Functional Activation and Connectivity under the Influence of Oxytocin: An Explorative Study using Functional Magnetic Resonance Imaging

Claire L Poppy

Master of Philosophy in Psychology (Cognitive Neuroscience)
Department of Psychology
UNIVERSITY OF OSLO

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Author: Claire L Poppy http://www.duo.uio.no/

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Acknowledgments/Statement of Involvement

This study took place as part of a larger project ('the Oxytocin Project'), sponsored by OptiNose AS, and based at the Norwegian Centre for Mental Disorders Research at Oslo University Hospital. The project was a randomised, placebo-controlled double blind, double-dummy four period cross-over study with the purpose of investigating differential effects of oxytocin according to the method of administration or dosage – intranasal 8 or 24 IU versus 1IU administered as a slow intravenous infusion. Effects were measured in the fMRI BOLD response, eye-tracker, pupillometry, cognitive responses and a number of physiological measures including cortisol, vasopressin and heart rate variability in healthy male volunteers. The project also trialled a new nasal device ('Optinose') that has shown increased uptake in previous preliminary trials. This thesis, "*Functional Activation and Connectivity under the Influence of Oxytocin: An Explorative Study using Functional Magnetic Resonance Imaging*" is located within the main project, but draws upon the fMRI data only. In recognising the role of OptiNose AS as a sponsor of the project, please note that the work undertaken in this thesis was sponsored by OptiNose AS, and all intellectual property arising in this work belongs to OptiNose AS.

Joining the project at its inception in July 2013, I contributed towards its progress and was one of four individuals responsible for its daily coordination. Duties involved designing and programming of the behavioural task in EPrime 2.0; recruitment and screening of participants; overseeing the pilot study (not described in this paper); nasal examination (rhinometry); administration of intranasal oxytocin; collection of saliva; collection of ECG and eye-tracker/pupillometry data; assisting radiographers during scanning; administration of anxiety tests (STAI); management of project resources; and data entry. Blood samples were taken by nurses who were attached to the project and radiographers were responsible for operation of the MRI scanner. The other members of the project are given in the acknowledgements below. Whilst the project, from which the data for this thesis was drawn, was sponsored by OptiNose AS, my contribution as a master's student to the project was voluntary, and analyses have been conducted independently.

This study and thesis, located within a large clinical trial, necessitates the recognition of many individuals who have each played a part in supporting the research process. First and foremost, I am grateful to my supervisor Professor Lars T Westlye who, from the onset, encouraged me to participate in the project. In so doing, he provided me with a fantastic opportunity to develop my research skills within the field of cognitive neuroscience, but

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Abstract

The neuropeptide oxytocin (OT) has received much interest in recent years and has been associated with increased trust and other prosocial behaviours. A plethora of studies have attempted to understand the full extent of its influences on the human brain and its potential as a treatment of socially-related psychiatric disorders. Neuroimaging studies have contributed, particularly in implicating mechanisms related to the amygdala. Nevertheless, many such studies have failed to consider the influence of other regions or neural networks.

The current randomised, placebo-controlled, double-blind, four period, cross-over clinical study investigated the effects of 8 and 24 international units (IU) of OT, administered intranasally (IN), and 1 IU of intravenous (IV) in healthy males (N=16) during a facial emotion sensitivity task (happy/angry/neutral faces vs shapes) using functional magnetic resonance imaging (fMRI). Graph theory-based network analysis was conducted using fast eigenvector centrality mapping (fECM) as a data-driven explorative approach to investigate functional connectivity. The task events were subjected to general linear analysis in order to explore patterns of functional activation.

We observed no significant main effect of oxytocin condition on ECM. We found a reduction in differential processing between neutral ambiguous faces and shapes, and between happy and angry faces, under the influence of OT. In addition, we found that IN administration of OT proved to have a greater influence on cortical activity than IV administration, and that a lower dosage of IN OT (8IU) had a greater influence over neuronal activation than a higher dosage (24IU). Further research should investigate the influence of OT with lower dosages, particularly in recruiting endogenous production of OT, independent of method of administration, and to utilise fECM with a larger sample size.

Keywords: oxytocin, fMRI, fast eigenvector centrality mapping (fECM), IN, IV

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Abbreviations

Anterior cingulate cortex	ACC
Anterior insular	AI
Anterior medial temporal lobe	aMTL
Anterior temporal lobe	ATL
Arginine vasopressin	VP
Autism spectrum disorder	ASD
Alcohol Use Disorder Identification Test	AUDIT
Blood brain barrier	BBB
Boundary based registration	BBR
Brodmann area	BA
Cluster index	CI
Dynamic causal modelling	DCM
Default mode network	DMN
Drug Use Disorder Identification Test	DUDIT
Echo planar imaging	EPI
Eigenvector centrality mapping	ECM
Electrocardiogram	ECG
Emotion Stimuli Model	ESM
Explanatory variable	EV
Family-wise error rate	FWER
fast Eigenvector centrality mapping	fECM
Fast spoiled gradient echo	FSPGR
Flip angle	FA
Functional MRI of the brain	FMRIB
FMRIB’s Expert Analysis Tool	FEAT
FMRIB’s Local Analysis of Mixed Effects	FLAME
FMRIB’s Linear Image Registration Tool	FLIRT
FMRIB’s Non-Linear Image Registration Tool	FNIRT
Functional magnetic resonance imaging	fMRI
Fusiform face area	FFA
Fusiform gyrus	FFG

Generalised anxiety disorder	GSAD
General linear modelling	GLM
Globus pallidus	GP
Inferior frontal gyrus	IFG
Inferior temporal cortex	IT
International units	IU
Intranasal	IN
Intravenous	IV
Intrinsic connectivity network	ICN
Juxtapositional Lobule	JPL
Large dense-core vesicles	LDCV
Lateral occipital cortex	LOC
Magnetic resonance imaging	MRI
Middle frontal gyrus	MFG
Middle temporal gyrus	MTG
Medial orbitofrontal cortex	mOFC
Medial prefrontal cortex	mPFC
Multivariate Exploratory Linear Optimized Decomposition into Independent Components	MELODIC ICA
Mini-International Neuropsychiatric Interview	MINI
Montreal Neurological Institute	MNI
Motion correction and FLIRT	MCFLIRT
NEO Personality Inventory-R	NEO
Nucleus accumbens	NAcc
Optinose Nose-to-Brain device	N2B
Oxytocin	OT
Reaction time	RT
Reading the mind in the eyes test	RMET
Rest-functional magnetic resonance imaging	r-fMRI
Resting state networks	RSN
Region of interest	ROI
Sample	P
Signal-to-noise ratio	SNR

Sodium chloride	NaCl
State-Trait Anxiety Inventory	STAI
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Superior temporal cortex	STC
Superior temporal gyrus	STG
Superior temporal lobule	STL
Superior temporal sulcus	STS
Supramarginal gyrus	SMG
Task-functional magnetic resonance imaging	t-fMRI
Temperament and Character Inventory	TCI
Ventral tegmental area	VTA
Visual Analogue System	VAS
Visual Stimuli Model	VSM
Wechsler Abbreviated Scale of Intelligence	WASI
White matter	WM

Introduction

The neuropeptide oxytocin (OT) is synthesised in the parvocellular and magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus. Thereafter, it is released directly into the bloodstream via projections to the neurohypophysis (posterior pituitary gland) from where it has an impact upon a number of physiological processes including uterine contraction during childbirth, milk release in mothers during lactation, reductions in some forms of stress and pair-bonding, in a number of different species. Though OT is responsible for a number of physiological processes, neurons also project centrally where they act as neuromodulators, influencing the actions of neurotransmitters**.** In humans, OT receptors are found throughout the brain, specifically the amygdala, ventromedial hypothalamus, septum, nucleus accumbens and brainstem.

Early research into OT focused upon animal studies where centrally-acting OT was linked with social behaviours in mammals, albeit with species-specific effects (Insel, 2010), most notably with prairie (*microtus ochrogaster*) and montane (*microtus montanus*) voles (Pedersen, Ascher, Monrie & Prange, 1982; Pedersen & Prange, 1979). Recent studies, however, have begun to focus upon its effects in humans. This has led it to become associated with a number of prosocial behaviours including increased trust in strangers (Kosfeld, Heinrichs, Zak, Fischbacher & Fehr, 2005; Theodoridou, Rowe, Penton-Voak & Rogers, 2009), higher scores on 'mind-reading' tasks involving interpretation of gaze (Domes, Heinrichs, Michel, Berger & Herpertz, 2007b), altered eye gaze (Guastella, Mitchell & Dadds, 2008) and increased recognition of facial expressions (Schulze et al., 2011). Yet it has also been associated with envy and gloating (Shamay-Tsoory et al., 2009) and increased in-group favouritism/out-group aggression (De Drue et al., 2010). At the same time it has been linked with psychiatric disorders with reductions in the symptoms of anxiety (Labuschagne et al., 2010), autism (Domes et al., 2013) and schizophrenia (Pedersen et al., 2011) (for a full review see Bakermans-Kranenburg & van IJzendoorn, 2013; Meyer-Lindenberg, Domes, Kirsch & Heinrichs, 2011).

A number of different hypotheses have been developed to account for the processes underlying the influences of OT. No definitive conclusions have been reached, however, and a number of issues have been identified which might explain inconsistencies in findings. These include individual differences such as gender or psychiatric history, contextual factors, differences between paradigms and the emotional valence of the stimuli presented, dosage and

method of administration, aside from the very complex nature of OT and neuropeptides in general (Landgraf & Neumann, 2004).

Human studies have used physiological methods and neuroimaging to explore more fully the influences of OT (Bethlehem, van Honk, Auyeng & Baron-Cohen, 2013). Many studies in neuroimaging (Petrovic, Kalisch, Singer & Dolan, 2008; Labuschagne et al., 2010), however, have focused upon regions of interest, most often the amygdala, based upon the early and well-founded work of Kirsch and colleagues (2005). Given the dynamic nature of neuropeptides and how they function (see Appendix A), it seems problematic in identifying a simple region-based explanation of their effects on social behaviours. Moreover, in identifying a region of interest, it neglects other methods which might identify important subsidiary regions and networks (Bethlehem et al., 2013).

Though recent interest in the possible effects of OT have popularised it in the media, the mechanisms underlying its application have more serious and important implications for translational neuroscience. OT promises to alleviate the symptoms of many different types of psychiatric illness and, therefore, it is important to establish how its effects are manifested, and from this, which patients would benefit most from its use. Given the continued uncertainties in the literature, this study adopts a more explorative network approach through eigenvector centrality mapping (ECM) in order to identify regions showing drug induced alterations in global connectivity during a facial emotion sensitivity task which may have previously gone unseen. At the same time, linear modelling of the elements in the task allow for exploration of functional activation and the effects of oxytocin upon valence-specific emotion recognition; in this study, happy and angry faces. Finally, the study design allows for consideration of IN dosage (8IU vs 24IU) and method of administration (IN vs IV) to explore the possibility of differential influences upon cortical activity and to answer unresolved issues in the literature.

Rationale

This paper begins with an outline of the human OT literature including inconsistencies in findings. Studies utilising neuroimaging are presented thereafter, illustrating a particular focus upon the amygdala and highlighting the need for a focus across the entire brain. A final section considers the merits of examining functional connectivity and the use of graph theory.

Towards an understanding of the influence of OT in humans. Animal studies of attachment and bonding have contributed heavily to our current understanding of the effects of

OT (Insel & Shapiro, 1992; Pedersen et al., 1982; Pedersen & Prange, 1979). Just as earlier animal studies focused upon social bonds, interest in OT and humans has focused upon its social influences. One of the earliest studies (Kosfeld et al., 2005), for example, found that males who had received 24 IU of intranasally administered OT, were more likely to trust a partner in an economic game than those who had received a placebo dosage. Similarly, Zak, Stanton and Ahmadi (2007) found that participants who received 40IU of OT were 80% more likely to be generous than their control counterparts in an economic task. OT was also found to increase altruistic behaviour (De Dreu et al., 2010) and emotional empathy (Hurlemann et al., 2010). Such studies led to the hypothesis that OT promoted *pro-social behaviours*. This hypothesis was further supported by research which demonstrated that OT increased ratings of facial trustworthiness and attractiveness (Theodoridou et al., 2009), enhanced the ability of participants to infer the mental state of others based upon viewing only the eye region of a series of faces (Domes et al, 2007b), and it increased the number of fixations and gaze time towards the eye region (Guastella et al., 2008) possibly allowing for increased emotion recognition.

Though an increasing number of studies supported the view that OT enhanced pro-social behaviours not all findings were entirely consistent. In 2009, Shamay-Tsoory and colleagues found that OT increased envy and gloating in an economic task which promoted social comparison whilst later studies found that OT increased in-group favouritism but also defensive aggression to out-groups (De Drue et al., 2010; De Dreu**,** Greer, Van Kleef, Shalvi & Handgraaf, 2011). Shamay-Tsoory et al. (2009) argued that the link between OT and maternal aggression in rodents (Bosch et al., 2004) was testimony to the fact that OT does not only promote positive social interactions and went on to propose, instead, that it increases the *social salience* of stimuli per se which in turn leads individuals to attend more to social cues, be they positive or negative. Pupillometry data has produced more direct evidence of this process, demonstrating that OT increases the salience of social stimuli and recruits increased attentional resources in order to process it (Prehn et al., 2013; Tollenaar, Chatzimanoli, van der Wee & Putman, 2013).

Whilst emotional face processing and trust have been the two focal concerns within the literature, an increasing range of other studies have emerged - from social cognition and behaviour, to attachment, from aggression to anxiety, fear conditioning and fear extinction. Neuroimaging studies have also provided new insights (discussed below). At the same time OT's influences on the psychiatric population has been investigated and includes a broad range of disorders including social phobia (Heinrichs et al., 2006), schizophrenia (Feifel et al.,

2010), depression (Cardoso, Orlando, Brown, Joober & Ellenbogen, 2013a), borderline personality disorder (Simeon et al., 2011), social anxiety disorder (Hoge et al., 2012), Williams syndrome (Dai et al., 2012) and autism (Andari et al., 2010), with some translational possibilities.

In an attempt to interpret the literature five hypotheses have been proposed. The *social reward hypothesis* (Evans, Shergill & Averbeck, 2010; Izuma, Daisuke & Sadato, 2008) suggests that social behaviours are motivated by reward, linked to the relevant brain regions, in particular the striatum, whilst the *affiliative-motivational hypothesis* founded on the early work of Carter (1998), Depue and Morrone-Strupinsky (2005) and Taylor et al. (2006) purports that OT encourages affiliation towards others, also through reward pathways. As noted above, Shamay-Tsoory et al. (2009) proposed the *social salience hypothesis*, the view that OT enhances the attention to all social stimuli regardless of valence. In addition, Kemp and Guastella (2010, 2011) proposed the *social approach/withdrawal hypothesis* which suggests that OT enhances approach behaviours, for example, during happy or angry interactions, whereas it dampens withdrawal behaviours such as during threatening situations (Domes, Steiner, Porges & Heinrichs, 2013; Radke, Roelofs & de Bruijn, 2013). Finally, OT is thought to reduce stress and to have an *anxiolytic effect* (Bale, Davis, Auger, Dorsa & McCarthy, 2001; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). For example, a recent study found that OT reduced anxiety after social rejection and promoted continued trust (Cardoso, Ellenbogen, Serravalle & Linnen, 2013b). Indeed, Churchland and Winkielman (2012) argue that most discrepancies in the literature can be accounted for via this hypothesis.

An alternative approach to the inconsistencies in the literature is a focus upon contextual factors and individual differences (Bartz, Zaki, Bolger & Ochsner, 2011b). Such factors may be defined as sex and hormonal status, attachment style, childhood trauma, or the presence of psychiatric symptoms and genetic variation which may influence sensitivity to and interpretation of emotional significance/salience of a situation (Olff et al, 2013). A number of studies lend support to such a perspective with OT having differential effects depending upon the population being tested. OT, for example, increased social cognition in depressed patients with higher but not lower attachment anxiety (MacDonald, MacDonald, Wilson & Feifel, 2011); negative cognitive self-appraisal was reduced in high trait anxiety individuals but low trait participants were unaffected (Alvares, Chen, Balleine, Hickie & Guastella, 2011); and those who were less anxiously attached reported their mothers being

more caring under the influence of OT whilst high anxiously attached reported mothers being less caring (Bartz et al., 2010). Further, Bartz et al. (2011a) tested patients with borderline personality disorder with 40 IU and a control group of healthy individuals and found differential effects depending on childhood trauma and attachment style. OT has also been associated with ceiling effects in healthy individuals or even adverse influences. For example, healthy participants over-rated the emotional intensity of facial expressions (Cardoso, Ellenbogen & Linnen, 2012). Also, whereas performance of less socially competent individuals on the reading the mind in the eyes test (RMET) improved, healthy controls were unaffected (Luminet, Grynberg, Ruzette & Mikolajczak, 2011), and in a pupillometry study, Leknes et al (2013) noted that the influence of OT depended upon performance of individuals at baseline.

A further problematic issue in studying the effects of OT has been concerned with the blood brain barrier (BBB). This is an endothelial layer of cerebral blood vessels which prevents many substances entering the extracellular fluid surrounding the brain. Though it is permeable by some molecules, it is unlikely that large hydrophilic molecules such as OT can cross (Evans, Dal Monte, Noble & Averbeck, 2013; Ruhle, Russell, Ermisch & Landgraf, 1992). IN administration, however, has proven to be particularly effective in studies of OT since it has been shown to allow neuropeptides such as insulin, vasopressin, melanocortin (Born et al., 2002) and OT (Szeto et al., 2011) to cross the barrier in minute quantities. The exact pathway of entry is yet to be fully understood but it is now generally believed that extra-axonal and extra-systemic direct pathways must exist between the nasal and cranial cavities, olfactory epithelia, other brain regions and the cerebral spinal fluid contained in the arachnoid space (Veening & Olivier, 2013).

Whilst a popular choice of administration in OT studies, the use of IN administration may provide real problems when considering the translational application of OT. Specifically, this is related to whether patients are able to effectively self-administer over a period of time and the likelihood of consistent dose delivery (MacDonald & Feifel, 2013). This may be a particular problem for children and certain psychiatric patients and, for this reason, this study aims to consider the possibility of using alternative methods of administration.

Though the size of OT molecules might prohibit it from crossing the BBB, there is some suggestion that other mechanisms may allow for OT administered peripherally to enter

the brain or that such a conclusion reflects methodological inadequacies (Zlokovic, 1990). It may be that OT binds to peripheral receptors which initiate feedback in the brain which in turn has observed effects on behaviour. Indeed, davunetide, another peptide, has been shown to enter the brain via systemic circulation in rats rather than direct nose-to-brain pathways (Morimoto, De Lannoy, Fox, Gozes & Stewart, 2009) and in a study of peripheral administration of anxiety, OT reduced anxiety levels (Ayers, Missig, Schulkin & Rosen, 2011). The authors suggest that some, but not all, central effects of OT may result from peripheral administration. This coheres with a study which found that binding sites are up-regulated in specific areas, responding to exogenous or endogenous cues which can include processes in the periphery (Viero et al., 2010). In humans, IV administration has been used effectively in patients diagnosed with autism with reductions in repetitive behaviours (Hollander et al., 2003) and increases in social cognition (Hollander et al., 2007). Thus the possible use of IV or other administration such as aerosols (Modi, Connor-Stroud, Landgraf, Young & Parr, 2014) remains an open question (MacDonald & Feifel, 2013), but one worth further consideration given the possible disadvantages of IN administration and the possibility of inconsistent dosage for self-administering clinical populations. Another possibility is the use of nasal devices designed for optimal uptake, with maximal inhalation and minimal loss via the throat. Such devices may lead to a more consistent dosage and overcome the problems envisaged by some (for a full discussion of nasal drug delivery devices, see Djupesland, 2012).

A further unanswered question in the literature is that of dosage (Harris & Carter, 2013) – what is necessary and what is safe? Typically, studies have used 20-40 IU but some have used less (Goldman, Gomes, Carter & Lee, 2011) and some more (Epperson, McDougle & Price, 1996). A particularly important consideration is whether there is an optimal dosage of OT (McDonald & Feifel, 2013), particularly given its therapeutic possibilities, and the consequences for chronic treatment. Most studies in the OT literature involve a single dose which, in therapeutic terms, might be referred to as *acute* treatment. Yet therapeutic treatment is likely to be long term and *chronic*, and in some cases the two even have opposing effects as a result of changes in tolerance to the drug (ibid). Long term treatment may also be necessary for a drug to become effective.

Three animal studies have examined chronic treatment with IN administration of OT (Bales et al., 2013; Huang et al., 2014; Peters, Slattery, Uschold-Schmidt, Reber & Neumann, 2014), all suggesting differential effects depending upon whether the treatment was acute or

chronic. A small number of human clinical studies with schizophrenic patients (Feifel et al., 2010; Pedersen et al., 2011; Modabbernia et al., 2013), and children/young teenagers diagnosed with autism spectrum disorder (ASD) (Anagnostou et al., 2014; Sikich et al., 2013, cited in Young, 2013; Tachibana et al., 2013), have shown improvements in symptoms using between 8 and 40 IU of OT for up to 6 months, with no adverse effects. These studies are a promising start but clearly chronic treatment warrants further investigation.

