	❍
MDMA (ecstasy) (2)	❍	❍	❍	❍	❍
LSD (3)	❍	❍	❍	❍	❍
Mushroom (4)	❍	❍	❍	❍	❍
Ketamine (5)	❍	❍	❍	❍	❍
Heroin (6)	❍	❍	❍	❍	❍

Q29 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 behavior.

	I strongly disagree (1)	I quite disagree (2)	I neither agree or disagree (3)	I quite agree (4)	I strongly agree (5)
1- I am often confused about what emotion I am feeling (1)	❍	❍	❍	❍	❍
2- It is difficult for me to find the right words for my feelings (2)	❍	❍	❍	❍	❍
3- I have physical sensations that even doctors don't understand (3)	❍	❍	❍	❍	❍
4- I am able to describe my feelings easily (4)	❍	❍	❍	❍	❍
5- I prefer to analyze problems rather than just describe them (5)	❍	❍	❍	❍	❍

6- When I am upset, I don't know if I am sad, frightened, or angry (6)	❍	❍	❍	❍	❍
7- I am often puzzled by sensations in my body (7)	❍	❍	❍	❍	❍
8- I prefer to just let things happen rather than to understand why they turned out that way (8)	❍	❍	❍	❍	❍
9- I have feelings that I can't quite identify (9)	❍	❍	❍	❍	❍
10- Being in touch with emotions is essential (10)	❍	❍	❍	❍	❍
11- I find it hard to describe how I feel about people (11)	❍	❍	❍	❍	❍

12- People tell me to describe my feelings more (12)	❍	❍	❍	❍	❍
13- I don't know what's going on inside me (13)	❍	❍	❍	❍	❍
14- I often don't know why I am angry (14)	❍	❍	❍	❍	❍
15- I prefer talking to people about their daily activities rather then their feelings (15)	❍	❍	❍	❍	❍
16- I prefer to watch « light » entertainment shows rather than psychological dramas (16)	❍	❍	❍	❍	❍

17- It is difficult for me to reveal my innermost feelings, even to close friends (17)	❍	❍	❍	❍	❍
18- I can feel close to someone, even in moments of silence (18)	❍	❍	❍	❍	❍
19- I find examination of my feelings useful in solving personal problems (19)	❍	❍	❍	❍	❍
20- Looking for hidden meanings in movies or plays distracts from their enjoyment (20)	❍	❍	❍	❍	❍

Q30 The following questions ask you about your habitual use of various types of alcoholic drinks. Please consider your drinking for the last six months in answering the questions and take your time to give an accurate answer to each question. 1. On how many days per week did you drink wine (at least one small glass)?

- ❍ 0 (1)
- ❍ 1 (2)
- ❍ 2 (3)
- ❍ 3 (4)
- ❍ 4 (5)
- ❍ 5 (6)
- ❍ 6 (7)
- ❍ 7 (8)

Q33 2. On those days you did drink wine, about how many glasses (pub measure) did you drink?

- ❍ 0 (1)
- ❍ ….
- ❍ 500 (501)

Q32 3. How many glasses (pub measure) of wine did you have in a week, in total?

_____ Glasses of wine in a week (1)

Q35. 4. On how many days per week did you drink beer (at least half a pint)?

- ❍ 0 (1)
- ❍ 1 (2)
- ❍ 2 (3)
- ❍ 3 (4)
- ❍ 4 (5)
- ❍ 5 (6)
- ❍ 6 (7)
- ❍ 7 (8)

Q36 5. On those days you did drink beer, about how many pints did you typically have?

❍ 0 (1)
❍ ….
❍ 500 (501)

Q38 6. How many pints of beer did you drink in a week, in total?

_____ Pints of beer in a week (1)

Q40 7. On how many days per week did you drink only spirits (whiskey, vodka, gin etc) ?

❍ 0 (1)
❍ 1 (2)
❍ 2 (3)
❍ 3 (4)
❍ 4 (5)
❍ 5 (6)
❍ 6 (7)
❍ 7 (8)

Q41 8. On those days you did drink spirits, about how many shots did you typically have?