In animal studies, there is also some indication that behavioural effects are dosage-dependent (Bales et al., 2007; Kramer, Cushing & Carter, 2003; Windle, Shanks, Lightman & Ingram, 1997) and similar conclusions have been reached in studies with humans. Hall, Lightbody, McCarthy, Parker & Reiss (2012), for example, found that in patients with Fragile X syndrome, 24 IU led to increased eye gaze but had no effect on salivary cortisol levels, whilst 48 IU reduced salivary cortisol and had no impact on gaze. Dosage-dependent effects have also been found with emotion recognition in schizophrenic patients (Goldman et al., 2011) and cortisol levels in healthy participants taking strenuous exercise (Cardoso et al., 2013a). A study comparing emotion recognition in young men with ASD, and which compared 18 and 24 IU, found no dosage-effect but this may be due to the lower dosage being given to younger participants and thus the two conditions of the two doses were not entirely comparable (Guastella et al., 2010). The same might be said of a more recent study of young people with ASD (Dadds et al., 2014) where dosage (12IU vs 24IU) was based upon weight. Furthermore, no improvements were witnessed in emotion recognition but the study took place over 4 days and, in light of the duration of the studies outlined above, it is possible that effective treatment may need a longer duration. Such studies would suggest that the commonly used 24IU may not be the only effective dosage or even the optimal one (Bakermans-Kranenburg & van IJzendoorn, 2013) and, therefore, as with several issues regarding the influences and administration of OT, further investigation is necessary. In comparing two dosages (8IU and 24IU), this study will contribute towards a better understanding of the influences of differential dosage and the part this might play in inconsistencies in the literature described earlier.

Investigating the influence of OT upon neuronal recruitment. Neuroimaging studies have played a significant role in increasing our understanding of the influences of OT (for a full overview, see Appendix B) and have contributed to the hypotheses described above. In particular, such studies have identified the impact of OT upon the amygdala in

stress reduction. Based upon a sound body of research (Adolphs & Tranel, 2003; LeDoux, 2000; McCarthy, McDonald, Brooks & Goldman, 1996), Kirsch et al (2005) conducted the first neuroimaging study of OT and investigated whether it would modulate amygdala activation, thereby influencing those networks involved in the processing of fearful stimuli. Fifteen male participants received 27 IU of OT, administered intranasally, and were, thereafter, scanned whilst performing a matching task with a series of threatening images (angry vs fearful, faces vs scenes). Subsequently whole brain and region of interest (ROI)-analysis were performed. It was found that OT reduced activation for all threatening stimuli, but especially social stimuli (faces vs scenes). It also had an impact on the functional connectivity between the amygdala and brainstem. That OT had an impact on all threatening stimuli led the authors to conclude that OT led to anxiolytic effects by reducing amygdala activation. Later studies supported such conclusions (Baumgartner et al., 2008; Riem et al., 2011), also implicating reward pathways.

The role of OT in modulating the fear response via attenuation of activity in the amygdala is focal in a number of neuroimaging studies but what is less clear is whether OT has an influence over only threatening stimuli, social stimuli in general, or both. Whilst angry and fearful faces and scenes have been found to be influenced by OT administration (Kirsch et al., 2005), activity in the right amygdala has been found to be reduced for happy and sad faces (Domes et al., 2007a), suggesting that OT reduces arousal to social stimuli in general. Likewise, another study involving female participants (Lischke et al., 2012), found that OT had an impact upon perception of threating scenes - some social, others not, whilst individuals diagnosed with generalised anxiety disorder (GSAD) and with hypoactivity in the amygdala (Labuschagne et al., 2010), had reduced amygdala activation to fearful faces, but with no impact on healthy controls. Further, OT did not have an impact on happy or angry faces thereby suggesting that its effect was to reduce the stress experienced expressly for fear rather than all emotions. The authors suggest that faces with angry expressions, whilst threatening, are more difficult to interpret and produce a more variable response. It may be that it is the combination of relevant social stimuli and fear processing which is important since OT has been found to reduce activation in the amygdala for direct gaze fearful faces compared with averted gaze fearful faces (Petrovic et al., 2008). However, nociceptive painful stimuli, that is, a threatening but non-social stimuli, has been found to produce a reduction in the amygdala when subjects anticipate experiencing pain (Singer et al., 2008). This was the case only for participants categorised as selfish during a trust game compared with those who were

prosocial. Such a conclusion falls in line with the issue of individual differences raised earlier, and further demonstrates difficulties which exist within the OT literature.

The second hypothesis to dominate the neuroimaging literature has been the social salience hypothesis, the view that OT modulates motivation for socially salient stimuli. In the first OT study to combine eye-tracker with fMRI, Gamer et al. (2010) were able to investigate neural activation in conjunction with fixation upon different regions of the face. They found increased activation in the right posterior amygdala on trials where participants moved their attention to the eye region from another part of the face. This finding coincided with increased functional connectivity between the amygdala and superior colliculi, the region associated with shifts of attention to relevant targets. They also found reduced activation in the amygdala for fearful stimuli, but increased activity for happy faces. As a result, they suggested that social salience rather than the valence of the stimuli per se was important, a view which coheres with the findings of Domes et al. (2007a, 2010). Further support was found by Domes et al. (2013) when patients with ASD completed a neutral face matching task, that is, one using socially non-threatening stimuli. OT increased activation in the amygdala, arguably because it increased participants' attentional resources to the social salience of the faces.

It is possible that the effects of OT occur as a result of the close connections between the oxytocinergic and dopaminergic systems (Rilling et al., 2012). OT was found to have an impact upon the caudate nucleus, a region associated with feedback and cooperation, in a study of cooperation and trust using the Prisoner's Dilemma game (ibid). Under the influence of OT, the caudate nucleus and reciprocity were increased, as well as activation in the left amygdala. Worthy of note, both the amygdala and caudate nucleus are targets of the mesolimbic dopamine system and thus, once again, OT may exert its influence via the reward pathways. Finally, the study established that there was increased connectivity between the amygdala and insular, a region associated with awareness of subjective feelings. This led the authors to suggest that OT may make the amygdala more sensitive to subjective feelings and thus, more responsive to salient stimuli.

It is possible that the anxiolytic and social salience hypotheses are not incompatible. Fathers who observe images of their own child, a familiar child or an unknown child, elicit differential cortical activation with reductions for images of their own child and an unknown child (Wittforth-Schardt et al., 2012). Specifically, when fathers viewed their own child

functional activation and connectivity was reduced in the globus pallidus (GP), medial orbitofrontal cortex (mOFC) and ventral tegmental area (VTA) suggesting that the salience of the image was modulated by the reward pathways of the brain. For the unknown child, the hippocampus, superior temporal cortex (STC) and posterior insular showed reduced activity implying that their salience was effected by the novelty of the stimuli. The authors suggest that the role of OT is to down regulate physiological responses to salient cues in order to promote psychological well-being, and in so doing it reduce experiences such as stress and pain perception. At the same time they suggest that such an account also explains the effects OT seems to have in promoting social approach behaviours and reducing fear of betrayal.

In summary, neuroimaging studies have given us a general understanding that OT is likely to influence activity in the amygdala via projections to reward pathways in the midbrain and the dopaminergic systems. We also know that it has an impact on perception of and response to social and threatening stimuli via activation in the amygdala though the nuances of this are unresolved. There is some indication that OT may reduce arousal in general rather than only for threatening stimuli. Not included in the discussion above, neuroimaging studies have also shown that activation in the amygdala of females tends to increase under the influence of OT, whilst it is reduced in males (Domes et al., 2010; Lischke et al., 2012).

Whilst there has been much focus on the amygdala, it is clear that the influence of OT goes beyond this region and it may be that investigation of these other areas will provide a better understanding of its influences. Many of the studies begin with whole brain analysis but then create a ROI (see Appendix B). This approach allows for exploration of connections between the ROI and other regions in the brain but fails to establish how different regions may operate collectively, that is, it neglects a network approach. Several studies have followed Kirsch et al's lead and focused upon activation in the amygdala, but others have been applied, for example, Baumgartner et al. (2008) created ROIs for the amygdala, striatum, anterior cingulate cortex (ACC) and midbrain whilst Singer et al. (2008) did so for the amygdala, anterior insular (AI) and ACC. All studies have been hypothesis-driven, based upon existing literature and a particular finding is anticipated, an approach which encourages the use of ROIs. Few have taken an explorative approach, perhaps with the exception of Pincus et al. (2010) and Labuschagne et al. (2011) who utilise only whole brain analysis without developing any specific region for further investigation.

Very few of the neuroimaging studies to date explicitly set out to examine the functional connectivity between different regions of the brain. Those which do all adopt a seed-based approach to functional connectivity thereby looking at connectivity between a specific region and other areas within the brain**.** Such studies have shown us that connectivity is influenced by OT - between the amygdala and the reward pathways in the midbrain (Kirsch et al., 2005), between the amygdala and the region controlling attentional shifts, the superior colliculi (Gamer et al., 2010), between the left nucleus accumbens (NAcc) and right amygdala (Atzil, Hendler & Feldman, 2011), between the amygdala, insular, ACC and inferior frontal gyrus (IFG), areas associated with emotional processing and decision-making or inhibition/aversion (Striepens et al., 2012), between the reward pathways of the GP, mOFC and VTA, and between the hippocampus, STC and AI, areas associated with social and emotional processing (Wittforth-Schardt et al., 2012), between the amygdala and AI, ACC and inferior temporal cortex (IT) (Rilling et al., 2012), between the amygdala, OFC, ACC, supramarginal gyrus (SMG), middle temporal gyrus (MTG), precuneus and hippocampus regions associated with attachment and reward (Riem et al., 2012), between the amygdala, ACC and medial prefrontal cortex (mPFC) (Sripada et al., 2013)**,** between posterior cingulate, postcentral gyrus and cerebellum involved in touch and self-referential processing (Riem et al., 2013), between the amygdala, rostral ACC and mPFC (Dodhia et al., 2014) and between amygdala, insular and dorsal ACC (Gorka et al., 2014). Thus OT appears to influence connectivity between social and emotional processing, cognition and reward pathways; eight of the eleven studies are task-based. As Bethlehem et al. note, however, *"Using a seed-based hypothesis-driven approach, any changes in regions that have an indirect effect will likely have been missed"* (2013:9).

More than simply understanding which regions may be influenced by OT and how this occurs, whether via the amygdala the dopaminergic system, or some other pathway, knowledge of the different regions activated may contribute towards a better understanding of the networks throughout the brain. Furthermore, it may give some indication as to the mechanisms underlying social responses. OT, as a neuropeptide, does not function in the same way as a classic neurotransmitter (see Appendix A). It is stored in large dense-core vesicles (LDCVs), it has a half-life of approximately 20 minutes overcoming both spatial and temporal constraints, it can be released from dendrites as well as by the soma of the neuron and, release, once initiated can be self-perpetuating through a positive feedback loop, leading to a continued behavioural response for a sustained period of time (Landgraf & Neumann,

2004). Furthermore, this mechanism is thought to occur through a process of activity-based priming such that the nature of the initialising stimulus is crucial for the release of OT and the behaviours associated with its release. Ludwig and Leng's (2006) suggest,

> *Priming is a conditional persistent functional re-wiring of neural networks, and such re-wiring, which is achieved through peptide actions at receptors expressed at diverse sites following a hormone-like signal within the brain, might underlie peptide-dependent changes in behaviour (p. 134).*

Whilst Ludwig and Leng (2006) refer to lactation in rats and other animals with the stimulus being the pups first suckling action on the mother's teat, Bethlehem et al (2013) postulate that in humans those regions which are activated by OT may also be dependent on the nature of the stimulus, for example, a fearful face or the sound of a crying baby. Most importantly, the actions of neuropeptides are thought to occur as part of a dynamic neural network, once again emphasising the need for an explorative approach in examining the influence of OT on the human cortex, and demanding a possible retreat in the existing hypothesis-driven tradition until firmer conclusions are drawn.

Functional connectivity and graph theory. Brain connectivity as revealed using neuroimaging, be it structural, functional or effective, may be understood as "*….the study of interactions between distinct brain areas*" (Smith, 2012:1257). In this study the focus is upon functional connectivity, that is, "*… statistical dependencies between spatially segmented neuronal events*" (Stephan & Friston, 2010:446). Thus connectivity is inferred based on fMRI time signal correlations (Joyce, Laurienti, Burdette & Hayasaka, 2010) rather than being measured directly. In examining connectivity between regions it is possible to identify networks, thereby offering a more dynamic model than focusing only on where and when activation takes place (Roebroeck, Formisano & Goebel, 2011). Such networks may reflect a synchronisation of activity across populations of neurons (Basar et al., 2001) and in a classic review of studies that investigated synchronisation, Varela et al (2001) suggested that the entire brain consists of several such networks, referring to the brain as a 'brainweb'.

Research conducted into neural networks, has mainly used resting state fMRI (r-fMRI), that is, the measurement of brain activation when it is not involved in a specific experimental task. In such a state, it is possible to measure correlations between regions exhibiting spontaneous low frequency (<0.1 Hz) fluctuations in the fMRI signal, suggesting

that the two are functionally connected (Biswal et al, 2010). Typically the default mode network (DMN) (Raichle et al., 2001) is identified but many others resting state networks (RSN) have also been found (Douchet et al., 2011). Furthermore, irregularities in these networks have been observed in neuropsychiatric disorders or brain injury (Gorges et al., 2013; Hoekzema et al., 2013; Keehn, Shih, Brenner, Townsend & Muller, 2013; Mayer, Manell, Ling, Gasparovic &Yeo, 2011; Rosanova et al., 2012; Shen, Wang, Lui & Hu, 2010). There is evidence, however, that task-positive networks also exist, otherwise referred to as intrinsic connectivity networks (ICN) (Seeley et al., 2007). A number of ICNs have been identified for various cognitive processes (Laird, Fox, Eickhoff & Turner, 2011). For example, cognition (left lateralised to Broca and Wernicke's area), inhibition (the right inferior frontal gyrus), and working memory, (the lateral prefrontal and parietal cortices). Moreover, a study of the Brain Map database which contains imaging of almost 30,000 subjects found a strong correspondence between resting state and active networks (Smith et al., 2009). The authors noted that this correspondence, *"… implies that the resting brain's functional dynamics are fully utilising the set of functional networks as established by the brain over its range of possible tasks"* (p. 13043). Such commonalities suggest that research of neural networks need not exclude task-fMRI (t-fMRI).

A number of studies suggest that connectivity between brain regions warrants further investigation. Kennedy and Adophs (2012) argue in favour of a network approach to the role of the amygdala in social cognition since studies of those with difficulty in social function, for example, prosopagnosia, Williams syndrome and Capgras syndrome, do not find a single structure alone is responsible for their difficulties. Even the case of Phineas Gage (van Horn et al., 2012) may now be understood in terms of damage to white matter and neural circuitry, and poor social cognition in schizophrenics may be related to attenuated connectivity between the amygdala and insula, but increased connectivity with the pre-frontal cortex may indicate a compensatory role for top-down processing (Mukherjee et al., 2012). Similarly, those with ASD have reduced connectivity in the social brain regions, the amygdala, superior temporal sulcus (STS), MTG, fusiform gyrus (FFG), mPFC and the IFG. A better understanding of brain connectivity is essential in order to understand how cognition and emotion are integrated (Pessoa, 2008).

Functional connectivity has been typically analysed using seed-based correlation analysis or independent component analysis (Zuo et al., 2010). The former being more hypothesis-driven whilst the latter allowing for a more data-driven approach. Other methods

of analysis have included principle component analysis, clustering, pattern classification, structural equation modelling, dynamic causal modelling (DCM), and Granger causality. In recent years, however, graph theory, the study of graph topology and its characteristics, has been applied to 'small world networks' (Watts & Strogatz, 1998), the phenomenon that most nodes within a network are easily reached within a small number of steps and that the number of steps is a function of the number of nodes. The inception of graph theory dates back to 1736 with Leonhard Euler's published solution to the Problem of the Seven Bridges of Konigsberg proving that some of the bridges must be crossed more than once. Modern day graph theory, however, may be applied to a number of networks including the brain. Local clustering of nodes are thought to create functional segregation within the network via short paths and highly connected nodes throughout the brain, otherwise known as hubs or 'the rich club' (Van den Heuvel & Sporns, 2013). Secondly, the hubs establish global links between local clusters ensuring integration of information throughout the whole network (Bullmore & Sporns, 2009).

A network approach departs from earlier ways of conceptualising the brain as consisting of different regions, but instead it can be viewed as an interconnected system (for reviews see van Straaten & Stam, 2013; Telesford, Simpson, Burdette, Hayasaka & Laurienti, 2011). Using graph theory, nodes are often known as 'centrality metrics' and are likely to influence the flow of information from one part of the brain to another. Information flows along edges which are defined by the functional connectivity between the nodes or vertices – mathematically, the correlation of the time-series produced during neuroimaging. Typically, methods of analysis within graph theory involve two stages, firstly identifying the 'nodes' through parcellation of grey matter, and secondly, estimating the connections or 'edges' between the nodes in relation to the time-series of the nodes identified using fMRI, thereby describing the connectivity between regions. Measures of centrality can be degree (number of connections), closeness (distance between nodes), betweeness (shortest geodesic path), subgraph (the number of walks beginning and ending at a particular node), leverage (nodes which are more connected than others), Katz (the influence of an actor within a given network), or eigenvector (how frequently a particular node is revisited). It is the latter centrality metric which is adopted in this study, otherwise known as Eigenvector Centrality Mapping (ECM) - *"Mathematically, eigenvector centrality is a positive multiple of the sum of adjacent centralities and is based on the philosophy that a node is more central if its neighbours are also highly central"* (Joyce et al., 2010:1).

Aims of the present study

Neuropeptides, including OT, function in a way which makes it difficult to study them, for example, lacking temporal and spatial specificity (Landgraf & Neumann, 2004). Animal studies of OT have established that it is involved in bonding between parents and their offspring (Pedersen & Prange, 1979) and between mates (Young, Young & Hammock, 2005). It has also been found to have an anxiolytic effect (Bale et al., 2001). Studies in humans are less conclusive; it has been found to influence social cognition (De Dreu et al., 2010; Kosfeld et al., 2005; Zak et al., 2007) though issues of dosage and method of administration may contribute to differential findings in the literature (Harris & Carter, 2013; MacDonald & Feifel, 2013). Neuroimaging studies have focused largely upon the amygdala and task-related connectivity (Domes et al., 2007a; Kirsch et al., 2005; Petrovic et al., 2008) throughout the cortex with some recent studies investigating the influence of the dopaminergic system (Groppe et al., 2013; Wittforth-Schardt et al., 2012). Such studies have typically adopted a ROI-approach to analysis and, in so doing, have adopted an *a priori* hypothesis based upon previous literature. Given that neuropeptides are notoriously difficult to study, and given that research into the effects of OT is in its infancy, this study adopts a more explorative approach. Furthermore, recent literature has demonstrated the importance of ICNs in neuropsychiatric disorders (Keehn et al., 2013) and a correspondence between task-related behaviours and specific networks (Laird et al., 2005, 2009). It is possible, therefore, that other networks or hubs (Van den Heuvel & Sporns, 2013) may exist which have yet to be identified.

This study sought to explore activation and connectivity during t-fMRI whilst the participants were actively involved in a social task. In order to do this, healthy males were recruited and participated in t-fMRI, the task being typical of those used within the OT literature and involving identification of facial emotions (Domes et al., 2014; Gorka et al., 2014; Labuschagne et al., 2010, 2011). Further, the study design allowed for the exploration of the influences of intravenously (1IU) and intranasally (8IU and 24 IU) administered OT upon cortical activity and connectivity in order to address whether dosage or method of administration had an impact upon the influence of OT. As already noted, eigenvector centrality mapping (ECM) was selected for analysis (see below). This provides a good method for investigating areas which may be influenced by OT but have previously been missed. To our knowledge this is the first study to use ECM in the study of t-fMRI. For this reason, additional whole brain analysis was also conducted using linear modelling which allowed for the specific features of the task to be modelled.

Research questions

As an explorative study, we sought to examine whether:

1. There is differential functional connectivity under the influence of OT compared with placebo during a facial emotion sensitivity task.
2. There is differential neuronal recruitment across the brain under the influence of OT, compared with placebo, for faces vs shapes, and angry vs happy faces, during a facial emotion sensitivity task.
3. Dosage of OT (8 vs 24 IU) and method of administration (IN vs IV) have a differential influence on brain activation.

There is increasing evidence that OT influences functional connectivity across different cortical regions (Gorka et al., 2014; Rilling et al., 2012, 2014; Wittforth-Schardt et al., 2012). We predict that OT will differentially influence centrality between key regions/hubs throughout the brain compared with placebo, such nodes being specific to the task (Bethlehem et al., 2013). Further, we predict that OT will lead to differential functional activation in response to faces vs shapes, and between emotional expressions (happy vs angry faces) during the task (Domes et al., 2010; Labuschagne et al., 2010; Phan, Fitzgerald, Pradeep & Tancer, 2006).

It has been demonstrated that OT reaches the brain via intranasal administration with consequences for central processing (Szeto et al., 2011). It remains less clear whether peripheral administration has the same effects (Morimoto et al., 2009; Zlokovic, 1990). For this reason, we anticipate that intranasal administration is likely to have a greater impact than intravenous administration but that an effect of IV administration is not ruled out (Hollander et al., 2003, 2007). It is not possible to predict which dosage will have the greatest effect on neuronal recruitment. Many studies utilize 24 IU (Dodhia et al., 2014; Domes et al., 2014; Rupp et al., 2014), but there are no consistent findings in terms of dosage (Bakermans-Kranenburg & van IJzendoorn, 2013). This may indeed reflect the nature of the treatment (acute vs chronic) or the method of administration such that it is not possible to control for consistent dosage. This study, therefore, in utilizing a new nasal device (see Appendix C) with optimal uptake, might offer evidence that a lower dosage than 24 IU is equally effective.

Methods

Participants

Participants were drawn from the male student population of the University of Oslo, Norway. In total, 57 individuals responded during the recruitment phase and were assessed for eligibility using a telephone screening protocol. Of these, 25, aged 18-35, were fully screened at the hospital for a period of 2 hours each. Of these, 7 did not meet the criteria for the project (see below), and were excluded. In total, 18 participants began testing, 2 of whom withdrew from the project having participated in at least one session. A total of 16 participants (aged 20-30 years, mean=24 years, SD=3.27) completed all four sessions. Participants were compensated for their involvement per session including screening.

Exclusion from the study was established through a series of medical and psychological assessments. The exclusion criteria included a self-reported history of psychiatric illness based upon the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998); self-reported evidence of alcohol or drug abuse using the Alcohol Use Disorder Identification Test (AUDIT) (Saunders, Aasland, Babor, de la Fuente & Grant, 1993) and the Drug Use Disorder Identification Test (DUDIT) (Berman, Bergman, Palmstierna & Schlyter, 2005); and an IQ below 75 points as measured by the Wechsler Abbreviated Scale of Intelligence (WASI) (Weschler, 1999). Furthermore, participants received a short medical in which their height and weight were measured, as well as their heart rate, blood pressure, oral temperature, respiration rate and electrocardiogram (ECG), to ensure no clinically significant results as judged by a physician. A basic haematology assessment was conducted to establish that participants' values were within the normal range.

Participants were also screened using auditory rhinometry to establish whether or not there was evidence of nasal septal deviation which might hinder the uptake of OT via the nasal cavities. Auditory rhinometry is a procedure in which sound waves are sent through the nasal cavity via the nostril. This produces a reading which reflects the opening in the cavity and can give an accurate indication of any blockages caused by structural problems such as a septal deviation or by a temporary condition such as congestion. During screening participants were also given training of the nasal device which would be used to self-administer the study drug (OT or placebo). This device, known as the Optinose Nose-to-Brain (N2B) device, adopts a different technique to traditional nasal sprays (see Appendix C).

Written informed consent was obtained from all participants before testing and ethical approval was given by the Regional Committee for Medical and Research Ethics (*REK* sør-øst) and the Norwegian Medicines Agency (*Statens legemiddelverk*). The study was monitored by Smerud Medical Research, a contract research organisation (CRO, www.smerud.com), which also coordinated the study randomisation and blinding.

Study Design

The project followed a randomised, placebo-controlled double blind, double-dummy four period cross-over design. Participants were randomised into one of four treatment sessions, using a four-period four-treatment Latin square (ACDB, BDCA, CBAD, DABC in a 4:4:4:4 ratio). Conditions were as follows:- A=8IU IN; B=24IU IN; C=1IU IV; D= placebo. On each visit participants received both IN and IV administration with at least one placebo dosage per session. Thus participants received a combination of either OT (8IU or 24IU) administered intranasally, placebo (either IN or IV) and 1IU (administered intravenously) (see Figure 1 below). IN OT was supplied by Sigma-Tau Farmaceutica SA, Funchal, Portugal. IN OT was delivered in sodium chloride (NaCl) whereas the placebo was NaCl alone. All study personnel and participants were blind to the condition being tested since a dedicated nurse was responsible for the mixing of the IV study drug and Smerud Medical Research distributed the IN study drug. A washout period of at least 6 days between sessions was implemented and testing took place at the same time of day each time to cohere with the circadian rhythms of the participants and the possible influence this might have upon the naturally occurring concentration of OT in the body and uptake of the drug. This study draws upon data obtained during all four conditions.

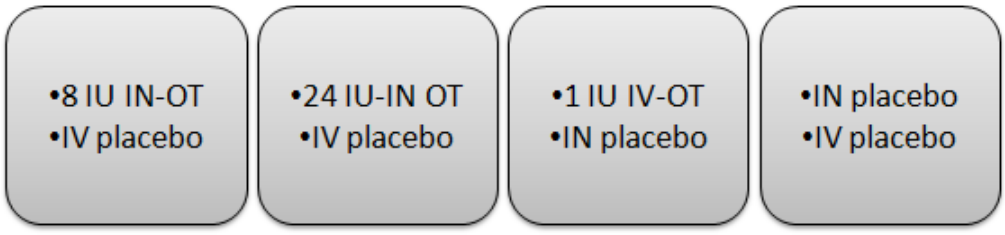


Figure 1: Randomised conditions

Materials

A number of variables were tested for the project. Data was collected in the form of functional magnetic resonance imaging, both during an facial emotion processing task and at rest, eye-tracker, pupillometry, ECG, respiration rate, blood and saliva samples (OT, vasopressin, cortisol), The State-Trait Anxiety Inventory, y form (STAI) (Spielberger, Gorsuch, Lushene, Vagg & Jacobs, 1983), the Temperament and Character Inventory (TCI) (Cloninger, 1993), and the NEO Personality Inventory-R (NEO) (Costa & McCrae, 1992). This study draws only upon the t-fMRI data as outlined previously.

Cognitive fMRI task. Participants viewed a *facial emotion sensitivity task*, programmed in EPrime 2.0 (Psychology Software Tools, Inc., Pittsburgh), in which a selection of images from the Karolinska Directed Emotion Faces database (Lundqvist, Flykt & Öhman, 1998) were presented (see Figure 2 below) followed by two questions relating to the image they had viewed. Male and female faces, each depicting happy, angry or neutral expressions were used in the task. Whilst the neutral faces offered a control condition, the happy and angry faces offered the opportunity to explore the emotion valence-specific effects of OT. The facial stimuli were presented within an ellipsoid with the background altered to control for the luminance of the whole image. This measure was necessary to ensure changes in luminance were not a confounding factor in the pupillometry data. In addition to facial stimuli, images of geometric shapes (square/triangle) of different colours (yellow/blue/green) on a black background were used as non-social stimuli in order to test for differential effects of OT on social and non-social stimuli, respectively. All images were presented at 3 randomised locations along the y-axis such that either the eyes, nose or mouth were centred in the screen. This was for the purpose of the eye-tracker data to enable evaluation of eye movement from the centred fixation point to different regions of the face.

The task followed an event-related design with trials presented in a pseudo-randomised fashion ensuring that no trial condition (gender/emotion/question) was repeated more than twice in a row as this might have an impact on the BOLD response. Once all possible combinations were established and repetitions removed the order was randomised in MS Excel 2013 and then presented sequentially within EPrime. The task consisted of 5 blocks of 20 trials, each approximately 4 min in duration with a resting period of 20 ms between each block; the total duration of the task being approximately 21 minutes. Each trial started with a fixation cross of 3000 ms duration followed by stimulus presentation (face or

shape) for 1000 ms followed by a black screen for 3000 ms. Next, the subject was prompted to respond to the first question, followed by the second using a visual analogue scale (VAS) rated from 1-5, the location of the cursor being randomised per trial. The face stimuli consisted of 10 possible faces (5 male, 5 female), each presented 6 times – once per emotion (happy/angry/neutral) and once per emotion question (happy/angry). Hence, the task included 60 facial trials (10 x 3 x 2). Furthermore, it included 20 non-social trials (shapes) and 20 null trials. In null trials, which were also pseudo-randomly distributed among the other trials, the stimulus was simply replaced by a fixation cross and no questions were given. Each of the four sessions included 10 new faces in order to avoid cross-over memory effects.

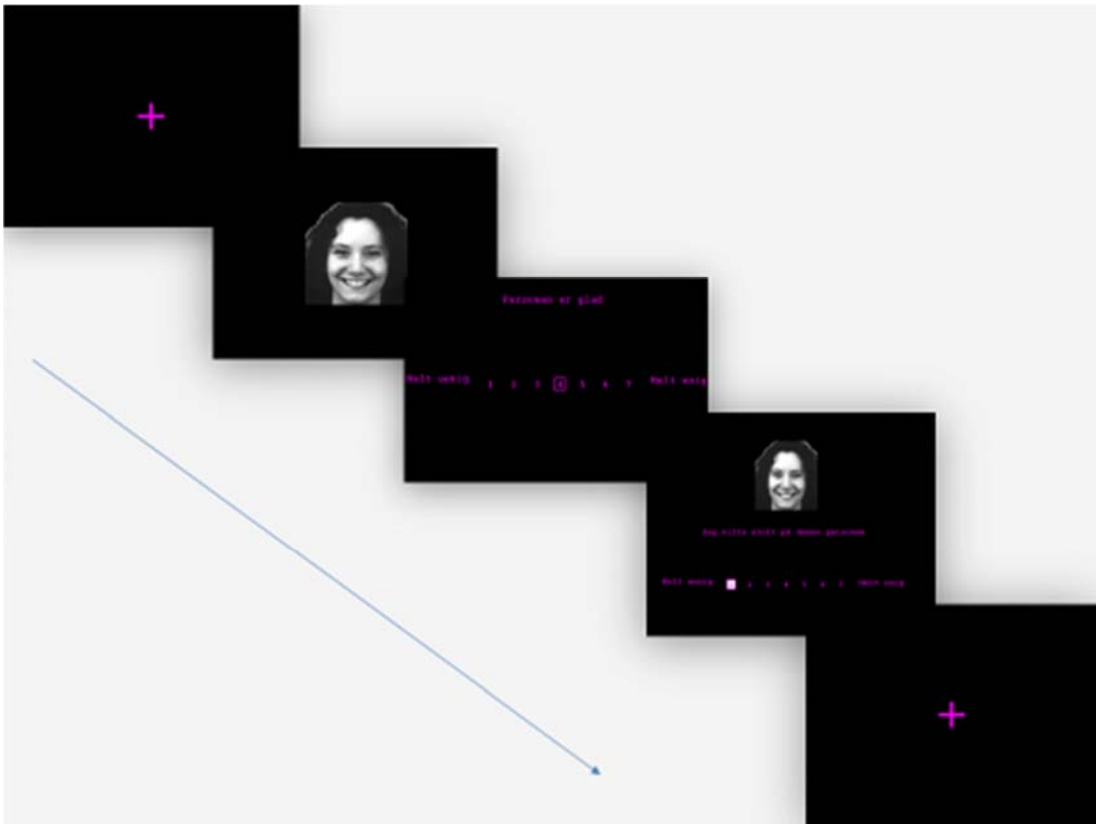


Figure 2: Facial emotion sensitivity task. Fixation point followed by face (happy/angry/neutral), followed by two questions:- Question 1: How happy is this person? Question 2: How much would you trust this person?

Stimuli was presented before participants received one of two randomly presented questions. In the social condition, questions were either *"How happy is this person?"* or *"How angry is this person?"* Thus, they were asked to make such judgements in order to elicit the sensitivity to recognition of facial emotions. The comparable questions in the non-social condition were *"How blue is this colour?"* or *"How yellow is this colour?"*. A second

question, always the same, was presented thereafter in which subjects were presented with the face a second time and asked to rate *"How much would you trust this person?"* The comparable question in the non-social condition being *"How much do you like this colour?"* Participants used a response grip (Nordic imaging Lab, Bergen, Norway, http://www.nordicneurolab.com) in their left hand to make their responses. Stimuli were presented through VisualSystem goggles (Nordic Imaging Lab, Bergen, Norway, http://www.nordicneurolab.com).

Procedure

Each testing session began with a pre-scanning phase in which participants received a brief re-assessment of the criteria used to exclude participants during screening. They first completed the STAI in order to establish a baseline of anxiety before auditory rhinometry was performed in order to check their nasal cavities for signs of congestion. They were also retrained with the Optinose N2B device. A nurse then conducted a brief medical assessment in which participants were again weighed and their blood pressure was measured. They were also asked to confirm that they had not consumed alcohol or caffeine in the previous 24 hours.

All participants were then guided to the scanning room for a 2 min baseline reading of their heart rate (ECG) and respiration rate. This was conducted whilst the participant was lying on the scanner bed but before the machine was in operation. Immediately afterwards, participants were provided with eye-tracker goggles and assisted by the radiographer in making the adjustments to the camera so that they would be able to see the task stimuli clearly during testing. Following this, they performed a short practice session of the cognitive task (different faces from those used in the testing sessions) so that they could become accustomed to the task instructions, speed of responding and the controls. They were then disconnected from the respiration belt and ECG sensors and guided out of the scanning room.

The second phase of the session involved administration of a venflon into the arm of the participant by the project nurse after which baseline samples of both blood and saliva (P1) were taken to establish basic haematology as well as OT, vasopressin and cortisol levels. The participant then received an IV (500 ml) and self-administered the IN study drug simultaneously using the nasal device (3 puffs in each nostril, each 4 IU, administered in alternating order under close supervision and whilst looking into a mirror). Following the infusion (approximately 20 minutes), blood and saliva samples were taken again (P2) thus recording the immediate levels of the study drug in the participant's system. Following this

participants returned to the scanner room and were reconnected to the ECG and respiration belt. The radiographer assisted them with the goggles and prepared them for scanning whilst the nurse and study staff took blood and saliva sample P3.

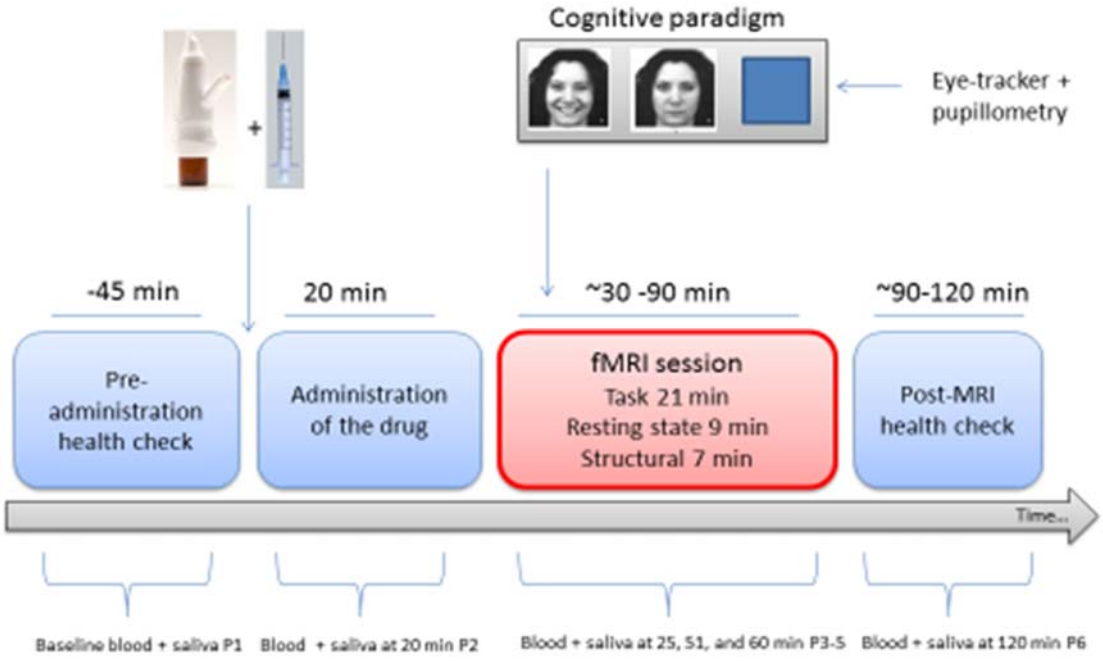


Figure 3: Study Procedure. Prior to administration of the study drug, participants received a health check and baseline blood samples were taken. This was followed by administration of the drug (IN and IV). Thereafter participants were scanned. The session ended with a final blood sample and observation period to ensure no adverse effects were experienced.

The sequence of the scanning was determined based on the anticipated pharmaco-kinetics of OT, and consisted of (1) 21 min fMRI during task performance, (2) 9 min fMRI during resting state and (3) T1-weighted structural MRI. The cognitive task was run during the first fMRI sequence, approximately 40 minutes after receiving the treatment drug. For the resting state sequence, participants were instructed to lie in the dark with their eyes open. Further blood and saliva samples were taken in each of the breaks between sequences, i.e. after the t-fMRI sequence (P4) and following resting state (P5). ECG was recorded during the full duration of the scan session and hard triggered at the beginning and ending of each sequence in order to facilitate the analyses.

Once the scanning sequences had been completed the participant left the scanning room and waited for a short period until 120 minutes had elapsed from administration of the study drug in order to allow for observation and reporting of any adverse events related to study administration, typically a period of approximately 20 minutes. Thereafter, sample P6

was taken and participants completed the STAI a second time. Total session length was typically 3 hours, thus a total of 12 hours per participant across all sessions.

MRI acquisition

MRI data was obtained on a standard 8 channels whole-head coil on a 3T General Electric Signa HDxt scanner (GE Healthcare, Milwaukee, WI, USA) at Oslo University Hospital Ullevål. The MR sequence used for fMRI was a T2*-weighted echo-planar imaging (EPI) sequence with the following parameters: repetition time (TR) = 2400 ms, echo time (TE) = 30 ms, flip angle (FA) = 90 degrees, field of view = 64, 48 axial slices, voxel size = 3.2 x 3.75 x 3.75 mm. In each session, one run comprising 528 volumes was collected during emotion task performance, resulting in a scan time of 21 minutes. In addition, an identical MRI sequence was used during a resting-state run with a duration of 9 minutes, yielding 232 dynamic fMRI volumes. The imaging protocol also consisted of a fast spoiled gradient echo (FSPGR) T1-weighted sequence with the following parameters: TR = 7.8 ms, TE = 2.956 ms, inversion time (TI) = 450 ms, FA = 12 degrees, 166 sagittal slices, and voxel size of 1.2 x 1.0 x 1.0 mm. In the present study, the T1-weighted sequence was used for co-registration purposes.

MRI preprocessing and analysis

Initial processing of T1-weighted data was conducted using FreeSurfer (http://surfer.nmr.mgh.harvard.edu) (Dale, Fischl & Sereno, 1999) in order to extract the brain from the skull and other non-brain tissues. Thereafter, the first five volumes were discarded to allow for signal equilibration. Pre-processing of fMRI data was conducted using FEAT (FMRIB's Expert Analysis Tool) version 6.0 (Smith et al., 2004), part of the Functional MRI of the brain (FMRIB) Software Library (FSL) 5.0.1 (http://www.fmrib.ox.ac.uk/fsl) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). This included motion correction (head movement and cardiac/respiratory artefacts) and improving the signal-to-noise ratio (SNR) using pre-whitening and spatial smoothing; a Gaussian kernel of FWHM of 7.0 mm was applied. A high pass filter of 100 s was adopted with a signal loss threshold of 10. Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC ICA) (Beckmann & Smith, 2004) was also included for automated denoising (see below). FMRIB's Linear Image Registration Tool (FLIRT and MCFLIRT) (Jenkinson,

Banner, Brady & Smith, 2002) was utilized in order to register each participant's fMRI data to a standard space (MNI-152) via the T1-weighted volume. After applying Boundary Based Registration (BBR), images were registered to each participants own structural image, and further processed using FMRIB's Non-Linear Image Registration Tool (FNIRT) (Andersson, Jenkins & Smith, 2010) to a resolution of 2x2x2 mm.

Additional noise reduction was conducted using FIX 1.05 (FMRIB's ICA-based X-noisefier (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIX) (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014**)**. This is a novel tool for further cleaning components in fMRI data following processing with MELODIC. Using training files based upon actual data or standard files included in the package, FIX removes noisy components from the 4D fMRI data before further analysis. For the t-fMRI here, the standard file was used on a subset of the files and compared to manual classification. Since there appeared to be a high degree of correspondence, the standard file was then applied to all 64 data sets (16 participants x 4 conditions). A visual inspection was conducted on all data thereafter.