❍ 0 (1)
❍ ….
❍ 500 (501)

Q42 9. How many drinks of spirits did you have in a week, in total?

_____ Drinks of spirits in a week (1)

Q43 10. On how many days per week do you drink alcopops (Hooch, Bacardi Breezer etc.)?

❍ 0 (1)
❍ 1 (2)
❍ 2 (3)
❍ 3 (4)
❍ 4 (5)
❍ 5 (6)
❍ 6 (7)
❍ 7 (8)

Q44 11. On those days you drink alcopops, about how many bottles do you typically have?

❍ 0 (1)
❍ ….
❍ 500 (501)

Q45 12. How many bottles of alcopops do you have each week, in total?

_____ Bottles of alcopops in a week (1)

Q39 13. When you were drinking, how fast did you drink? (Here, a drink is a glass of wine, a pint of beer, or a shot of spirits, straight or mixed.)

❍ 7 or more drinks per hour (1)
❍ 6 drinks per hour (2)
❍ 5 drinks per hour (3)
❍ 4 drinks per hour (4)
❍ 3 drinks per hour (5)
❍ 2 drinks per hour (6)
❍ 1 drink per hour (7)

Q47 14. How many times have you been drunk in the last 6 months of drinking? (we mean loss of co-ordination, nausea, and/or inability to speak clearly, or blackout)

❍ 0 (1)
❍ ….
❍ 500 (501)

Q48 15. What percentage of the times did you get drunk?

_____ (1)

Q50 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. During most situations I am aware of:

	Never (1)	Occasionally (2)	Sometimes (3)	Usually (4)	Always (5)
1. swallowing frequently (1)	❍	❍	❍	❍	❍
2. a ringing in my ears (2)	❍	❍	❍	❍	❍
3. an urge to cough to clear my throat (3)	❍	❍	❍	❍	❍
4. my body swaying when I am standing (4)	❍	❍	❍	❍	❍
5. my mouth being dry (5)	❍	❍	❍	❍	❍
6. how fast I am breathing (6)	❍	❍	❍	❍	❍
7. watering or tearing of my eyes (7)	❍	❍	❍	❍	❍
8. my skin itching (8)	❍	❍	❍	❍	❍
9. noises associated with my digestion (9)	❍	❍	❍	❍	❍

10. eye fatigue or pain (10)	❍	❍	❍	❍	❍
11. muscle tension in my back and neck (11)	❍	❍	❍	❍	❍
12. a swelling of my body or parts of my body (12)	❍	❍	❍	❍	❍
13. an urge to urinate (13)	❍	❍	❍	❍	❍
14. tremor in my hands (14)	❍	❍	❍	❍	❍
15. an urge to defecate (15)	❍	❍	❍	❍	❍
16. muscle tension in my arms and legs (16)	❍	❍	❍	❍	❍

17. a bloated feeling because of water retention (17)	❍	❍	❍	❍	❍
18. muscle tension in my face (18)	❍	❍	❍	❍	❍
19. goose bumps (19)	❍	❍	❍	❍	❍
20. facial twitches (20)	❍	❍	❍	❍	❍
21. being exhausted (21)	❍	❍	❍	❍	❍
22. stomach and gut pains (22)	❍	❍	❍	❍	❍
23. rolling or fluttering my eyes (23)	❍	❍	❍	❍	❍
24. stomach distension or bloatedness (24)	❍	❍	❍	❍	❍

25. palms sweating (25)	❍	❍	❍	❍	❍
26. sweat on my forehead (26)	❍	❍	❍	❍	❍
27. clumsiness or bumping into people (27)	❍	❍	❍	❍	❍
28. tremor in my lips (28)	❍	❍	❍	❍	❍
29. sweat in my armpits (29)	❍	❍	❍	❍	❍
30. sensations of prickling, tingling, or numbness in my body (30)	❍	❍	❍	❍	❍
31. the temperature of my face (especially my ears) (31)	❍	❍	❍	❍	❍
32. grinding my teeth (32)	❍	❍	❍	❍	❍