Statistical analyses

Statistical analysis was conducted for functional connectivity using Eigenvector Centrality Mapping (ECM) whilst functional activation was analysed using linear modelling. In both sets of analyses, the significance level for the P-value (the probability of obtaining a test statistic result as extreme as that observed) was set at $p<0.05$.

Functional Connectivity: Eigenvector Centrality Mapping (ECM). ECM is a parameter free and data-driven method and, since each voxel in the brain is treated as a node in order to establish which are visited most often, the entire brain is part of the analysis. In recent years this approach has been used solely with r-fMRI (Hardmeier et al. 2012; Joyce et al., 2010; Lohmann et al., 2010; Meinzer, Lindenberg, Antonenko, Flaisch & Floel, 2013; Wink, de Munck, van der Werf, van den Heuvel & Barkhof, 2012; Zuo et al. 2012) though the close connection between networks at rest and in active states (Laird et al., 2011; Smith et al., 2009) would suggest ECM may be a fruitful approach to t-fMRI as well.

Centrality of a node is a definition of the prominence of that node within a network (Sporns, Honey & Kotter, 2007). According to graph theory, eigenvector centrality refers to

the prominence of a node within the network as defined by its relationship to neighbouring nodes. Whilst the centrality of a node increases with its connection to other nodes, it increases further if it is connected to other nodes which have many connections of their own. The entire network of connections can be described by a *binarised adjacency matrix*. When applied to the brain the adjacency matrix involves the mapping of tens of thousands of voxels per individual making this a time-consuming and computationally-costly process with implications for the resolution of the images. Fast ECM (fECM), introduced by Wink et al. (2012), overcomes this by utilizing eigenvector estimates based on the data instead of constructing an adjacency matrix. fECM involves computation of matrix-vector products in order to create a projection matrix by utilizing the 'Power Iteration Method' (Goub & van der Vorst, 2000). From this, the eigenvector with the largest value is identified. This is repeated until a convergence criterion is reached, typically after 10 iterations (Wink et al., 2012). In running fECM, we computed ECM-maps for each participant and each of the four conditions (64 data sets) before merging them into a single 4D file. We then computed mean ECM-maps across conditions in order to identify major brain hubs.

Permutation testing. 4D files were subjected to permutation testing to test the effect of OT condition upon centrality. This was done using FSL's *randomise* (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise (Winkler, Ridgway, Webster, Smith & Nichols, 2014). Randomise provides non-parametric permutation testing which controls the familywise error rate (FWE) and allows statistical inferences to be made upon data where the null distribution is unknown (Nichol & Holmes, 2002).

We set up a repeated measures GLM for individual data sets. Subject means were regressed out and 6 paired t-test contrasts were added in order to contrast the conditions with each other and with placebo. Two additional contrasts were set up to compare OT (all three conditions collapsed) with placebo. An F-test was also included in the model to test for the main effect of condition. Each contrast yielded 5000 permutations (alpha=0.05), corrected for multiple voxel-wise comparisons (Nichols & Holmes, 2002). We used group exchangeability blocks to account for within-subject repeated measures design, that is, permutations were performed within subject only. Further, threshold-free cluster enhancement (TFCE) was applied to allow for identification of the clusters without the need for arbitrary cluster-threshold prior to testing. An inclusion mask containing only voxels present in all data sets was produced. The Harvard-Oxford Cortical and Subcortical Atlases (Harvard Center for

Morphometric Analysis), part of FSL, was used to establish anatomical labels for local maxima.

Functional Activation: Linear modelling. General Linear Model (GLM) analysis was conducted on 64 data sets (16 participants x 4 conditions) in order to examine the possible influence of OT on brain activity during the facial emotion sensitivity task. By inputting the time course of a given task design, it is possible to estimate brain activation and, thereafter, to test whether or not observed activation matches this model. Briefly, fMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) as described above. First level (time-series) analysis was conducted using FILM (FMRIB's Improved Linear Model) in order to create the initial model followed by higher level analysis using FLAME (FMRIB's Local Analysis of Mixed Effects).

Each condition (8IU, 24IU and 1IU IV) and all events in the task (explanatory variables) (EVs) were used as predictors in the model. We tested two models, the first (referred to as the Visual Stimuli Model or VSM) modelling faces compared with shapes, and the second (Emotion Stimuli Model or ESM) modelling emotional stimuli (happy/angry/neutral faces) compared with shapes or compared with each other. The questions in the task were included in both models. Firstly, we combined all four conditions (8IU IN/24IU IN/1IU IV and placebo) to examine the main effects of task across all participants and time-points in each model. Secondly, we conducted a repeated measures analysis to examine the group effects of individual conditions compared with both placebo and with each other, in both models. The latter included both a series of pairwise contrasts and F-tests in order to test for main effects of condition.

Arising from the contrast vectors created in the model, Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z>2.3$. All clusters were corrected for multiple comparisons with a corrected cluster significance threshold of $p=0.05$ (Worsley, 2001). A visual inspection of the statistical maps for each volume was conducted using the Harvard-Oxford Cortical Structural brain atlas. Thereafter, using coordinates derived from the cluster indexes, significant regions of activation were identified for all local maxima. To cross check the identified regions, coordinates were converted to the Talairach system, using Signed Differential Mapping, (www.sdmproject.com/utilities/?show=Coordinates) and

thereafter, processed with the Talairach Client (www.talairach.org/client.html) (Lancaster et al., 1997, Lancaster et al., 2000) (see Appendix D).

Results

Eigenvector Centrality Mapping (ECM)

Figure 4 shows the group mean map. Briefly, we observed high centrality in brain regions consistent with resting-state literature (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006; van den Heuvel & Sporns, 2013) namely, in the precuneus, insular, PFC, SMG, SPL, STG, MTG, FFG, lingual gyrus, and in the ACC and posterior cingulate.

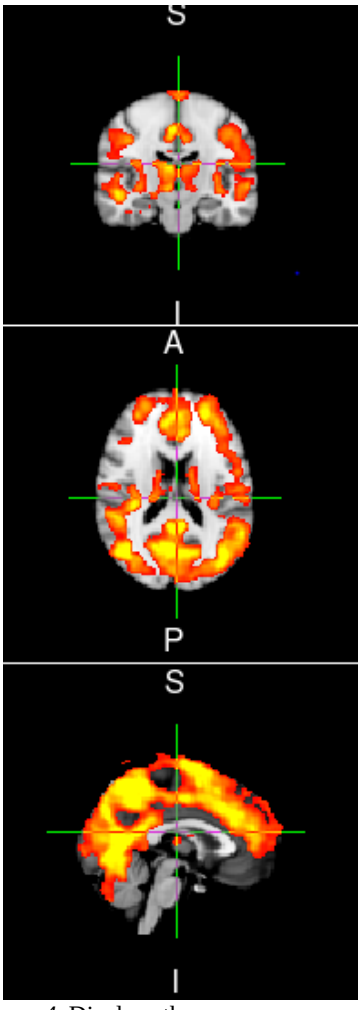


Figure 4. Displays the group mean map, established when mapping the entire sample with a cluster generating threshold of t>2.13 (p<0.05, corrected). Images are displayed in radiological convention.

Permutation testing revealed no significant main effect of OT condition with the F-tests ($p<0.05$, corrected). Since this is an explorative study we also aimed to investigate trends within the data and, therefore, conducted pairwise comparisons, corrected for multiple comparisons ($p<0.05$, corrected), but found no significant differences in centrality between OT conditions.

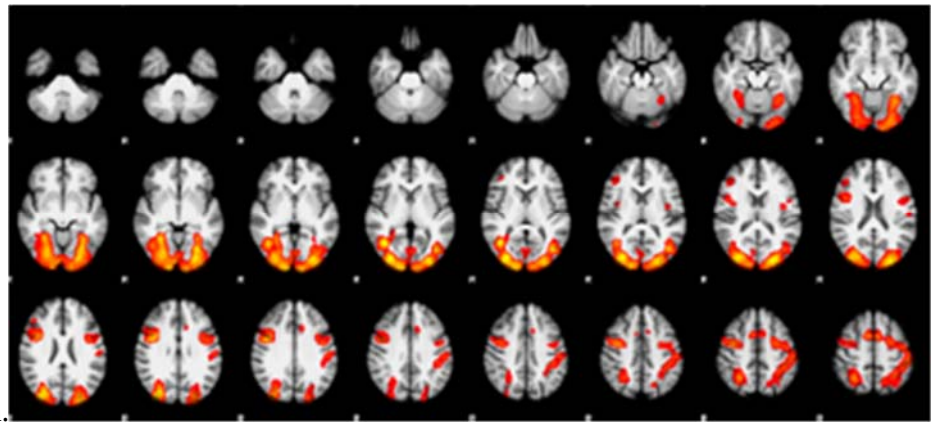
Conventional fMRI task-based analysis (Linear Modelling)

The following section presents the main effects for both the experimental and OT conditions using linear modelling.

Main effects of experimental conditions. We initially took an exploratory approach to examine the effects of the task across all participants and all conditions. Specifically, we examined the effects of faces and shapes in the visual stimuli model (see Figures 5 and 6 below), and angry, happy and neutral faces in the emotion stimuli model (see Figure 7, 8 and 9 below).

Visual Stimuli Model. We observed increased neuronal recruitment for faces (*faces*) compared to baseline (which included all fixation points), in three clusters (see Figure 5a below) – peak values were found in the right lateral occipital cortex (LOC) and middle temporal gyrus (MTG) ($p<0.001$, corrected), left juxtapositional lobule (JPL), superior frontal gyrus (SFG), paracingulate gyrus ($p<0.001$, corrected), precentral gyrus, IFG and MFG ($p=0.002$, corrected). Other regions included the left FFG, lingual gyrus, right cingulate gyrus, and left SMG. We also observed reduced neuronal recruitment in three further clusters – peak values in the local maxima were found in the right hippocampus and amygdala ($p<0.001$, corrected), right paracingulate gyrus, frontal medial cortex, and cingulate gyrus ($p=0.029$, corrected) and right insular, Heschl's gyrus, and planum polare ($p=0.046$, corrected). Additionally, reduced activation was found in the left FFG, and cingulate gyrus (Figure 5b).

a.



b.

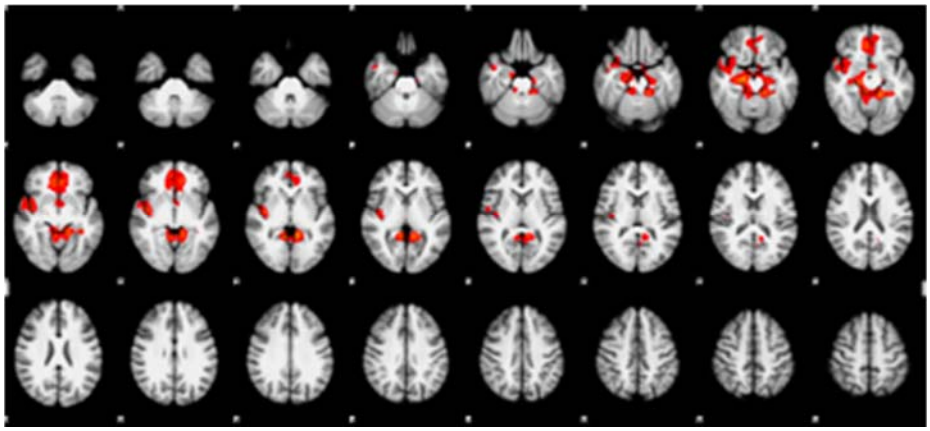
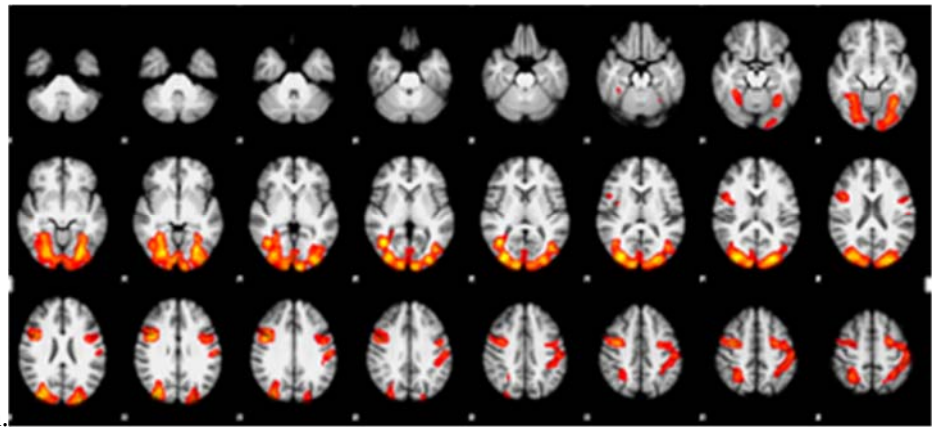


Figure 5: Maps display clusters that showed differences in activation for *faces compared to baseline*, thresholded at p<0.05, corrected. a) Map displaying regions with increased activation. b) Map displaying regions with reduced activation. Images are displayed in radiological convention.

We observed increased neuronal recruitment for shapes (*shapes*) compared to baseline in three clusters (see Figure 6a below) – peak values were found in the right LOC and MTG (p<0.001, corrected), left postcentral gyrus and SMG (p<0.001, corrected), and right precentral gyrus, IFG and MFG (p<0.004, corrected) but activation was also found in regions which included the left FFG, LOC and MFG. Neuronal recruitment was, however, reduced in three clusters (Figure 6b) – right hippocampus and amygdala (p<0.001, corrected), right cerebellum (p=0.003, corrected), and right paracingulate gyrus, frontal medial cortex and cingulate gyrus (p=0.037, corrected), as well as in the left FFG, LOC and cingulate gyrus. The right LOC, FFG and occipital pole (p<0.001, corrected) and left LOC and occipital pole (p=0.003, corrected) showed increased activation in faces compared with shapes (*faces>shapes)*.

a.



b.

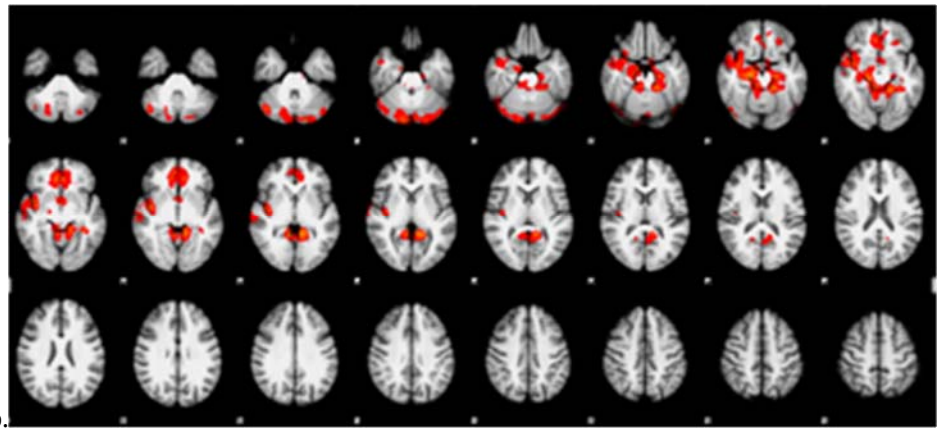
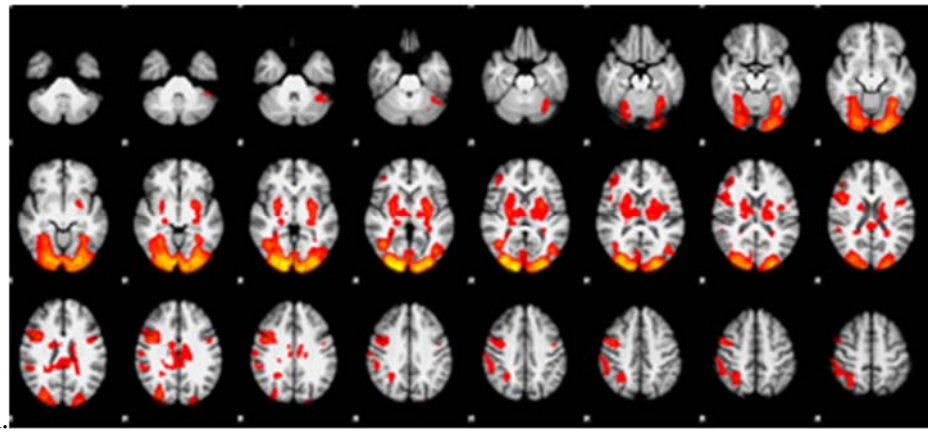


Figure 6: Maps display clusters that showed differences in activation for *shapes compared to baseline*, thresholded at p<0.05 corrected. a) Map displaying regions with increased activation. b) Map displaying regions with reduced activation. Images are displayed in radiological convention.

Emotion Stimuli Model. We observed an effect of happy faces (*happy*) compared to baseline with increased activation in two clusters (see Figure 7a below) with peak values in the right occipital pole, intracalcarine cortex, lingual gyrus and LOC (p<0.001), right precentral gyrus, IFG and MFG (p<0.001, corrected). Additional regions included the left LOC, FFG, and left and right putamen. Reduced activation was found with peak values in the right frontal medial cortex, paracingulate gyrus (p<0.001, corrected), right pre and postcentral gyri (p=0.001, corrected) and left precentral gyrus and SFG (p=0.043, corrected), as well as in the right hippocampus and amygdala (Figure 7b).

a.



b.

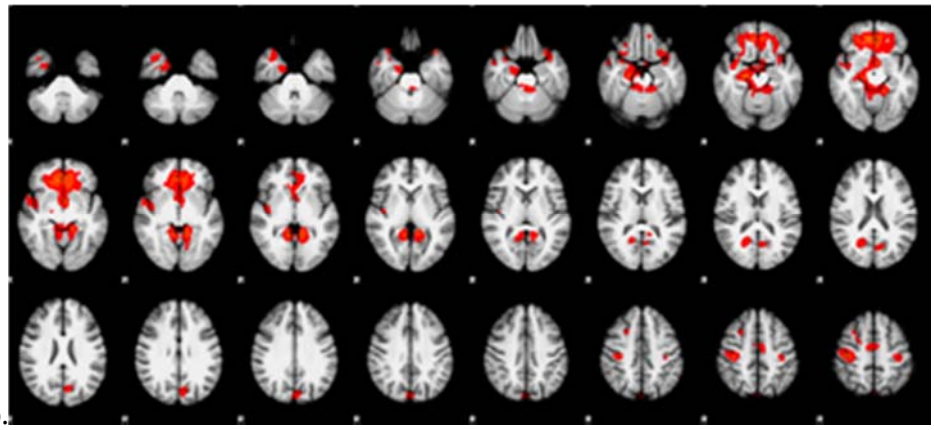
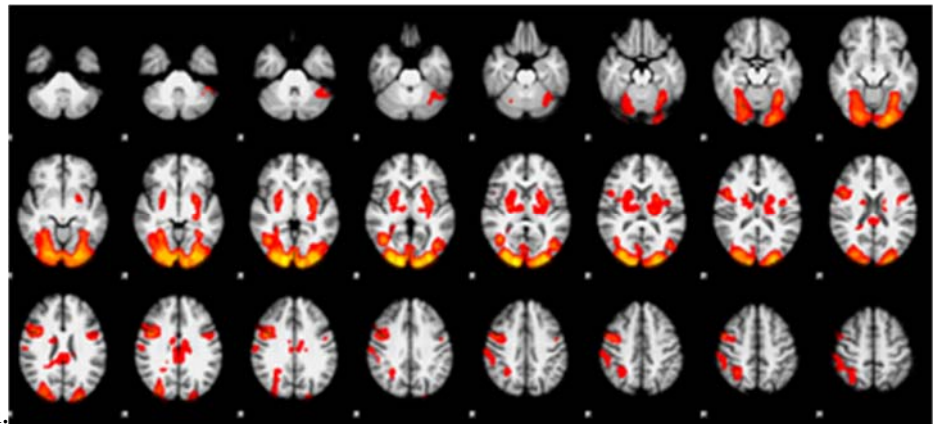


Figure 7: Maps display clusters that showed differences in activation *happy faces compared to baseline*, thresholded at p<0.05 corrected. a) Map displaying regions with increased activation. b) Map displaying regions with reduced activation. Images are displayed in radiological convention.

We also observed an effect of angry faces (*angry*) compared to baseline with increased activation in the right occipital pole, intracalcarine cortex, lingual gyrus, LOC (p<0.001, corrected) and right precentral gyrus, IFG, MFG (p<0.001, corrected), as well as the left LOC, and right putamen (see Figure 8a below). We also observed reduced activation in the left lingual gyrus, cingulate gyrus and parahippocampal gyrus (p<0.001, corrected) and right pre and postcentral gyri (p=0.006, corrected), in addition to regions which included the right hippocampus and amygdala (Figure 8b).

a.



b.