33. general jitteriness (33)	❍	❍	❍	❍	❍
34. muscle pain (34)	❍	❍	❍	❍	❍
35. joint pain (35)	❍	❍	❍	❍	❍
36. fullness of my bladder (36)	❍	❍	❍	❍	❍
37. my eye movements (37)	❍	❍	❍	❍	❍
38. back pain (38)	❍	❍	❍	❍	❍
39. my nose itching (39)	❍	❍	❍	❍	❍
40. the hair on the back of my neck "standing up" (40)	❍	❍	❍	❍	❍
41. needing to rest (41)	❍	❍	❍	❍	❍
42. difficulty in focusing (42)	❍	❍	❍	❍	❍
43. an urge to swallow (43)	❍	❍	❍	❍	❍

44. how hard my heart is beating (44)	❍	❍	❍	❍	❍
45. feeling constipated (45)	❍	❍	❍	❍	❍

Q52 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 During stressful situations I am aware of:

	Never (1)	Occasionally (2)	Sometimes (3)	Usually (4)	Always (5)
46. vascular responses such as my face becoming flushed or pallid, or feeling faint. (1)	❍	❍	❍	❍	❍
47. body posture shifts such as being hunched over, head down, and knees locked. (2)	❍	❍	❍	❍	❍
48. muscle tone or tremor such as arms and legs feeling weak, hands shaking, and lips quivering. (3)	❍	❍	❍	❍	❍
49. breathing more rapidly and shallowly, and having difficulty in catching my breath. (4)	❍	❍	❍	❍	❍

50. digestive responses including gastric distress, gas, cramps, and diarrhea. (5)	❍	❍	❍	❍	❍
51. difficulty in paying attention with my mind wondering or day dreaming. (6)	❍	❍	❍	❍	❍
52. difficulties in sensory abilities such as problems hearing, seeing, smelling, or feeling touch. (7)	❍	❍	❍	❍	❍
53. emotional problems such as more frequent feelings of depression, frustration, rage, or anger. (8)	❍	❍	❍	❍	❍
54. difficulty organizing my thoughts. (9)	❍	❍	❍	❍	❍

55. difficulty speaking clearly and understandably. (10)	❍	❍	❍	❍	❍

Q51 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

beendeveloped to measure how your autonomic nervous system reacts , using the following 5-point scale, rate yourself on each of the statements below.

	Never (1)	Occasionally (2)	Sometimes (3)	Usually (4)	Always (5)
56. I feel nauseous. (1)	❍	❍	❍	❍	❍
57. I have difficulty coordinating breathing and eating. (2)	❍	❍	❍	❍	❍
58. My nose is runny, even when I am not sick. (3)	❍	❍	❍	❍	❍
59. When I am eating, I have difficulty talking. (4)	❍	❍	❍	❍	❍
60. My heart often beats irregularly. (5)	❍	❍	❍	❍	❍
61. When I eat, food feels dry and sticks to my mouth and throat. (6)	❍	❍	❍	❍	❍

62. I have "sour" stomach. (7)	❍	❍	❍	❍	❍
63. I feel like vomiting. (8)	❍	❍	❍	❍	❍
64. I feel shortness of breath. (9)	❍	❍	❍	❍	❍
65. I have difficulty coordinating breathing with talking. (10)	❍	❍	❍	❍	❍
66. When I eat, I have difficulty coordinating swallowing, chewing, and/or sucking with breathing. (11)	❍	❍	❍	❍	❍
67. I have a persistent cough that interferes with my talking and eating. (12)	❍	❍	❍	❍	❍

68. I drool, especially when I am excited. (13)	❍	❍	❍	❍	❍
69. I gag from the saliva in my mouth. (14)	❍	❍	❍	❍	❍
70. I produce a lot of saliva even when I am not eating. (15)	❍	❍	❍	❍	❍
71. I have difficulty adjusting my eyes to changes in illumination. (16)	❍	❍	❍	❍	❍
72. I have chest pains. (17)	❍	❍	❍	❍	❍
73. I gag when I eat. (18)	❍	❍	❍	❍	❍

74. When I talk, I often feel I should cough or swallow the saliva in my mouth. (19)	❍	❍	❍	❍	❍
75. I am constipated. (20)	❍	❍	❍	❍	❍
76. I have indigestion. (21)	❍	❍	❍	❍	❍
77. After eating I have digestive problems. (22)	❍	❍	❍	❍	❍
78. I have diarrhea. (23)	❍	❍	❍	❍	❍
79. When I breath, I feel like I cannot get enough oxygen. (24)	❍	❍	❍	❍	❍
80. I have difficulty controlling my eyes. (25)	❍	❍	❍	❍	❍

81. I get dizzy when urinating or having a bowel movement. (26)	❍	❍	❍	❍	❍
82. I have trouble focusing when I go into dimly or brightly illuminated places. (27)	❍	❍	❍	❍	❍

Q53 IV: STRESS STYLE 1 Each of us responds differently to stressful events and conditions. The Stress Style 1 Scale evaluates your style of responding to stress. Using the following 5-point scale, rate yourself on each

of the statements below. When I am emotionally stressed because of a specific problem:

	Never (1)	Occasionally (2)	Sometimes (3)	Usually (4)	Always (5)
83. I approach the problem head-on. (1)	❍	❍	❍	❍	❍
84. I withdraw. (2)	❍	❍	❍	❍	❍
85. I know that things will be better later, so I wait until I feel better before acting. (3)	❍	❍	❍	❍	❍
86. I know that things will go better if I act immediately. (4)	❍	❍	❍	❍	❍
87. I feel mental tension. (5)	❍	❍	❍	❍	❍
88. I feel frustrated. (6)	❍	❍	❍	❍	❍
89. I feel insecure. (7)	❍	❍	❍	❍	❍
90. I feel aimless. (8)	❍	❍	❍	❍	❍

Q54 V: STRESS STYLE 2 Each of us responds differently to stressful events and conditions.The Stress Style 2 Scale evaluates your style of responding to stress. Using the following 5-point scale, rate yourself on each of the statements below. When I am emotionally stressed because of a specific problem:

	Never (1)	Occasionally (2)	Sometimes (3)	Usually (4)	Always (5)
91. I feel dizzy. (1)	❍	❍	❍	❍	❍
92. I have difficulty speaking. (2)	❍	❍	❍	❍	❍
93. I feel a tingling in my face. (3)	❍	❍	❍	❍	❍
94. I feel my blood sugar drop. (4)	❍	❍	❍	❍	❍

Q55 VI: HEALTH HISTORY INVENTORY I experience, have experienced, or have been diagnosed as having:

	Never (1)	Mild (2)	Moderate (3)	Severe (4)	Debilitating (5)
95. migraine headaches (1)	❍	❍	❍	❍	❍
96. gastric distress or digestive problems (2)	❍	❍	❍	❍	❍
97. arthritis (3)	❍	❍	❍	❍	❍
98. hypertension (4)	❍	❍	❍	❍	❍
99. hopeless unhappiness (5)	❍	❍	❍	❍	❍
100. clinical depression (6)	❍	❍	❍	❍	❍
101. bulimia (7)	❍	❍	❍	❍	❍
102. anorexia (8)	❍	❍	❍	❍	❍
103. obesity (9)	❍	❍	❍	❍	❍
104. asthma (10)	❍	❍	❍	❍	❍