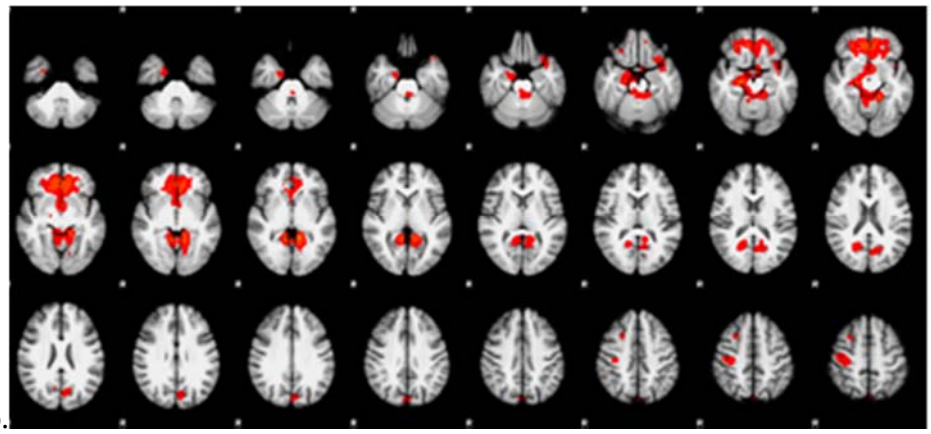
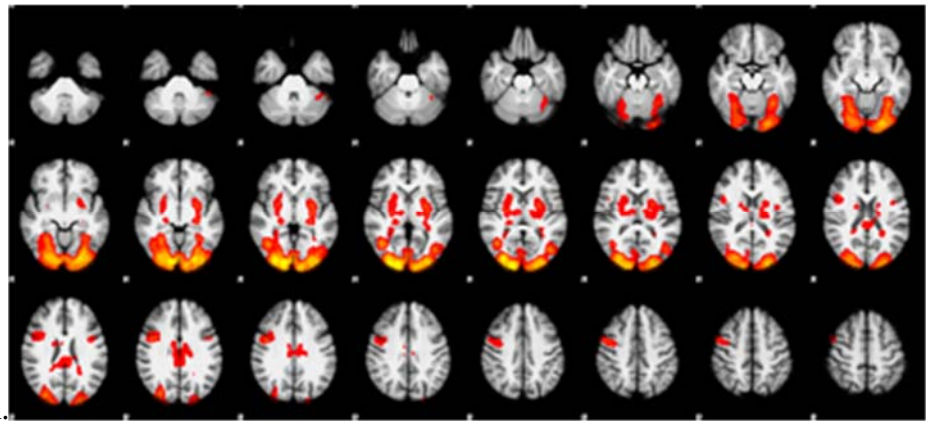


Figure 8: Maps display clusters that showed differences in activation for *angry faces compared to baseline*, thresholded at p<0.05 corrected. a) Map displaying regions with increased activation. b) Map displaying regions with reduced activation. Images are displayed in radiological convention.

Activation to neutral faces (*neutral*) compared to baseline was increased in the right occipital pole and LOC (p<0.001, corrected), left putamen (p<0.001, corrected), right precentral gyrus, IFG and MFG (p=0.031, corrected) as well as regions which included the left FFG and LOC (see Figure 9a below). It was, however, reduced in the right parrahippocampal gyrus, hippocampus, amygdala (p<0.001, corrected), right pre and postcentral gyri (p=0.003, corrected), left precentral gyrus and SFG (p=0.013, corrected) (Figure 9b).

a.



b.

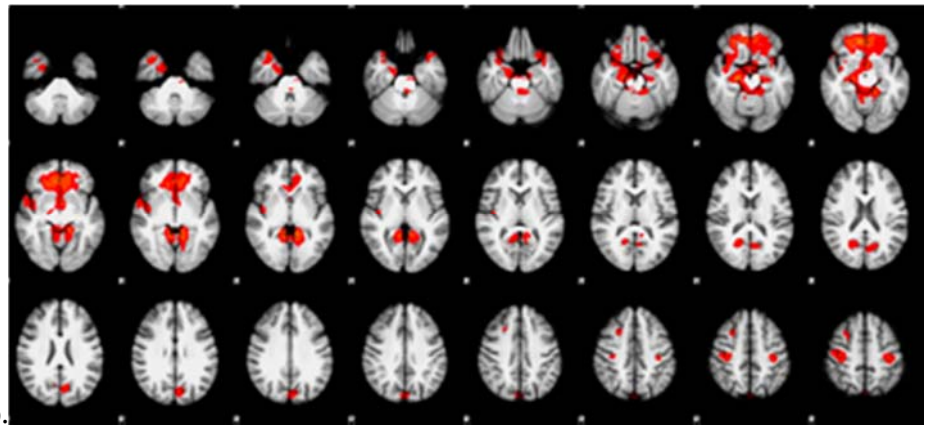


Figure 9: Maps display clusters that showed differences in activation for *neutral faces compared to baseline*, thresholded at p<0.05 corrected. a) Map displaying regions with increased activation. b) Map displaying regions with reduced activation. Images are displayed in radiological convention.

There was no effect for happy faces compared with angry (*happy>angry*), happy and neutral (*happy>neutral*) or angry and neutral (*angry>neutral*).

Main effects of OT conditions. The F-tests revealed no main effect for OT condition. Thereafter, we investigated the outcomes of the pairwise comparisons. These have been corrected for multiple comparisons across space (p<0.05, corrected). Comparisons of multiple *contrasts* and *conditions* invariably lead to an increased risk of Type I errors as well, but remain uncorrected. The aim of this study was to take an exploratory approach in order to examine possible influences of OT upon task activation, particularly for faces and for angry/happy faces. To this end, we report the results of the pairwise comparisons below but with circumspect interpretation. Note that localisation of activation, based upon the spatial

distribution of the statistical maps, is reported for the peak local maxima but detailed findings for all maxima are also presented (see Appendix D) in order to demonstrate bilateral effects and the hierarchy of activation within the clusters.

The three oxytocin conditions (8IU IN, 24 IU IN and 1IU IV), all influenced activation in the brain for at least one contrast though the extent of this influence varied greatly. Further, 8IU led to significant changes in neuronal activity in 18 clusters whereas in condition B, 24IU, 12 clusters and IV, 3. Figure 10 illustrates the extent of the influence of 8IU, in this case 8IU<placebo for the collapsed contrast *Q* (Questions 1 and 2), whilst Figure 11, illustrates Happy>Angry for 1IU IV<placebo (Figure 11a) and 24IU IN<placebo (Figure 11b).

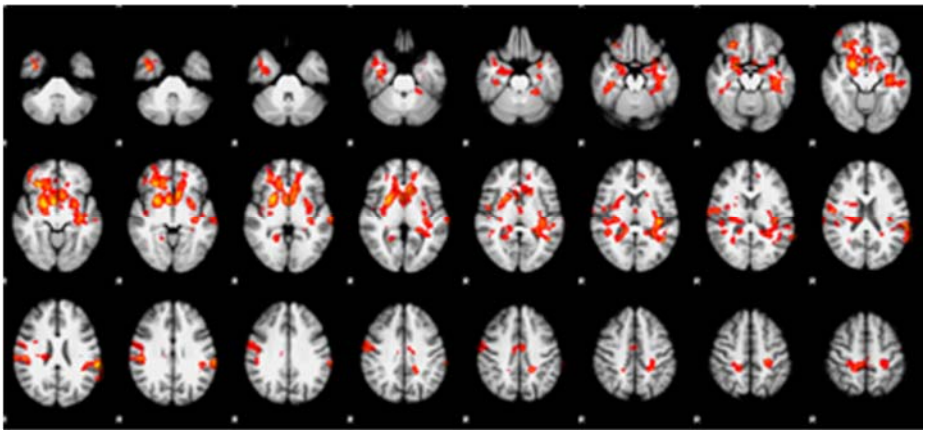
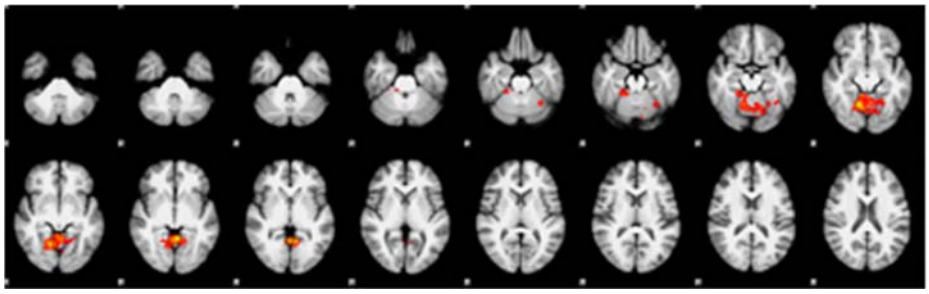


Figure 10: Map displays clusters that showed reduced activation for *questions compared to baseline* (collapsed Q1 and Q2, visual stimuli model), (8IU IN<placebo) thresholded at $p<0.05$ corrected. Images are displayed in radiological convention.

a.



b.

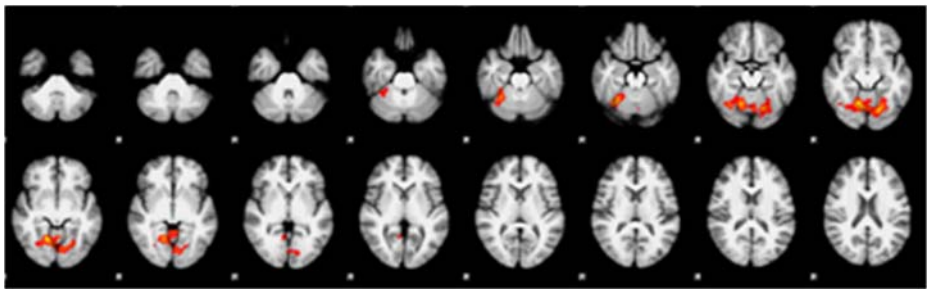


Figure 11: Maps display clusters that showed differences in activation for *happy>angry faces*, thresholded at p<0.05 corrected. a) Map displaying regions with reduced activation (1IU IV<placebo). b) Map displaying regions with reduced activation (24IU IN<placebo) (p=0.003). Images are displayed in radiological convention.

Visual stimuli (faces and shapes). We observed reduced task activation for faces only (*faces*) in the left LOC (p=0.026, corrected) and the right LOC, precuneus and cuneal cortex (p=0.036, corrected) following 24IU compared with 8IU IN (see Table 1 below). For shapes only (*shapes)* we observed reduced task activation in the left cerebellum following 1IU IV compared with placebo (p=0.034, corrected). The reduction for visual stimuli (*faceshape*) was observed in the left LOC following 24IU compared with 8IU IN (p=0.043, corrected). We also observed reduced task activation in the left cerebellum following 1IU IV compared with placebo (p=0.046, corrected) and in the left FFG, temporal occipital fusiform cortex, LOC and IT (see Appendix D, Table 8).

We found a reduction in the difference between neuronal activation for faces and shapes (*face>shape*) in the left LOC and FFG (visual stimuli model) (p=0.026, corrected), and in the left LOC and occipital pole (emotion stimuli model) (p=0.016, corrected) following 8IU IN compared with placebo. We also observed a reduction in the left MFG, frontal pole and the IFG following 24IU IN compared with 1IU IV (p=0.004, corrected).

Table 1: Cluster Index (CI) – Visual Stimuli Model (p=0.05, corrected)

COPE/ Event	Condition	Location	Cl	Voxel	(x,y,z)	Peak	P
Face VSM	24IU< 8IU	L LOC	2	1361	-24,-76,38	3.9	0.026
	24IU<8IU	R LOC, precuneus, cuneal cortex	1	1267	20,-68,40	3.67	0.036
Shape VSM	1IU IV<placebo	No label found (cerebellum)*	1	1290	-42,-58,-40	3.75	0.034
Faceshape VSM	24IU< 8IU	L LOC	1	1218	-32,-76,38	3.65	0.043
Faceshape VSM	1IU IV<placebo	No label found (cerebellum)*	1	1196	-42,-58,-40	3.78	0.046
Face>Shape VSM	8IU<placebo	L LOC, FFG	1	1336	-36,-84,26	3.24	0.026
Face>Shape ESM	8IU<placebo	L LOC, occipital pole	1	1506	-38,-88,26	3.36	0.016
Face>Shape ESM	24IU<1IU IV	L MFG, frontal pole, IFG	1	1967	-44,36,24	3.66	0.004
Neutral>Shape ESM	8IU<placebo	L LOC	1	1488	-34,-82,26	3.84	0.014
Neutral>Shape ESM	8IU< 24IU	R postcentral gyrus	1	1140	64,-12,42	3.67	0.047
Neutral>Shape ESM	8IU<1IU IV	R SMG, SPL, angular gyrus, postcentral gyrus	1	2220	38,-40,42	3.5	0.001
Neutral>Shape ESM	24IU< placebo	L LOC, SPL	1	1130	-22,-66,48	4.03	0.049
Neutral>Shape ESM	24IU<1IU IV	L frontal pole, MFG	1	1382	-44,38,24	4.06	0.02

* Talairach client (see Appendix D)

We observed a reduction in the difference between neuronal recruitment for neutral faces and shapes (*neutral>shape*) in the left LOC following 8IU IN compared with placebo (p=0.014, corrected). Also, a reduction in the right postcentral gyrus following 8IU compared with 24IU IN (p=0.047, corrected) (see Table 1 above). We also observed a reduction in the right superior parietal lobule (SPL), SMG, angular gyrus and postcentral gyrus following 8IU IN compared with 1IU IV (p=0.001, corrected) (see Table 1). Further, a reduction in the left LOC and SPL (p=0.049, corrected) following 24IU IN compared with placebo, and in the left MFG and frontal pole (p=0.02, corrected) following 24 IU IN compared with 1IU IV.

Emotional stimuli (happy/angry/neutral). We observed reduced task activation for happy faces (*happy*) in the right precuneus, cingulate gyrus, and the intra and supracalcarine cortices, otherwise identified as Brodmann's area 30 using the Talairach client following 24IU IN compared with placebo (p=0.017, corrected) (see Table 2 below). Other regions in the maxima also identified the right lingual gyrus and cerebellum (Appendix D). We also observed reduced task activation for neutral faces (*neutral*) in the left cerebellum following 24 IU IN compared with placebo (p=0.015, corrected).

Table 2: Cluster Index (CI) – Emotion Stimuli Model (p=0.05, corrected)

COPE/ Event	**Condition**	**Location**	**Cl**	**Voxel**	**(x,y,z)**	**Peak**	**P**
Happy face ESM	24IU< placebo	R precuneus, cingulate gyrus, subcalcarine cortex, intracalcarine cortex	1	1332	18,-52,-10	3.49	0.017
Neutral face ESM	24IU< placebo	No label found (left cerebellum)*	1	1400	-34,-68,-44	3.7	0.015
Happy>Angry ESM	8IU<placebo	L LOC, temporal occipital fusiform cortex, IT, FFG	2	1618	-4,-56,-14	3.41	0.014
	8IU<placebo	R white matter (WM) (no grey matter identified)	1	1276	24,-18,42	3.54	0.042
Happy>Angry ESM	24IU< placebo	R lingual gyrus, temporal occipital fusiform cortex	1	2206	14,-60,-12	3.39	0.003
Happy>angry ESM	1IU IV<placebo	No label found (left cerebellum)*	1	1874	-4,-50,-4	3.54	0.007

* Talairach client (see Appendix D)

We found the difference between happy and angry faces (*happy>angry*) was attenuated in the left LOC, the FFC and FFG, and the IT following 8IU IN compared to placebo (p=0.014, corrected). A second cluster (p=0.042, corrected) arose in white matter in the right hemisphere but no grey matter was identified in the peak value in the cluster. Other local maxima (Appendix D), however, were attenuated in the pre and postcentral gyri, the JPL and the cingulate cortex. Further, a reduction was observed in the right lingual gyrus and temporal occipital fusiform cortex following 24IU IN compared with placebo (p=0.003, corrected). A reduction was also observed following 1IU IV compared with placebo with the

peak value in the cerebellum (p=0.007, corrected) and the right lingual gyrus (see Appendix D, Table 8). However, we found no significant changes in neuronal recruitment between any emotional stimuli (happy or angry faces) and non-emotional stimuli (neutral faces or shape).

Questions. We also conducted exploratory analysis into the influence of OT on the questions asked during the task. We observed reduced task activation for question 1 (*"How happy/angry is this person?"*) (*Q1*) in the left SPL and SMG (p=0.028, corrected) following 8IU, compared with placebo (see Table 3 below). We also observed reduced task activation in the left pre and postcentral gyri (p<0.001, corrected) following 24IU IN compared with placebo. Other regions in the local maxima (Appendix D) include activation in the insular cortex, SMG and the IFG. Further, we observed reduced task activation in the left frontal pole, otherwise identified as the SFG (Talairach client) (p=0.016, corrected) (see Table 3 below) following 24 IU IN compared with 1IU IV.

Table 3: Cluster Index (CI) - Questions (p=0.05, corrected)

COPE/ Event	Condition	Location	Cl	Voxel	(x,y,z)	Peak	P
Q1 VSM	8IU<placebo	L SPL, SMG	1	1941	-28,-44,36	3.69	0.028
Q1 VSM	24IU< placebo	L pre and postcentral gyri	1	3724	-56,-6,24	4.19	<0.001
Q1 VSM	24IU<1IU IV	L frontal pole	1	2228	-34,52,8	3.6	0.016
Q2 VSM	8IU<placebo	R putamen	2	3285	22,4,-10	4.19	<0.001
	8IU<placebo	L amygdala, hippocampus	1	1404	-16,-8,-16	3.68	0.041
Q2 VSM	8IU<1IU IV	R amygdala, pallidum	1	2553	20,-8,-10	4.02	0.002
Q2 ESM	8IU<1IU IV	L frontal pole	2	4393	-18,56,16	3.84	0.000
	8IU<1IU IV	R IT(pos), MTG(pos)	1	1601	48,-28,-14	3.48	0.021
Q VSM	8IU<placebo	R frontal pole, frontal orbital cortex	3	10494	30,34,-6	4.19	0.000
	8IU<placebo	R pre and postcentral gyri, precuneus)	2	4209	6,-34,66	4.21	<0.001

	8IU<placebo	L postcentral gyrus, SMG, opercularum	1	2320	52,-22,26	3.69	0.012
Q VSM	8IU<1IU IV	L pre and postcentral gyri, central opercularum	2	6573	-50,-6,20	3.91	0.000
	8IU<1IU IV	R subcallosal cortex, accumbens	1	3093	10,14,-10	3.63	0.003
Q VSM	24IU<1IU IV	L frontal orbital cortex, frontal operculum, temporal pole, IFG	1	2902	-46,20,-10	3.87	0.004

We observed reduced task activation for question 2 (*"How much would you trust this person?"*) (*Q2*) in the right putamen (p<0.001, corrected) and left amygdala and hippocampus (p=0.041, corrected) following 8IU IN compared with placebo. Within the cluster, attenuated task activation was observed in the frontal orbital cortex, MTG and superior temporal gyrus (STG). We also found reduced task activation in the right amygdala and pallidum (visual stimuli model) (p=0.002, corrected) following 8IU IN compared with 1IU IV (see Figure 12 below), and in the left frontal pole (emotion stimuli model) (p<0.000, corrected) and right IT and MTG (p=0.021, corrected) also following 8IU IN compared with 1IU IV (see Table 3 above).

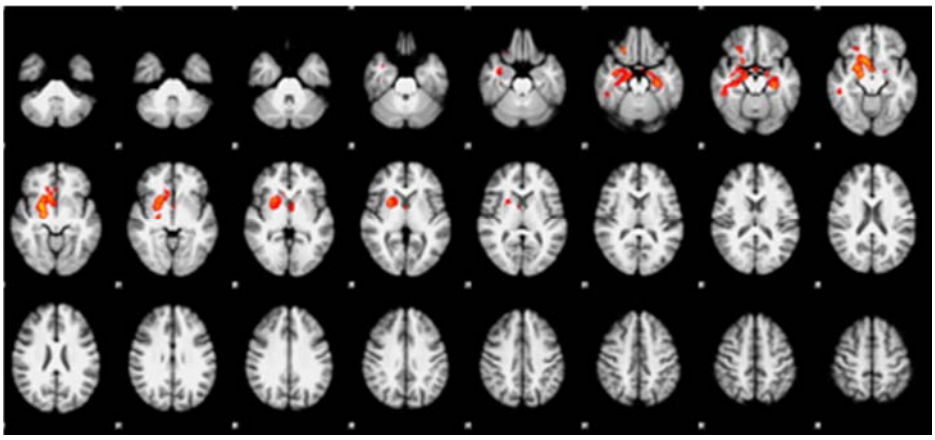


Figure 12. Map displays clusters that showed reduced activation for *question 2 compared to baseline* (visual stimuli model), (8IU IN<1IU IV) thresholded at p<0.05, corrected. Images are displayed in radiological convention.