105. endocrine problems (e.g., thyroid, adrenal, or gonadal hormone dysfunction) (11)	❍	❍	❍	❍	❍
106. eczema (12)	❍	❍	❍	❍	❍
107. edema (13)	❍	❍	❍	❍	❍
108. back problems (14)	❍	❍	❍	❍	❍
109. diabetes (15)	❍	❍	❍	❍	❍
110. epilepsy (16)	❍	❍	❍	❍	❍
111. cancer (17)	❍	❍	❍	❍	❍
112. hypoglycemia (18)	❍	❍	❍	❍	❍
113. heart disease (19)	❍	❍	❍	❍	❍
114. stroke (20)	❍	❍	❍	❍	❍
115. gastric & duodenal ulcers (21)	❍	❍	❍	❍	❍

116. psychiatric disorders (22)	❍	❍	❍	❍	❍
117. pneumonia (23)	❍	❍	❍	❍	❍
118. heart attack (24)	❍	❍	❍	❍	❍
119. motion sickness (25)	❍	❍	❍	❍	❍
120. premenstrual syndrome (26)	❍	❍	❍	❍	❍
121. severe menstrual cramps (27)	❍	❍	❍	❍	❍
122. post-partum depression (28)	❍	❍	❍	❍	❍

Q57 DEMOGRAPHICS ANDHEALTH BEHAVIOR SURVEY1. Perceived physical fitness

❍ Very fit (1)
❍ fit (2)
❍ Average fitness (3)
❍ Unfit (4)

Q58 2. Current substance use (alcohol, drugs, cigarettes)

- ❍ No use (1)
- ❍ Slight social use (2)
- ❍ Great social use (3)
- ❍ Abuse (4)
- ❍ Severe abuse (5)

Q59 3.a Smoking (number per typical day) of cigarettes

Q60 3.b Smoking (number per typical day) of cigares

Q61 3.c Smoking (number per typical day) of pipes

Q62 4. Alcohol Units (1/2pt small glass wine= 1/2 beer pint = 2cl of spirit = 1 unit) per typical week

Q63 5. Is there any strenuous physical activity on your current job?

- ❍ Yes (1)
- ❍ No (2)

Q64 6. Do you engage in regular physical exercise for recreation off the job.

- ❍ Yes (1)
- ❍ No (2)

Q65 Congratulations!

You have finished the questionnaire. Thank you very much for your help.

If you would like a chance to win £25 please enter your email address below, this will be kept separate from your response data.

Q67 You have completed an online study exploring the relationship between emotional regulation and social drinking. We would like to ask if you are willing to take part in similar paid studies? If you would be willing to be contacted with further information regarding this study, please tick the case. The researcher responsible for this new study is Sophie Betka and can be contacted on s.betka@bsms.ac.uk.

Thank you very much, Have a nice day.

- ❍ Yes, I would like to be contacted with further information regarding the study. (I understand that I am only giving consent to be contacted with further information about particular studies, and that I am not obliged to participate.) (1)
- ❍ No, thank you. (2)

Q68 DEBRIEFING

If you have any question about the questionnaire, please feel free to contact Sophie Betka on s.betka@bsms.ac.uk

Please find some information about alcohol and drug abuse.

Risks associated with heavy drinking

Excessive alcohol consumption can affect your physical and mental health, your work, and your social and personal relationships. You are also more likely to find yourself in dangerous situations if you have been drinking a lot, as alcohol affects your judgement and you may do things that you would not consider doing when sober. Health risks associated with heavy drinking include: liver disease (cirrhosis), anaemia and nutritional disease, pancreatitis, heart muscle damage, alcoholic dementia, high blood pressure, stroke, coronary heart disease, heartbeat irregularities and psychiatric disorders (e.g. depression, personality disorders, sexual problems, hallucinations and memory loss).

Binge drinking

Binge drinking is defined as drinking eight or more units of alcohol in one session if you are a man, and more than six units in one session if you are a woman. Studies are starting to reveal that drinking a large amount of

alcohol over a short period of time may be significantly worse for your health than frequently drinking small quantities.

Recommendations

To reduce health risks from drinking, the Department of Health recommends that:

- Adult males should drink no more than 3-4 units of alcohol a day

- Adult females should drink no more than 2-3 units of alcohol a day

One unit is defined as 7.9g (or 10mls) of pure alcohol. This is roughly equivalent to half a pint of beer, cider or lager, a 25ml (pub) measure of spirit such as vodka, whisky or gin, a 50ml (pub) measure of fortified wine

such as port or sherry, or a small (125ml) glass of wine, at 8% AbV (Alcohol by Volume).

* The above information is taken from the NHS Direct website; see further details:

http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=10

If you believe that you are a heavy drinker and you are concerned about it, you should ask your GP for help. Also, there are the following support services on campus:

University of Sussex Health Centre: http://www.sussex.ac.uk/aboutus/campus/health

University of Sussex Psychological and Counselling Service: http://www.sussex.ac.uk/sas/1-4-6.html

Additional Links and Services

Find out more information about drug abuse and misuse:

Young Person's Substance Misuse Service in Brighton & Hove: ru-ok? www.areyouok.org.uk is the young person's substance misuse service for Brighton & Hove. ru-ok? provides free, confidential help and advice to young people (and their families) because of problems with drugs, alcohol or legal highs. Available Monday to Friday 9am-5pm (Call (01273) 293966)

Support for the families, friends and carers of substance users: PATCHED offers free and confidential advice, information and support for the families, friends and carers of substance users in Brighton & Hove. PATCHED offers

carer's needs assessments, counselling for individuals and for families, 'outreach' and educational groups. Available 10am-10pm every day.

(Visit us at 9 The Drive, Hove BN3 3JE; Call our helpline on 0800 085 4450)

Find out more information about local and national organisations from Think Drink Drugs

Addaction UK - a leading UK charity working solely in the field of drug and alcohol treatment; Tel: 0207 2515860

Alcohol Brief Intervention Service - The CRI Alcohol Brief Intervention Service offers free information and advice on drinking at safer levels to anyone aged 16 and over; Tel: (01273) 823026 9am-5pm weekdays or email bis@sussexpartnership.nhs.uk

Information Sheet – Study 2

Impact of oxytocin on emotional regulation in binge drinkers: fMRI investigations

You are being invited to take part in a research study. 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. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

This study aims to improve our understanding of the relationship between social drinking and emotional regulation.
In this study, we are investigating the effects of oxytocin, which is a hormone naturally secreted by the organism, on the emotional regulation and on mind - body interactions. This study focuses on both behavioural and cerebral levels.
The study will also involve a brain scan (MRI) so we can measure the effects of oxytocin on brain activity, during three differents tasks.

2. Why have I been chosen?

You have been chosen as you are a healthy control participant and we wish to study emotional processing and body-mind interactions.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

You will undergo three separate test days. The first session is a basline session. The protocol of the second and third sessions is identical and these two sessions will be separated by at least a week.

Session 1

Session 1 is a baseline session. You will be asked to complete some questionnaires regarding your medical history, drug-use history, weekly alcohol use, emotional awareness and experience of emotional neglect, at the laboratory. We will also measure your blood pressure and weight you. We will perform a blood sample and a (confidential) drug test from urine sample. Then you will perform two short behavioural tasks measuring your emotional perception. This session will last approximately an 1 hour and 30 minutes.

Session 2

1) You will fill out a MRI safety questionnaire and we will check medical history changes that might have occurred since the previous experiment.

2) An urinary drug test is performed and we will measure your blood pressure.

3) You will perform a short psychometric questionnaire.

4) You will inhale 40IU of oxytocin or 40IU of placebo.

5)You will receive some information about the three tasks you will have to perform later, then you will complete the same short psychometric questionnaire again.

6)You will be installed in the MR Imaging, physiological equipment will be applied, including application of finger sensors (pulse oximetry, blood pressure etc.).

7) You will perform an emotional task (evaluation of empathy) and a cognitive task (evaluating memory processes).

8) The interoceptive task will be perform outside of the scanner and will be followed by a short memory retrieval task (related to the cognitive task you will have to do in the scanner).