For questions 1 and 2 collapsed (*Q*), we found reduced task activation in the right frontal pole and frontal orbital cortex (p<0.000, corrected), the right pre and postcentral gyri and precuneus (p<0.001, corrected), and left postcentral gyrus, SMG and operculum (p=0.012, corrected) following 8IU compared with placebo. We also found attenuated

activation in the left pre and postcentral gyri and central operculum ($p<0.000$, corrected) and in the right subcallosal cortex and accumbens ($p=0.003$, corrected) following 8IU compared with 1IU IV. Reduced activation was also observed in the left frontal orbital cortex frontal operculum, temporal pole and the IFG ($p=0.004$, corrected) following 24IU compared with 1IU IV.

Discussion

In this study we investigated both functional activation and connectivity whilst performing a facial emotion sensitivity task. We used a network approach and the use of fECM to investigate centrality across the brain and, as predicted, found evidence of high degree of centrality in a number of hubs (precuneus, insular, PFC, SMG, SPL, STG, MTG, FFG, lingual gyrus, and in the ACC and posterior cingulate**)**. However, we found no main effect of OT condition upon centrality, nor any significant pairwise comparisons between each of the OT conditions. Further, we used general linear modelling to explore activation in relation to the specific elements of the task. We found differences in activation in those regions broadly responsible for visual object and face recognition, language processing, decision-making, sensation and movement, emotional processing, reward and memory, thereby demonstrating that the task was effective in recruiting neuronal activity in regions that would be expected for such a task. Using F-tests, however, there was no main effect of OT condition on task-related activation. We further analysed the data with paired-comparisons, corrected for multiple comparisons, and found a number of interesting findings. Specifically, under the influence of oxytocin we found decreased differential activation between faces and shapes (*faces>shapes*), between neutral faces and shapes (*neutral>shapes*), and between happy and angry faces (*happy>angry*), in the visual cortex and language processing regions.

We further investigated the differential impact of dosage (8IU and 24IU) and administration (IN vs IV). Whilst IN administration of oxytocin influenced neuronal recruitment in a number of cortical regions compared with both placebo and IV, there was some indication that OT administered intravenously can indeed influence emotion perception with attenuation of activation in the left FFG, LOC, IT and FFC for visual stimuli (*faceshape*), and attenuation of activation in the right lingual gyrus for the contrast *happy>angry*. Furthermore, dosage of IN administration appeared to be important with 8IU having a greater influence over neuronal activation than 24IU in terms of the number of

contrasts influenced and the cluster sizes. These findings have interesting implications for future research and are discussed below.

Functional Connectivity

In adopting a network approach, we have been able to identify functional connectivity between a number of regions during an emotion perception task. Centrality was identified in regions which included the precuneus, ACC, SMG, SPL, STG, FFG, lingual gyrus, insular, IFG, MFG and SFG, thereby mirroring findings from resting state literature (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006; van den Heuvel & Sporns, 2013). As the first study, to our knowledge, to adopt ECM with t-fMRI, we are able to demonstrate its validity as a measure of connectivity, and to contribute towards a developing literature in which ECM has been used with r-fMRI (Hardmeier et al. 2012; Joyce et al., 2010; Lohmann et al., 2010; Meinzer et al., 2013; Wink et al., 2012; Zuo et al. 2012).

Whilst exploring connectivity with t-fMRI is not without its problems since it is more susceptible to artefacts such as noise from cardiac and respiratory movement (Gonzalez-Castillo et al., 2012), it may be a useful approach to examining psychiatric populations (ibid). Tasks can be designed according to the symptoms of specific disorders and thus may be more sensitive to impairments in connectivity than r-fMRI. There are many ways in which to examine connectivity within the brain and local and global features of networks can differ according to the parcellation schemes adopted (Wang et al., 2008). This is particularly important for examination of t-fMRI (Gonzalez-Castillo et al., 2012); parcellation needs to be functionally meaningful and take account of interregional differences and reconfiguration of connectivity as a result of the task. ECM analysis, therefore, is especially useful for examining functional connectivity since it is conducted on a voxel-by-voxel basis.

Several studies have provided evidence indicating that OT influences connectivity (Dodhia et al., 2012; Gamer et al., 2010; Gorka et al., 2014; Kirsch et al., 2005; Riem et al., 2013; Rilling et al., 2012, 2014; Sripada et al., 2013; Wittforth-Schardt et al., 2012) though, using fECM, we did not find evidence for an effect. Existing studies, however, have typically used a seed-based approach to investigate connectivity between the amygdala and other specific regions. In this study we sought to take an explorative approach and, hence, as well as using ECM to examine whole brain networks, we sought to be data-driven. This is important from a methodological stance. Correction for multiple comparisons, for example,

necessitated by the high number of calculations whole brain analysis entails, are typically stringent and thus a larger effect size is required in order to survive such correction. Such techniques are necessary, however, since analysis of thousands of voxels increases the family-wise error rate (FWER) and the probability of making Type I errors (false positives) (Nichols, 2012). Extracting a ROI for seed-based analysis will give specific values for a much smaller number of voxels than whole brain analysis. Such an approach gives increased power and consequently may allow for identification of the influence of oxytocin in specific regions that is not possible with whole brain analysis. At the same time, such an approach is limited to activation in, or connectivity between, the chosen regions.

In addition to methodological considerations, it may also be the case that whole brain methods other than ECM may be more sensitive to the effects of oxytocin. It is possible that the definition of eigenvector centrality, that a node is defined by its centrality and that of its neighbours, may not cohere with the centrality present in the data in this study. For example, it is possible that a node may have many links but all may have low centrality, or that the nodes may be sparsely linked but have high centrality; these are issues for further investigation. Indeed, Telesford et al. (2011) suggest that choice of centrality measure depends very much upon the information that is flowing through the network. Thus, our study may indicate that ECM is not sensitive as an approach to the influence of OT but this is not to say that other methods of centrality will be unable to capture such influences.

Functional Activation

We were able to demonstrate the efficacy of the task with differential neuronal recruitment evident in several regions which included the FFG, LOC, MTG, IT, amygdala, hippocampus, putamen, cingulate cortex, paracingulate gyrus, lingual gyrus, SFG, MFG, and pre and postcentral gyri as would be expected of a face processing task. The following section outlines the three key findings in relation to impact of OT condition upon functional activation – (i) influences related to specific elements of the task, (ii) dosage-dependent effects, and (iii) method of administration effects.

Task-related effects. We investigated both *visual* stimuli (faces vs shapes; neutral face vs shape) and *emotional* stimuli (happy/angry faces vs neutral faces). We found that oxytocin, administered intranasally, led to a reduction in the differential task activation

between faces and shapes in the left LOC, FFG, and occipital pole (8IU IN<placebo) and left MFG, frontal pole, and IFG (24IU IN < 1IU IV). Of particular interest, we observed that oxytocin reduced the differential task activation between neutral, emotionally ambiguous faces and shapes in the left LOC (8IU IN<placebo), right postcentral gyrus (24IU IN<placebo), right SMG, SPL, angular gyrus, and postcentral gyrus (8IU IN<IV), left LOC and SPL (24IU IN<placebo), and left frontal pole and MFG (24IU IN<1IU IV), regions responsible for object and spatial recognition, language processing, sensation and cognition. Thus, under the influence of oxytocin in both intranasally-administered conditions, neutral faces and shapes were processed more similarly. These findings cohere with earlier studies that suggest oxytocin promotes attention to socially salient stimuli (Groppe et al., 2013; Wittforth-Schardt et al., 2012). It also coheres with findings that OT promotes accurate detection and appraisal of emotional social information (Guastella & MacLeod, 2012), given that the emotional status of the neutral face was ambiguous and hence, in this sense, equivalent to the shapes.

An important and related concern, no significant findings were observed between *emotions* (happy/angry) and *non-emotions* (faces in general, neutral faces, shapes), that is, the task activation under the influence of oxytocin remained distinct for emotional faces and non-emotional stimuli. This would support earlier findings that oxytocin supports identification and perception of *emotionally* non-ambiguous faces (Domes et al., 2007a; Ellenbogen, Linnen, Grumet, Cardoso & Joober, 2012; Guastella et al., 2010; Lischke et al., 2012; Marsh et al., 2010; Schulze et al., 2011), possibly through recruitment of attentional mechanisms that would not otherwise be utilised (Prehn et al., 2013, Tollenaar et al., 2013). The *superior colliculus*, a region located in the midbrain and associated with attention, and the *locus coeruleus*, a brainstem nucleus and part of the noradrenergic pathways which contains oxytocin receptors, and reliably used as a measure of attention and cognitive effort (Laeng, Sirois & Gredeback, 2012) have both been raised as possible neural sites for such processing (Gamer et al., 2010; Leknes et al., 2012).

In our data there was a reduction in differential task activation between happy and angry faces in the left LOC, temporal occipital fusiform cortex, IT and FFG (8IU IN<placebo), right lingual gyrus, temporal occipital fusiform cortex (24 IU IN<placebo) and the left cerebellum (1IU IV<placebo). Thus, under the influence of oxytocin in all conditions, albeit it in different regions, neuronal recruitment for happy and angry faces was more similar than compared with placebo. This may reflect a general emotional response as described

above, or instead a response to emotions which promote approach behaviours which may explain the distinction between emotional and neutral faces in our data. These findings are particularly interesting in relation to the withdrawal-approach hypotheses (Kemp & Guastella, 2010, 2011). Such a theory proposes that the influence of oxytocin is such as to increase approach and affiliative behaviours; what is processed as socially salient is closely connected with motivational mechanisms. There is some support for this argument in the literature (Domes et al., 2007b; Di Simplicio, Massey-Chase, Cowen & Harmer, 2009; Evans, Shergill & Averbeck, 2010; Leknes et al., 2012; Radke et al., 2013); by reducing the salience of threatening stimuli, oxytocin reduces the experience of anxiety and consequently, aversion to angry faces. This would suggest an evolutionary advantage since it allows for defensive behaviour without retreat.

Another consideration with regards to the effect of oxytocin on the task was upon the answering of the two questions. The first, randomly presented, asked participants how happy or angry they perceived the face. This question was followed by a second, in which they were asked to rate how much they were likely to trust the person they had just observed. We found differential task activation for these two questions. The first question recruited the left SPL, SMG (8IU IN<placebo), left pre and postcentral gyri (24IU IN<placebo), and left frontal pole (24IU IN<1IU IV). The second question, however, led to reduced task activation in the left amygdala and hippocampus, and right putamen (8IU IN<placebo), right amygdala and pallidum, left frontal pole, right IT and MTG (8IU IN<1IU IV). This suggests that the question of trust involved memory, recognition of faces, and emotional processing. It also demonstrates that the amygdala was only significantly effected when participants were asked how much they would *trust* a person but not significantly influenced by oxytocin in the *evaluation* of emotion per se (question 1). This would suggest that the influence of oxytocin on the amygdala may be nuanced and has implications for other oxytocin studies where its influence in emotional processing has been considered but differential paradigms applied.

As already noted, the use of differential paradigms makes it somewhat difficult to draw any conclusions from previous oxytocin studies (for a meta-analyses see van IJzendoorn & Bakermans-Kranenberg, 2012; Shahrestani, Kemp & Guastella, 2013). In considering facial stimuli and emotional expressions, neuroimaging studies have involved matching tasks (Kirsch et al., 2005; Labuschagne et al., 2010; Gorka et al., 2014), detecting gender (Domes et al., 2007a), detecting location of a face on a screen (Petrovic et al., 2008), and evaluating emotional arousal (Domes et al., 2010). Three studies have involved evaluation similar to

that used in 'question 1' here (Domes et al., 2014; Gamer et al., 2010; Labuschagne et al., 2012). Of these three studies, however, one considered the role of the amygdala in individuals with Asperger's syndrome and did not separate out individual emotions in analysis (Domes et al., 2014), one considered fearful and happy faces and the influence of oxytocin on the amygdala (Gamer et al., 2010) and one examined the influence of oxytocin on the mPFC/ACC in patients with GSAD (Labuschagne et al., 2012). Thus, in reality, despite attempts here to adopt a paradigm similar to those previously used, the paradigms also differed with this study. Consequently, it is somewhat difficult to draw upon existing literature, a criticism levelled at the oxytocin literature in general, as noted earlier.

Dosage-dependent effects. The three oxytocin conditions (8IU IN, 24 IU IN and 1IU IV), all had an impact on brain activation for at least one contrast though the extent of this influence varied from one condition to the next. Whilst most significant findings were found for intranasally administered oxytocin compared with either placebo or IV, 8IU IN proved to have a greater impact in terms of the number of contrasts it influenced and the extent of the cluster sizes compared with 24 IU. We also observed some specific differences between the two dosages of intranasal oxytocin. We found that 8 IU reduced differences in task activation between neutral faces and shapes in the postcentral gyri compared with both 24IU and with 1IU IV (see Table 1). We also found that 24 IU reduced activation for faces in the left and right LOC compared with 8IU, and in visual stimuli in general (*faceshape*) in the left LOC compared with 8IU. The differential effects of dosage upon specific regions is an interesting issue to pursue further. For example, 8IU OT was the only dosage to influence question 2 with attenuated activation in the right putamen and left amygdala and hippocampus (8IU<placebo), left frontal pole, and right amygdala, pallidum, IT and MTG (8IU IN<1IU IV).

There is no empirical evidence that a higher dosage is more effective than a lower dosage (van IJzendoorn et al., 2012) and it may be that higher doses lead to the occupation of vasopressin receptors, thereby inducing unpredictable social consequences (Bakermans-Kranenburg & van IJzendoorn, 2013). Arginine vasopressin (VP), like OT, is a nonapeptide, differing only in two amino acid sequences. It is also synthesised in the hypothalamic paraventriculuar and supraoptic nuclei, and it is possible for VP and OT to interact with each other's receptors (Gimpl & Fahrenholz, 2001). It is also associated with social behaviours,

though typically more aggressive in nature than OT (Donaldson & Young, 2008). Its functional and structural commonalities, and cross-reactivity, especially in higher dosages (Neumann & Landgraf, 2012), would imply that VP may be a confounding variable where OT dosage is particularly high; whether 24IU IN may be defined as particularly high remains unclear. The effectiveness of the 8IU dosage in this study compared with 24IU may reflect difficulties with a higher dosage though 24 IU showed significant results in several paired comparisons in this study, and is typically effective in other OT studies (Domes et al., 2013; Riem et al., 2012). Alternatively, it is possible that a lower dosage is simply more effective in recruiting endogenous oxytocin release leading to a positive feedback loop (Barraza & Zak, 2009; Ludwig & Leng, 2006) and a general enhanced effect across contrasts.

Method of administration. Of interest, following IV administration of oxytocin, we observed reductions in task activation for visual stimuli (*faceshape*) in regions associated with object and face processing (left FFG, fusiform cortex, LOC and IT) compared with placebo. We also observed reductions in differential activation for happy and angry faces in the right lingual gyrus and brainstem (see Figure 11a above). Such reductions were observed in several regions (Appendix D, Table 8) but with the peak z values being found in the cerebellum. These results cohere with those established elsewhere in the data. As shown above (Table 2), we observed reduced task activation in the left LOC for visual stimuli (*faceshape*) following 24IU compared with 8IU, whilst for happy and angry faces (*happy>angry*) task activation in the right lingual gyrus was influenced following 24IU IN compared with placebo (Table 3) (see Figure 11b above). Thus, sufficient quantities of oxytocin may reach the brain when administered peripherally in order to stimulate endogenous release of oxytocin (Landgraf & Neumann, 2004) that would otherwise only be evoked via behavioural stimulation (Ludwig & Leng, 2006).

It is possible that peripheral administration of OT stimulates the brain's natural production of OT even though reaching the brain in very small quantities. On a cautionary note, the number of contrasts in which IV was significant and the extent of the regions influenced were few. Further, as already noted, paired-comparisons are more susceptible to Type I errors. As with earlier research (Hollander et al., 2003, 2007) it would seem that findings in relation to IV OT should be treated with trepidation but not dismissed. More importantly perhaps, the finding that IV administered OT may reach the brain and influence

emotion perception, in conjunction with the finding that a lower dosage of IN administered oxytocin is more effective, begs the questions - what is a 'sufficient' amount of oxytocin? how might the natural production of oxytocin within the brain be better utilised in such experiments independent of method of administration?, and what might be the translational consequences of this?

Limitations

The main limitation of this study was the small sample size (N=16). FMRI studies are vulnerable to lower power given the number of comparisons involved, a poor signal-to-noise ratio, and differences in the anatomy between participants. A larger sample size can increase the power of the study by countering the effect of multiple comparisons. The impact of stringent techniques used in correction are likely to have had an impact on the fECM analysis and may have led to the null findings with regards to the influence of oxytocin condition upon centrality in this study. These issues, however, make it challenging to interpret a null result and for this reason replication with a larger sample is recommended. F-tests are also particularly sensitive to correction techniques, and again this may account for the lack of a main effect using the GLM model of the task. In this study, we also report paired-comparison t tests. Though not an approach which is recommended (Simmons, Nelson & Simonsohn, 2011) since such results are susceptible to Type I errors (false positives), the data here survived correction for multiple comparison. Further, significance levels were particularly high, many reaching $p<0.001$. Moreover, the explorative nature of this study warrants the reporting of trends and findings for further investigation.

With regard to the study design, it was both demanding and invasive for the participants. Every effort was made to ensure that they were well-informed, physically comfortable, and that they felt safe (only 2 participants withdrew from the study during the testing phase) but the level of care given may also have influenced outcomes. This is worthy of note given that this study investigated emotion perception. Exposure to an emotional stimulus may stimulate endogenous production of oxytocin (Barraza & Zak, 2009), independently of that received via intranasal or intravenous administration. Whilst we controlled for the levels of care received by limiting physical contact (Morhenn, Park, Piper & Zak, 2008), we cannot rule out that this may have had differential effects on the participants. Not providing them with this level of care, however, could equally have introduced

confounding factors by increasing anxiety levels (MacDonald et al., 2013). A further concern with the study design is that we failed to control for handedness with all participants using the handgrip in their left hand though all received training with the handgrip and practised the task beforehand.

The conclusions reached are somewhat limited by the use of a novel nasal device; in this study we did not compare it with a traditional device. It is, therefore, not possible to generalise findings regarding dosage with any certainty. Nevertheless, previous studies have shown that the OptiNose N2B device reliably delivers significantly more of the study drug to the posterior regions of the nasal cavity than traditional devices, thereby enhancing the possibility for the drug to cross the BBB (Djupesland et al., 2006; Djupesland & Skretting, 2012) (see Appendix B). It is possible to surmise, therefore, that the effects of oxytocin in this study will lead to stronger and more sensitive effects than had we used a traditional device, though we are unable to substantiate these presumptions. The effectiveness of the device may thus have contributed to the differential effects of 8IU and 24IU IN.

Conclusions

To summarise, following administration of oxytocin during a facial processing task, we conducted exploratory investigations into functional connectivity and activation using t-fMRI. We observed high centrality in brain regions corresponding with known 'brain hubs', but found no main effects of OT condition on fECM. Using conventional fMRI analyses, we also found activation in regions of the brain typically associated with emotion perception but no main effect of OT condition. Thereafter, we conducted exploratory pairwise comparisons and found a reduction in differential processing between neutral emotionally ambiguous faces and shapes in the visual cortex and language processing areas, and a reduction between happy and angry faces. Thus, under the influence of oxytocin, brain activation for *angry* and *happy* faces was more similar, and at the same time, these were in contrast with *neutral faces/shapes*. We also found both dose-dependent effects with 8IU IN being more effective in neuronal recruitment than 24IU, and method-of-administration effects; there were some indications that 1IU IV influences activation but to a lesser extent than intranasally administered OT. Given the limitations of sample size and the consequences this had for analysis, we suggest that any interpretation of our findings should be treated with caution. Replication with a larger sample size would provide an opportunity to explore further the provisional findings presented here. Suggestions for future research include replication of the

study for analysis with fECM and other centrality measures; further exploration into whether IV administration is likely to offer an effective alternative to IN administration; and further investigation into optimal dosage, in particular, the possible benefits of using a lower rather than higher dosage. We emphasise the validity of using ECM with t-fMRI, particularly with a view to the advantages of exploring functional connectivity in neuropsychiatric populations during task performance.

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Appendices

Appendix A - The Biological Bases of OT

Neuropeptides such as OT, are a dynamic and complicated means of communication between cells, both centrally and in the periphery; such complexity contributes to difficulties in identifying their primary target/s and specific effects. They are primarily secreted by specific populations of neurons (and some glia) and, as with classical neurotransmitters, neuropeptides influence a range of processes but they are specific in a number of different ways.