The session will last 2 hours. Upon completion of all steps you will receive approximately £5 per hour, or equivalent course credits, or a combination of cash and course credits, and will be fully debriefed as to the purpose of the study.

Session 3

Session 3 is exactly the same session as the session 2. If you inhaled placebo in session 2 you will receive oxytocin in session 3 (and vice versa). You will repeat the same procedure and tasks as session 2.

5. What are the risks/side effects of any treatment received when taking part?

Drug:
Oxytocin is a hormone which has a number of different functions. The form of oxytocin we will give you is a nasal spray which is licensed in some countries to help women breast feed. The dose we intend to use has been used in a number of psychological studies in healthy male volunteers and no serious side effects have been reported.

Oxytocin leaves the bloodstream very quickly. It has a half-life (time taken for the amount present in the blood to fall to half its initial level) of only 3 minutes. However, experimental work suggests that the enhancement of trust and empathy caused by oxytocin can last significantly longer. The effects should have diminished by the end of the testing session, but we advise you not to drive for at least an hour after leaving the lab. Throughout the test session, the researcher will be available in case you feel unwell. Also, you can call the researcher at any time after leaving should any questions or concerns arise.

Possible side effects of oxytocin spray include nasal irritation, and rarely nausea and vomiting. There is also a risk of cardiac arrhythmia but this is extremely unlikely, nevertheless we will not be recruiting individuals with high blood pressure or a history of heart problems. If experienced, these side-effects should be short lived. If you do experience any problems due to oxytocin or at any point during the experimental procedure, you should immediately report this to the researcher who will stop the experiment. A medical professional will be on call during both sessions to deal with any adverse effects.

As mentioned, oxytocin might temporarily enhance feelings of trust and empathy. This effect is slight but significant and should wear off by the time you have left the lab. However we advise you to consider that your decision-making capacity might be affected in the hour following the test session.
You are free to withdraw from the study at any point and for any reason.

MRI:
The technique has been used for over 20 years in medicine and every year approximately 10 million people are scanned worldwide. There are no known side effects and MRI causes no pain or damage. However, some people find the MRI scanner noisy and claustrophobic, and it can be uncomfortable to lie still throughout the duration of the scan.

Pregnant women are excluded from participation in this study.
If you are a woman of childbearing age, we recommend you have a pregnancy test to exclude pregnancy before participation in this study. If you are a sexually active heterosexual woman of childbearing potential and have not been using an accepted method of birth control we advise that you do not participate.
Due to potential risks and potential harm to the unborn foetus, sexually active women of childbearing potential must use a reliable method of birth control

while participating in this study. Reliable methods of birth control are considered to be: abstinence (not having sex), oral contraceptives (the 'pill'), intrauterine device (IUD), depot contraceptive medication, tubal ligation ('tubes tied'), or vasectomy of the partner (with confirmed negative sperm counts) in a monogamous relationship (same partner). An acceptable, although less reliable method involves the careful use of condoms and a spermicidal foam or gel and/or a cervical cap or sponge. We encourage you to discuss this issue further with your GP if you have any questions.

6. What are the possible benefits of taking part?

There are no immediate benefits to you of taking part in this study. Although this research may not directly benefit you, it could result in better understanding of the relationship between the mind and the body and new ways of treating anxiety and other mental health symptoms.

7. What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the researchers who will do their best to answer your questions (see contact details below).

8. Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential.

9. What happens if an abnormality is picked up during the tests performed as part of the study?

If an abnormality is picked up on the brain scan, then your GP will be informed of this so that the appropriate medical care can be arranged. If an abnormaility is identified during the heart monitoring, we will advise you to seek further medical attention and we may, with your permission, contact you GP directly.

Part 2

1. What will happen if I don't want to carry on with the study?

You may withdraw from the study at any time without explaining why.

2. What will happen to the results of the research study?

The anonymized results of the research study will be presented internally at meetings, they will be written and up and published in a scientific journal, and presented at scientific conferences.

3. Who is organising and funding the research?

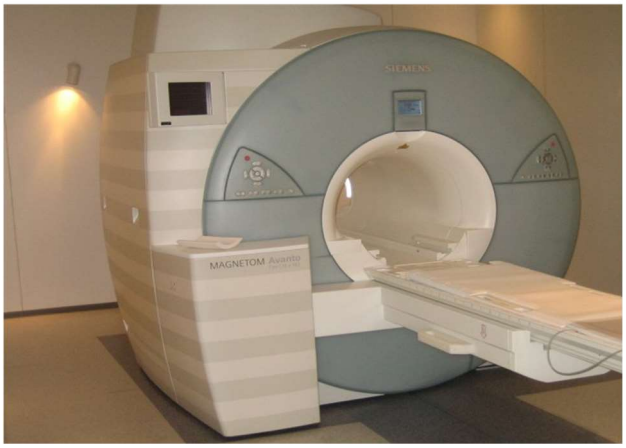
The research is organised by the Brighton and Sussex Medical School. The research is being funded by the European Research Council Grant research and the Society for the Study of Addiction.

4. Who has reviewed the study?

All research in the Brighton and Sussex Medical School is looked at by independent group of people, called a Research Ethics Committee, to protect your interests.

5. What can I expect in the MRI scanner?

The MRI examination is performed in a special room that houses the MR system or "scanner". The scanner consists of a circular magnetic tunnel which contains the radio coils. During your scan you will lie on a padded bed, which will move slowly into the scanner.



In preparation for the MRI examination, you will be asked to wear headphones or earplugs to protect your hearing as the scanner produces loud noises. These loud noises are normal and should not worry you.

A number of scans will be taken with a pause in between so do not be alarmed if the scanner goes quiet. The most important thing is to relax and try to keep still. It is not dangerous if you move, but the resulting pictures may be blurred. Some minor movement of your body is possible between the scans. The radiographer will be able to hear and see you throughout the session and you will be provided with a call button to alert them if you have any concerns. The scanning session will take approximately 1 hour.

6. **Complaints**
   If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (contact details are in part 1, section 11).

7. **Harm**
   In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the Brighton and Sussex Medical School, but you may have to pay your legal costs.

8. **Contact Details:**

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:

Sophie Betka	**S.Betka@bsms.ac.uk**	**01273 876702**
Prof Hugo Critchley	**H.Critchley@bsms.ac.uk**	**01273 873818**

Consent Form – Study 2

brighton and sussex
medical school

CONSENT FORM

Title of Project: Impact of oxytocin on emotional regulation in binge drinkers: fMRI investigations
Name of Researcher: Prof Hugo Critchley
Session 1

	Please initial box
I confirm that I have read and understood the information sheet dated 09/10/2015 for the study *Impact of oxytocin on emotional regulation in binge drinkers: fMRI investigations.* I have had the chance to read the information and ask questions about the study and am satisfied with the answers I have been given.	☐
I understand that my participation in this study is voluntary and that I am free to stop at any time, and I do not have to give a reason for doing so. I understand that if I ask to stop the study my medical care and legal rights will not be affected in any way.	☐
Occasionally an external regulator or funding body may ask to look at the data for this study to check that it is being run correctly.	☐
I understand that relevant sections of my medical notes and data collected during the study, may be looked at as part of the research. I give permission for my medical notes and data to be used for this purpose.	☐
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.	☐
I understand that my interview will be recorded.	☐
I understand that samples of my blood will be taken and kept as part of this study and I give permission for them to be used for this purpose.	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of Participant	Date	Signature

Researcher to complete:

- I have explained the information in this document and encouraged the participant to ask questions and provided adequate time to answer them.

_____	_____	_____
Name of Researcher or Person Seeking Consent (If different from researcher)	Date	Signature

When completed: 1 copy for the participant; *1 copy (original) to be kept in medical notes.*

CONSENT FORM
VERSION NUMBER 2
DATE 09/10/2015