Classical neurotransmitters, packaged in synaptic vesicles, function by transmitting signals from one neuron to another via the region between the presynaptic cell of one neuron and the postsynaptic cell of another, otherwise known as the synaptic cleft, and are typically triggered with an action potential. Neuropeptides differ in that they can occur via gene expression, blood flow, synaptogenesis and glial cell morphology (Landgraf & Neumann, 2004). Moreover, the effects of neuropeptides are most often long-lasting rather than instantaneous since they have a half-life of approximately 20 minutes in the brain (2 minutes in plasma) and their transmission can occur over long distances – possibly even transported via cerebral spinal fluid with the hypothalamus, thalamus and midbrain being primary targets (Proescholdt, Hutto, Brady & Herkenham, 2000). Neuropeptide release can also be self-perpetuating, not requiring repetitive electrical stimulation and thereby allowing for a sustained effect over a period of time (Landgraf & Neumann, 2004). In addition, following secretion, they are modified by extracellular peptidases which can disable their bioactivity or sensitise a peptide to a particular receptor whilst desensitising it to another.

Whereas classical neurotransmitters are directed to axonal terminals (or synaptic boutons), neuropeptides may be released from other parts of the neuron depending on the location of the receptors. Pow and Morris (1989) discovered that since the vesicles which house the neuropeptide have access to various parts of the neuron and not only the axonal terminal, it is possible for release to occur at various locations. The nature of the release varies according to cortical region; the hypothalamic nuclei, the region where OT is synthesised (Moos, Freund-Mercier & Guerne, 1984), for example, prefers dendritic release whereas in the limbic areas axonal release is preferred. Furthermore, unlike classical neurotransmitters, neuropeptides are not reabsorbed into the soma following secretion, but instead regenerated through de novo synthesis.

Understanding the role and effects of neuropeptides is further problematized by the fact that a single neuropeptide can act upon many receptors and several neuropeptides may share a single receptor. Also, an area may have many OT receptors but connect with very few projections. A number of factors will influence these particular combinations including preference for dendritic or axonal release in varying brain regions, the presence of certain enzymes and experiences of stress (Landgraf & Neumann, 2004). That an exogenous stimuli, can alter the processing of neuropeptides demonstrates further difficulties in establishing a definitive understanding of their effects.

Appendix B: OT neuroimaging overview of the literature

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Kirsch et al. (2005)	Amygdala	13 males. Angry and fearful faces/scenes	27IU IN/ placebo	ROI/seed-based FC (amygdala)	Reduced activation in left amygdala for all stimuli, especially social	Amygdala, midbrain
Domes et al. (2007a)	Amygdala	13 males. Happy, fearful/neutral faces, 4 intensities each	24IU IN/ placebo	WBA/ROI	Reduced activation in right amygdala for all stimuli. Reduced activation in brainstem, superior temporal lobule (STL) and temporal poles	
Baumgartner, Heinrichs, Vonlanthen, Fischbacher & Fehr (2008)	Amygdala, striatum, ACC, midbrain	49 males. Social risk and trust games	24IU IN/ placebo	ROI	Reduced activation in amygdala and left caudate after receiving feedback	
Petrovic, Kalisch, Singer & Dolan (2008)	Amygdala, FFA, insular, ACC, OFC	30 males. Fear conditioned to direct or averted gaze male faces	32IU IN/ placebo	ROI	Reduced activation for fear-related faces and activation in the aMTL, ACC, amygdala and fusiform face area (FFA), especially for direct gaze	
Singer et al. (2008)	Amygdala, AI, ACC	20 males (with romantic partner). Tested for empathy for pain followed by Economic trust game	32IU IN/ placebo	ROI	No impact on empathy regions. Attenuated activation in amygdala for pro-social task for selfish participants	
Strathearn, Fonagy, Amico & Montague (2009)	n/a	30 mothers. Images of happy/sad/neutral own/other baby faces	Natural serum levels	WBA	Increased OT levels for own child's face, associated with activation in ventral striatum, hypothalamus and pituitary	

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Domes et al. (2010)	Amygdala	16 females. Arousal rating of angry/happy/sad/neutral faces (plus eye-tracker)	24IU IN/ placebo	WBA/ROI	Enhanced activity in medial and superior temporal cortex and the FFG (fearful faces), IFG and ventrolateral PFC (angry faces), IFG and FFG (happy faces)	
Gamer, Zurowski & Buchel (2010)	Amygdala, superior colliculi	46 participants. Fearful, happy or neutral faces (also eye-tracker)	24 IU IN/ placebo	WBA/ROI/ seed-based PPI (superior colliculi)	Increased frequency of gaze to eye region, enhanced activation in right amygdala	Amygdala, superior colliculi
Labuschagne et al. (2010)	Amygdala	18 males (GSAD). Emotion identification with fearful/angry/happy/neutral faces/shapes	24IU IN/ placebo	WBA/ROI	GSAD reactivity in amygdala to fearful faces compared with controls is eliminated	
Pincus et al. (2010)	n/a	16 female and 1 male. (8 depressed females). RMET task	40IU IN/ placebo	WBA	Increased activation ventromedial, amygdala, parahippocampal in controls. Superior middle frontal gyrus and insula in depressed.	
Atzil, Hendler & Feldman (2011)	NAcc, amygdala	23 mothers. Infant-related videos	n/a	WBA/ROI	Increased activation in left NAcc for mothers who synchronise responses, increase in right amygdala for intrusive responses	L NAcc, R amygdala
Labuschagne et al.(2011)	n/a	18 males (GSAD). Emotion identification task with sad/happy/neutral faces	24IU IN/ placebo	WBA	GSAD heightened activity to sad faces in mPFC and ACC, reduced with administration of OT	

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Riem et al. (2011)	Amygdala, thalamus, insular	42 females (32 from twin pairs). Cries of 2 day old baby	24IU IN/ placebo	WBA/ROI	Reduced activation in right amygdala, enhanced in insular and IFG	
Lischke et al. (2012)	Amygdala	14 females. Positive, negative and neutral scenes (also eye-tracker)	24IU IN/ placebo	WBA/ ROI	Increased activation in amygdala and left ATL to negative non-social stimuli	
Riem et al. (2012)	Amygdala, Striatum, Insular, IFG, ACC, OFC	42 females. Infant crying and laughter.	24IU IN/ placebo	WBA/ROI/PPI FC (ACC/OFC)	Reduced activation of amygdala for infant laughter	Amygdala, OFC, ACC, SMG, MTG precuneus, and hippocampus
Rilling et al. (2012)	Amygdala, caudate	85 males. Prisoner's Dilemma game	24IU IN/ placebo	WBA/ROI/ seed-based FC (amygdala)	Increased left amygdala activation and augmented caudate response to reciprocated cooperation	Amygdala, AI, ACC, IT
Striepens et al. (2012)	Amygdala, insular	70 males. Memory task with aversive or neutral images.	24IN IU/ placebo	WBA/ROI/ PPI FC (amgydala, left AI)	Reduced coupling left amygdala and left ACC for negative stimuli, increased coupling in right amygdala and left insular for neutral	Amygdala, insular, ACC, IFG
Wittforth-Schardt et al. (2012)	GP, hippo-campus	19 fathers. Photos of own, other and unknown child	24IU IN/ placebo	WBA/ROI/ seed-based FC (globus pallidus)	Reduced activation and FC of the left GP, mOFC and VTA when viewing own child. Hippocampus, STC and insular for unknown child	GP, hippo-campus, frontopolar cortex

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Domes (2013)	Amygdala	28 males (14 autistic patients). Direct/averted gaze, same/different faces/houses	24IU IN/ placebo	WBA/ROI	OT attenuated activation in right amygdala, FFA and inferior occipital gyrus in autistics compared with controls	
Groppe et al. (2013)	VTA	28 females. Social incentive delay task (reward – happy face/punishment – angry face)	26IU IN/ placebo	WBA/ROI	OT increased activation during anticipation of high social reward or punishment	
Riem et al. (2013)	Amygdala, insular, precuneus/ posterior cingulate, brainstem, cerebellum	42 females. Maternal love withdrawal questionnaire. (Resting state)	16IU IN/ placebo	WBA/seed-based correlation (brainstem/ cerebellum)	OT alters connectivity for neglected individuals between posterior cingulate cortex and brainstem	Posterior cingulate, postcentral gyrus, cerebellum
Sripada et al. (2013)	Amygdala	15 males. (Resting state)	24IU IN/ placebo	WBA/ROI/ seed-based analysis (amygdala)		Amygdala, ACC and mPFC
Voorthuis, Riem, van IJzendoorn & Bakermans-Kranenburg (2013)	Insular, IFG, STG, OFC, MTG	50 females. Infant facial expression task	16IU IN/ placebo	WBA/ROI	Increased IFG, MTG and STG activation but reduced task performance	

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Dodhia et al., (2014)	Amygdala, ACC, mPFC	18 males (GSAD). (Resting state)	24IU IN/ placebo	WBA/ROI/ seed-based FC (amygdala)	Increased connectivity amygdala, ACC and mPFC (normalising effect)	L and R amygdala, rostral ACC and mPFC
Domes, Kumbier, Heinrichs & Herpertz (2014)	Amygdala	14 males (Asperger's Disorder). Facial Emotion Recognition Task	24IU IN/ placebo	WBA/ROI	Increased task performance and increased amygdala reactivity to facial stimuli	
Gorka et al., (2014)	Amygdala, insula, ACC	17 males (GSAD). Emotional Face Matching Task with fearful/angry/ happy/neutral faces/shapes	24IU IN/ placebo	WBA/ROI/ seed-based PPI (anterior to middle cingulate and insula)	Increased connectivity between the amygdala – bilateral insula ad the amygdala-mid/dorsal ACC whilst processing fearful faces	Amygdala, insula, dACC
Riem, Bakermans-Kranenburg, Voorthuis & van IJzendoorn (2014a)	Insular, STG, IFG	50 females. Maternal love withdrawal questionnaire, RMET	16IU IN/ placebo	ROI	Increased activation in the insula, and also STG in neglected individuals	
Riem, Voorthuis, Bakermans-Kranenbug & van IJzendoorn (2014b)	L insula, L IFG, R amygdala	50 females. Baby cries (500 and 700 hz) and sick/bored categorisation	16IU/ placebo	ROI	Increased insula, amydala and IFG to 700 hz, reduced to 500 hz. Differential activation for sick/bored label.	

Authors	ROI/Seed	Design (participants, stimuli)	OT admin	Analysis	Findings	Coupled regions
Rilling et al. (2014)	Amygdala, caudate	73 females. Prisoner's Dilemma game	24IU IN/ placebo	WBA/ROI/seed-based FC (amygdala)	Reduced activation in amygdala or no effect	
Rupp et al. (2014)	Amygdala	59 women (29 postpartum). Neutral/negatively valenced, low/high arousal	24IU IN/ placebo	WBA/ROI	Reduced right amygdala activity for negative images in postpartum for OT and placebo, but only in OT group for nulliparous women. Also, activation in mid-brain, thalamus, right dorsolateral PFC, occipito-temporal cortex.	

* Hosanagar et al. (2012) – restricted access.

Appendix C – OptiNose Nasal Device

The BBB, an endothelial layer of cerebral blood vessels, serves to prevent toxins and larger molecules from reaching the brain. It has been shown, however, to allow some molecules to cross in minute quantities including OT via intranasal administration (Szeto et al., 2011). In humans, the nasal anatomy is such that substances entering the nostrils may pass via the nasal valve, to the anterior and upper posterior region where the nasal mucosa is innervated by both the olfactory and trigeminal nerves of the brain. Traditional nasal devices, however, are typically ineffective in transporting the drug (liquid/powder) to the upper posterior area, and any medication not deposited in the nasal cavity is often swallowed (Djupesland, 2013). It is, therefore, problematic in controlling for consistent dosage or to estimate the optimal dosage required for a particular treatment.

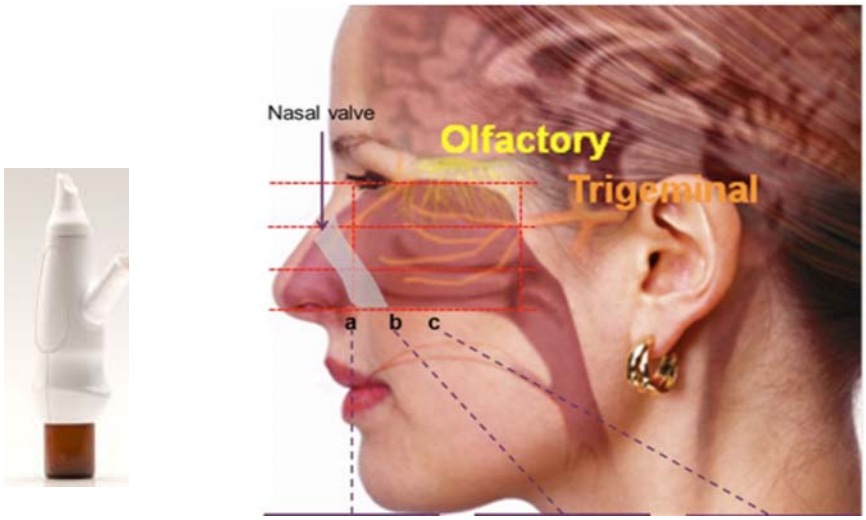

Figure 13: Optinose N2B device and anatomy of olfactory pathways (abc denoting possible pathways where OT can be lost with traditional nasal sprays).
Source: OptiNose AS.

A new device (see Figure 13), designed and produced by OptiNose AS, addresses the problems incurred by traditional devices and other methods (for a full review, see Djupesland, 2013). The primary objective of the main project, in which this current study is located, was to investigate the possible effects of OT (8IU and 24IU) administered with this novel device, *(OptiNose Breath-Powered™ Bi-Directional™ Nose-to-brain (N2B) liquid device*), compared with 1IU administered as a slow IV infusion. The device utilises the action of the soft palate

(venum) during the process of exhalation. In blowing into the device the user closes the soft palate, thereby separating the oral and nasal cavities. At the same time, this prevents loss of the drug into the gut via the throat or via lung inhalation, thereby promising improved tolerability of the drug (Djupesland, Skretting, Windern & Holand, 2004, 2006). The pressure created by blowing also propels the particles further into the upper regions of the nose and thus increases the potential for crossing the BBB. Early gamma studies have found less deposition of the drug in the lower regions of the nasal cavities, but greater in the higher regions, compared with standard nasal devices (Djupesland & Skretting, 2012; Djupesland et al., 2006).

The device comprises of a nasal spray pump with mouthpiece and a nozzle for insertion into the nostril. A glass vial containing the drug clicks into the lower part of the device. The user holds the device with two hands with their thumbs resting under the glass vial. The nozzle is inserted into the nostril in a vertical position and deeply enough to create a tight seal. This nozzle expands the nasal triangular valve at the lower part of the nasal cavities further supporting the transportation of the drug. The mouthpiece is placed firmly between the lips, slightly off centre since the nozzle is to be positioned vertically. The user takes a deep breath and blows with some effort into the mouth piece (for example, “with the effort needed to blow out 10 birthday candles”). At the same time s/he presses firmly on the vial with both thumbs to release the drug. The flow of air passes around the nasal septum and exits via the unused nostril.

Appendix D: Regions identified using MNI and Talairach coordinates

Table 4: Cluster Index - 8IU < placebo (p=0.05)

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
Q1 VSM	8IU<placebo	1	3.69	0.028	-28, -44, 36	L SPL, SMG, white matter (WM)	-27, -44, 36	L parietal lobe, sub-gyral, WM
		1	3.64		-48, -2, 22	L pre and postcentral gyri, IFG, WM	-46, -5, 23	L frontal lobe, precentral gyrus, WM
		1	3.5		-54, 6, 24	L precentral gyrus, IFG, WM	-51, 2, 25	L frontal lobe, IFG, BA9
		1	3.46		-54, 2, 24	L precentral gyrus, IFG, WM	-51, -2, 25	L frontal lobe, precentral gyrus, BA6
		1	3.31		-38, -38, 40	L SMG, SPL, postcentral gyri, WM	-37, -40, 36	L parietal lobe, sub-gyral, WM
		1	3.07		-64, -6, 28	L pre and postcentral gyri	-61, -9, 28	L frontal lobe, precentral gyrus, BA4
Q2 VSM	8IU<placebo	2	4.19	<0.001	22, 4, -10	R putamen, WM	19, 3, -4	R sub-lobar, lentiform nucleus, putamen
		2	4.17		24, 10, 4	R putamen, WM	21, 7, 9	R sub-lobar, lentiform nucleus, putamen
		2	4.15		18, 0, -10	R amygdala, pallidum, WM	16, -1, 5	R sub-lobar, lentiform nucleus, lateral globus pallidus
		2	3.88		4, 4, -10	R WM	3, 3, -4	R limbic lobe, ACC, BA25
		2	3.51		8, 16, -8	R subcallosal cortex, accumbens, WM	7, 14, -2	R sub-lobar, caudate, caudate head
		2	3.5		28, 36, -18	R frontal pole, frontal orbital cortex	25, 33, -8	R frontal lobe, IFG, BA 47
		1	3.68	0.041	-16, -8, 16	L amygdala, hippocampus	-16, -8, -11	L limbic lobe, parrahippocampal gyrus, BA 34
		1	3.52		-18, -4, -12	L amygdala, pallidum	-18, -4, -7	L sub-lobar, extra-nuclear, WM
		1	3.49		-22, 0, -12	L amygdala, putamen, WM	-21, -1, -7	L sub-lobar, extra-nuclear, WM
		1	3.33		-28, 2, -6	L putamen, WM	-21, -1, -1	L sub-lobar, lentiform nucleus, putamen
		1	3.11		-46, -12, -16	L MTG, STG, WM	-43, -11, -12	L temporal lobe, sub-gyral WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.07		-48, -10, -20	L MTG, STG, WM	-45, -9, -15	L temporal lobe, sub-gyral WM
Face>Shape VSM	8IU<placebo	1	3.24	0.026	-36, -84, 26	L LOC, FFG	-35, 82, 19	L occipital lobe, middle occipital gyrus, BA 19
		1	3.22		-38, -88, 26	L LOC, occipital pole, WM	-37, -86, 19	L occipital lobe, middle occipital gyrus, WM
		1	3.21		-28, -66, 46	L LOC, SPL, WM	-28, -67, 39	L parietal lobe, precuneus, WM
		1	3.21		-24, -66, 46	L LOC, SPL, WM	-24, -67, 39	L parietal lobe, precuneus, WM
		1	3.06		-34, -92, 26	L occipital pole, LOC	-33, -90, 19	L occipital lobe, middle occipital gyrus, BA 19
		1	2.98		-10, -68, 54	L LOC, precuneus, WM	-11, -70, 46	L parietal lobe, precuneus, WM
Q VSM	8IU<placebo	3	4.19	0.000	30, 34, -6	R frontal pole, frontal orbital cortex, WM	27, 30, 2	R frontal lobe, sub-gyral, WM
		3	4.06		8, 6, -4	R accumbens, caudate, WM	6, 4, 1	R sub-lobar, caudate
		3	4.03		6, 6, -10	R accumbens	5, 5, -4	R limbic lobe, ACC
		3	4.01		-62, -34, 22	L parietal operculum, STG, planum temporale, SMG	-59, -35, 20	L temporal lobe, STG
		3	4.0		22, 4, -10	R putamen	19, 3, -4	R sub-lobar, lentiform nucleus, putamen
		3	3.94		26, 10, -2	R putamen	23, 8, 4	R sub-lobar lentiform nucleus, putamen
		2	4.21	<0.001	6, -34, 66	R pre and postcentral gyri, precuneus,WM	4, -39, 60	R frontal lobe, paracentral lobe, BA5
		2	3.75		8, -22, 72	R precentral gyrus, SFG, WM	5, -29, 67	R frontal lobe, paracentral lobe, BA6
		2	3.39		-12, -38, 70	L pre and postcentral gyri, SPL, WM	-13, -43, 63	L parietal lobe, postcentral gyrus, WM
		2	3.22		-24, -16, 70	L precentral gyrus, SFG, WM	-24, -23, 65	L frontal lobe, precentral gyrus, BA4
		2	3.18		-20, -40, 40	L precuneus, cingulate gyrus (pos), WM	-20, -42, 36	L frontal lobe, sub-gyral, WM
		2	3.16		-32, -20, 68	L precuneus, postcentral gyrus, WM	-31, -26, 63	L frontal lobe, WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.69	0.012	52, -22, 26	L postcentral gyrus, SMG, operculum	47, -25, 26	R parietal lobe, inferior parietal lobule, WM
		1	3.46		54, -8, 18	L pre and postcentral gyri, operculum, WM	49, -11, 20	R parietal lobe, postcentral gyrus, WM
		1	3.28		16, -42, 12	L hippocampus, WM	14, -42, 11	R sub-lobar, corpus callosum, extra nucleus. WM
		1	3.21		42, -42, 12	L SMG, MTC, STG, angular gyrus, WM	38, -42, 12	R temporal lobe, STG, WM
		1	3.2		22, -50, 10	L precuneus, cingulate gyrus, supracalcarine cortex, WM	19, -49, 9	R sub-lobar, extra nucleus, WM
		1	3.14		66, -22, 24	L SMG, postcentral gyrus, parietal operculum, planum temporale, SMG, STG	60, -25, 25	R parietal lobe, inferior parietal lobule, WM
Face>Shape ESM	8IU<placebo	1	3.36	0.016	-38, -88, 26	L LOC, occipital pole	-37, -86, 19	L occipital lobe, middle occipital gyrus, BA19
		1	3.29		-34, -92, 26	L LOC, occipital pole	-33, -90, 19	L occipital lobe, middle occipital gyrus, BA 19
		1	3.22		-24, -66, 46	L LOC, SPL, WM	-24, -67, 39	L parietal lobe, precuneus, BA 7
		1	2.99		-28, -84, 36	L LOC, occipital pole, WM	-28, -83, 28	L occipital lobe, cuneus, BA 19
		1	2.96		-10, -68, 54	L LOC, precuneus, WM	-11, -67, 52	L parietal lobe, precuneus, WM
		1	2.89		-12, -64, 60	L LOC precuneus, SPL, WM	-13,-67, 52	L parietal, precuneus, BA 7
Happy>Angry ESM	8IU<placebo	2	3.41	0.014	-4, -56, -14	L LOC, temporal occipital FFC, IT, occipital FFG, WM	-5, -53, -13	L cerebellum, Anterior lobe, culmen
		2	3.24		8, -42, -18	Brainstem	6, -39, -16	Brainstem,midbrain
		2	3.23		-2, -66, -18	No label found	-3, -62, -18	L cerebellum, posterior lobe, declive
		2	3.1		12, -56, -4	R lingual gyrus, WM	10, -54, -4	R cerebellum, anterior lobe, culmen

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		2	3.03		-18, -56, -4	L lingual gyrus, precuneus, temporal temporal occipital FFC, occipital FFC	-18, -54, -5	L limbic lobe, parrahippocampal gyrus, BA19
		2	3.01		4, -56, -4	R lingual gyrus	3, -54, -4	R cerebellum, anterior lobe, culmen
		1	3.54	0.042	24, -18, 42	R WM	21, -22, 40	R limbic lobe, cingulate gyrus, WM
		1	3.39		24, -24, 46	R pre and postcentral gyrus, WM	21, -28, 43	R parietal lobe, sub-gyral, WM
		1	3.24		16, -14, 36	R cingulate gyrus, JPL, WM	13, -18, 35	R limbic lobe, cingulate gyrus, WM
		1	3.2		16, -8, 40	R cingulate gyrus, JPL, precentral gyrus, WM	13, -13, 39	R limbic lobe, cingulate gyrus, WM
		1	3.02		12, 0, 52	R JPL, cingulate gyrus, WM	9, -6, 51	R frontal lobe, MFG, WM
		1	2.86		38, -12, 36	R pre and postcentral gyrus, WM	34, -16, 36	R frontal lobe, sub-gyral, WM
Neutral>Shape ESM	8IU<placebo	1	3.84	0.014	-34, -82, 26	L LOC	-33, -80, 20	L occipital lobe, middle occipital gyrus, WM
		1	3.53		-34, -92, 26	L occipital pole, LOC	-33, -90, 19	L occipital lobe, middle occipital gyrus, BA 19
		1	3.39		-28, -84, 36	L LOC, occipital pole, WM	-28, -83, 28	L occipital lobe, cuneus, BA 19
		1	3.29		-26, -70, 36	L LOC, cuneal cortex, WM	-26, -70, 30	L parietal lobe, sub-gyral, WM
		1	3.12		-34, -56, 48	L SPL, angular gyrus, LOC, SMG, WM	-33, -58, 41	L parietal lobe, inferior parietal lobule, WM
		1	3.11		-30, -66, 46	L LOC, angular gyrus, SPL, WM	-29, -67, 39	L parietal lobe, precuneus, WM

Table 5: Cluster Index - 8IU < 24IU, 8IU < 1IU IV (p=0.05)

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
Neutral>Shape ESM	8IU<24IU	1	3.67	0.047	64, -12, 42	R postcentral gyrus	58, -17, 41	R frontal lobe, precentral gyrus, BA 4
		1	3.6		66, 2, 36	No label found	60, -3, 37	R frontal lobe, precentral gyrus, WM
		1	3.57		66, -2, 38	R precentral gyrus	60, -7, 39	R frontal lobe, precentral gyrus, BA 6
		1	3.46		62, 0, 40	R pre and postcentral gyri	56, -6, 41	R frontal lobe, precentral gyrus, WM
		1	3.3		56, -12, 52	R pre and postcentral gyri, WM	50, -18, 50	R parietal lobe, postcentral gyrus, WM
		1	3.12		48, -10, 48	R pre and postcentral gyri, WM	43, -15, 47	R frontal lobe, precentral gyrus, BA 4
Q2 VSM	8IU<1IU IV	1	4.02	0.002	20, -8, -10	R amygdala, pallidum, WM	18, -8, -5	R sub-lobar, lentiform nucleus, medial global pallidus
		1	3.91		24, 4, -8	R putamen, pallidum, WM	21, 3, -2	R sub-lobar, lentiform nucleus, putamen
		1	3.73		20, -2, -8	R putamen, pallidum, WM	18, -3, -3	R sub-lobar, lentiform nucleus, lateral globus pallidus
		1	3.64		-30, -24, -18	L parrahippocampal gyrus, hippocampus, WM	-29, -22, -15	R limbic lobe, parrahippocampal gyrus, WM
		1	3.62		10, 16, -10	R subcallosal cortex, frontal orbital cortex, accumbens, WM	8, 14, -3	R sub-lobar, caudate, caudate head
		1	3.43		24, 32, -20	R frontal orbital cortex, frontal pole	22, 30, -11	R frontal lobe, sub-gyral, WM
Q VSM	8IU<1IU IV	2	3.91	0.000	-50, -6, 20	L pre and postcentral gyri, central operculum, WM	-48, -9, 21	L parietal lobe, postcentral gyrus, WM
		2	3.89		-62, -30, 30	L SMG, postcentral gyrus, parietal operculum, planum temporale, WM	-59, -32, 27	L parietal lobe, inferior parietal lobule, BA 10
		2	3.84		-46, -10, 28	L pre and postcentral gyri, WM	-44, -13, 27	L frontal lobe, precentral gyrus, WM
		2	3.71		-68, -10, 14	L pre and postcentral gyri, STG	-4, -12, 15	L parietal lobe, postcentral gyrus, BA 43

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		2	3.71		-62, 6, 4	L precentral gyrus, IFG, temporal pole	-58, 4, 7	L frontal lobe, precentral gyrus, WM
		2	3.71		-52, -18, 44	L pre and postcentral gyri, SMG, WM	-50, -22, 41	L parietal lobe, postcentral gyrus, WM
		1	3.63	0.003	10, 14, -10	R subcallosal cortex, accumbens, WM	8, 12, -3	R sub-lobar, caudate, caudate head
		1	3.51		22, 2 -10	R putamen, amygdala, pallidum	19, 1, -4	R sub-lobar, lentiform nucleus, lateral globus pallidus
		1	3.48		20, -10, -8	R pallidum, amygdala, WM	18, -10, -4	R sub-lobar, extra-nuclear, WM
		1	3.44		50, -38, -18	R IFG, temporal occipital fusiform cortex, WM	45, -36, -15	R temporal lobe, FFG, WM
		1	3.34		-2, 4, 0	L WM	-3, 2, 4	L sub-lobar
		1	3.24		38, -26, -20	R temporal fusiform gyrus, parahippocampal gyrus, temporal occipital FFC, IFG, WM	34, -24, -15	R limbic lobe, parrahippocampal gyrus, WM
Neutral>Shape ESM	8IU<1IU IV	1	3.5	0.001	38, -40, 42	R SMG, SPL, angular gyrus, postcentral gyrus, WM	34, -43, 39	R parietal lobe, sub-gyral, WM
		1	3.32		38, -14, 46	R pre and postcentral gyri, WM	34, -19, 44	R frontal lobe, precentral gyrus, WM
		1	3.31		58, -33, 56	R SMG, parietal operculum, postcentral gyrus	52, -37, 52	R parietal lobe, inferior parietal lobule, BA 40
		1	3.13		46, -36, 52	R SMG, SPL, postcentral gyrus, angular gyrus, WM	41, -40, 48	R parietal lobe, inferior parietal lobule, BA 40
		1	3.12		42, -30, 62	R pre and postcentral gyri, SMG, SPL, WM	37, -35, 57	R parietal lobe, postcentral gyrus, BA 40
		1	3.09		56, 8, 36	R precentral gyrus, MFG, IFG, WM	50, 2, 38	R frontal lobe, MFG, BA 6
Q2 ESM	8IU<1IU IV	2	3.84	0.000	-18, 56, 16	L frontal pole, WM	-18, 49, 23	L frontal lobe, SFG,WM
		2	3.8		20, 20, 2	R putamen, caudate, WM	18, 17, 8	R sub-lobar, caudate, caudate body
		2	3.79		-6, 30, 2	L ACC, sub-callosal cortex, WM	-7, 26, 8	L sub-lobar, extra nuclear, corpus callosum, WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		2	3.52		12, 24, -8	R sub-callosal cortex, caudate, accumbens, WM	10, 21, -1	R sub-lobar, caudate, caudate head
		2	3.51		2, 34, 22	L ACC, paracingulate gyrus,	1, 28, 7	Inter-hemispheric
		2	3.47		-10, 18, -6	L accumbens, caudate, WM	-10, 16, 0	L sub-lobar, caudate, caudate head
		1	3.48	0.021	48, -28, -14	R IT(pos), MTG(pos), WM	43, -27, -10	R temporal lobe, sub-gyral, caudate head
		1	3.46		54, -40, 34	R SMG (pos), angular gyrus, parietal operculum, planum temporale, SMG (ant), WM	48, -42, 32	R parietal lobe, SMG, WM
		1	3.44		44, -24, -12	R MTG (pos), WM	40, -23, -8	R temporal lobe, sub-gyral, WM
		1	3.43		50, -32, -14	R IT(pos), MTG(pos), WM	45, -31, -10	R temporal lobe, sub-gyral WM
		1	3.38		58, -36, 6	R STG (pos), SMG(pos), MTG (pos), MTG (tempooccipital part), angular gyrus, WM	52, -36, 7	R temporal lobe, STG, WM
		1	3.32		60, -34, 10	R MTG (tempooccipital part), angular gyrus, WM	54, -35, 11	R temporal lobe, STG, WM

Table 6: Cluster Index - 24IU < placebo (p=0.05)

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
Q1 VSM	24IU<placebo	1	4.19	<0.001	-56, -6, 24	L pre and postcentral gyri, WM	-53, -9, 24	L frontal lobe, precentral gyrus, WM
		1	3.95		-58, -20, 44	L postcentral gyrus, SMG, WM	-55, -24, 41	L parietal lobe, postcentral gyrus, BA 1
		1	3.74		-40, -2, 2	L insular, WM	-40, -2, 2	L sub-lobar, insular, WM
		1	3.66		-54, 6, 24	L precentral gyrus, IFG, WM	-51, 2, 25	L frontal lobe, IFG, BA 9
		1	3.65		-54, 8, 16	L precentral gyrus, IFG, WM	-51, 5, 18	L frontal lobe, IFG, WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.57		-54, -28, 34	L postcentral gyrus, SMG, parietal operculum, WM	-51, -31, 31	L parietal lobe, inferior parietal lobule, WM
Happy ESM	24IU<placebo	1	3.49	0.017	18, -52, -10	R precuneus, cingulate gyrus, supracalcarine cortex, intracalcarine cortex, WM	15, -51, 9	R limbic lobe, posterior cingulate, BA 30
		1	3.41		4, -58, 50	R precuneus	2, -60, 44	R parietal lobe, precuneus
		1	3.31		6, -58, 0	R lingual gyrus, precuneus, intracalcarine cortex	4, -56, -1	R cerebellum, anterior lobe, culmen
		1	3.28		-2, -64, -48	No label found	-2, -57, -44	No label found (nearest L cerebellum)
		1	3.19		16, -66, -46	No label found	14, -59, -43	No label found (nearest R cerebellum)
		1	3.17		16, -64, -42	No label found	14, -58, -39	R cerebellum, posterior lobe, cerebellar tonsil
Neutral ESM	24IU<placebo	1	3.7	0.015	-34, -68, -44	No label found	-32, -61, -42	L cerebellum, posterior lobe, cerebellar tonsil
		1	3.54		14, -62, -40	No label found	12, -56, -37	R cerebellum, posterior lobe, cerebellar tonsil
		1	3.38		-6, -58, -52	No label found	-6, -51, -48	No label found (within 5 mm)
		1	3.37		6, -58, -50	No label found	5, -51, -46	No label found (nearest R cerebellum)
		1	3.19		0, -58, -44	No label found	-1, -52, -40	L cerebellum, posterior lobe, cerebellar tonsil
		1	3.19		36, -72, -28	No label found	32, -67, -27	R cerebellum, posterior lobe, tuber
Happy>Angry ESM	24IU<placebo	1	3.39	0.003	14, -60, -12	R lingual gyrus, temporal occipital FFC	12, -57, -12	R cerebellum, posterior lobe, declive
		1	3.26		30, -58, -20	R temporal FFC, FFG	30, -58, -20	R cerebellum, posterior lobe, declive

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.25		-18, -72, -14	L FFG, lingual gyrus, WM	-18, -68, -15	R cerebellum, posterior lobe, declive
		1	3.14		6, -68,-10	R lingual gyrus	4, -64, -11	R cerebellum, posterior lobe, declive
		1	3.13		10, -68, -12	R lingual gyrus, FFG	8, -64, -12	R cerebellum, posterior lobe, declive
		1	3.08		36, -62, -20	R temporal occipital FFC, FFG	32, -58, -19	R cerebellum, posterior lobe, declive
Neutral>Shape ESM	24IU<placebo	1	4.03	0.049	-22, -66, 48	L LOC, SPL, WM	-22, -67, 41	L parietal lobe, precuneus, WM
		1	3.26		-34, -84, 28	L LOC, occipital pole, WM	-33, -82, 21	L occipital lobe, middle occipital gyrus, WM
		1	3.13		-12, -64, 62	L LOC, precuneus, SPL, WM	-13, -67, 54	L parietal lobe, superior parietal lobule, BA 7
		1	3.1		-26, -70, 34	L LOC, WM	-26, -70, 28	L occipital lobe, sub-gyral, WM
		1	2.92		-18, -74, 40	L LOC, precuneus, cuneal, WM	-18, -74, 33	L occipital lobe, cuneus, WM
		1	2.85		-38, -94, 24	L occipital pole	-37, -91, 17	L occipital lobe, middle occipital gyrus, BA 19

Table 7: Cluster Index – 24IU < 8IU or 24IU < 1IU IV (p=0.05)

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
Face VSM	24 IU<8IU	2	3.9	0.026	-24, -76, 38	L LOC, WM	-24, -76, 38	L parietal lobe, precuneus, BA 7
		2	3.85		-30, -76, 38	L LOC, WM	-29, -76, 31	L occipital lobe, sub-gyral, WM
		2	3.34		-32, -70, 26	L LOC, WM	-31, -69, 21	L temporal lobe, MTG, WM
		2	3.19		-26, -62, 36	L LOC, SPL, WM	-26, -63, 30	L parietal lobe, sub-gyral, WM
		2	3.14		-30, -54, 36	L LOC, SPL, SMG, angular gyrus, WM	-29, -55, 31	L parietal lobe, sub-gyral, WM
		2	3.1		-34, -68, 38	L LOC, WM	-33, -68, 31	L parietal lobe, sub-gyral, WM
		1	3.67	0.036	20, -68, 40	R LOC, precuneus, cuneal cortex, WM	17, -69, 32	R occipital lobe, cuneus, WM
		1	3.5		22, -52, -6	R lingual gyrus, temporal occipital FFC, WM	19, -50, -6	R limbic lobe, parrahippocampal gyrus, BA 19
		1	3.48		24, -72, 38	R LOC, cuneal cortex, precuneus, WM	21, -72, 32	R occipital lobe, cuneus, WM
		1	3.25		22, -78, 42	R LOC, cuneal cortex, precuneus, WM	19, -78, 35	R parietal lobe, precuneus, BA 7
		1	3.21		20, -84, 42	R LOC, occipital pole, precuenus, WM	17, -84, 34	R occipital lobe, cuneus, BA 19
		1	3.14		26, -56, 18	R precuneus, supracalcarine cortex, cuneal cortex, WM	23, -56, 16	R temporal lobe, sub-gyral, WM
FaceShape VSM	24 IU<8IU	1	3.65	0.043	-32, -76, 38	L LOC, WM	-31, -76, 31	L occipital lobe, sub-gyral, WM
		1	3.54		-22, -74, 40	L LOC, precuneus, WM	-22, -74, 33	L occipital lobe, cuneus, WM
		1	3.32		-32, -70, 26	L LOC, WM	-31, -69, 21	L temporal lobe, MTG, WM
		1	3.17		-34, -68, 38	L LOC, WM	-33, -68, 31	L parietal lobe, sub-gyral, WM
		1	3.16		-38, -62, 38	L LOC, angular gyrus, SPL, WM	-37, -63, 32	L parietal lobe, angular gyrus, WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.07		-30, -54, 36	L SPL, angular gyrus, SMG, LOC, WM	-29, -55, 31	L parietal lobe, sub-gyral, WM
Q1 VSM	24 IU<1IU IV	1	3.6	0.016	-34, 52, 8	L frontal pole, WM	-32, 46, 15	L frontal lobe, SFG, WM
		1	3.46		-60, 10, 14	L precentral gyrus, IFG	-57, 7, 17	L frontal lobe, IFG, BA 44
		1	3.45		-54, 8, 18	L precentral gyrus, IFG,WM	-6, 4, 21	L sub-lobar
		1	3.43		-56, -4, 28	L pre and postcentral gyri, WM	-53, -8, 28	L frontal lobe, precentral gyrus, WM
		1	3.42		-56, -10, 34	L pre and postcentral gyri, WM	-53, -14, 33	L frontal lobe, postcentral gyrus, BA4
		1	3.39		-60, -8, 34	L pre and postcentral gyri, WM	-57, -12, 33	L frontal lobe, precentral gyrus, BA4
Q VSM	24 IU<1IU IV	1	3.87	0.004	-46, 20, -10	L frontal orbital cortex, frontal operculum, temporal pole, IFG	-43, 18, 4	L frontal lobe, IFG, BA 45
		1	3.77		-56, -2, 24	L pre and postcentral gyri, WM	-53, -5, 24	L frontal lobe, precentral gyrus, WM
		1	3.77		-50, 20, -10	L frontal orbital cortex, temporal pole, IFG, frontal operculum	-47, 18, -4	L frontal lobe, IFG, WM
		1	3.71		-54, -16, 44	L pre and postcentral gyri	-52, -20, 41	L parietal lobe, postcentral gyrus, WM
		1	3.66		-40, 26, -14	L frontal orbital cortex, WM	-38, 24, -7	L frontal lobe, IFG, WM
		1	3.64		-34, 36, -16	L frontal pole, frontal orbital cortex	-32, 33, -8	L frontal lobe, IFG, WM
Face>Shape ESM	24 IU<1IU IV	1	3.66	0.004	-44, 36, 24	L MFG, frontal pole, IFG, WM	-42, 30, 28	L frontal lobe, MFG, BA 9
		1	3.64		-50, 2, 18	L pre and postcentral gyri, IFG, WM	-47, -1, 20	L frontal lobe, IFG, BA 44
		1	3.6		-32, 16, 10	L frontal operculum, insular, central operculum, WM	-31, 12, 14	L sub-lobar, insular, BA 13
		1	3.58		-50, 10, 16	L IFG, precentral gyrus, WM	-47, 6, 18	L frontal lobe, IFG, BA 44
		1	3.39		-32, 28, 38	L MFG, SFG, WM	-31, 21, 40	L frontal lobe, MFG, BA 8
		1	3.18		-36, 26, 10	L frontal operculum, IG, WM	-30, 22, 15	L sub-lobar, insular, BA 13
Neutral>Shape ESM	24 IU<1IU IV	1	4.06	0.02	-44, 38, 24	L frontal pole, MFG, WM	-42, 32, 28	L frontal lobe, MFG, WM

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.53		-42, 36, 6	L frontal pole, IFG, MFG, WM	-40, 31, 12	L frontal lobe, IFG, WM
		1	3.32		-32, 16, 10	L frontal operculum, insular, central operculum, SM	-31, 12, 14	L sub-lobar, extra-nuclear, WM
		1	2.9		-34, 30, 34	L MFG, frontal pole, WM	-33, 23, 37	L frontal lobe, precentral gyrus, BA 9
		1	2.46		-30, 54, 10	L frontal pole, WM	-29, 48, 17	L frontal lobe, SFG, WM
		1	2.43		-6, 8, 8	L caudate	-7, 5, 12	L sub-lobar, caudate, caudate body

Table 8: Cluster Index - 1IU IV vs placebo (p=0.05)

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
Shape VSM	1IU IV<placebo	1	3.75	0.034	-42,-58,-40	No label found*	-40, -52, -37	L cerebellum, posterior lobe, cerebellar tonsil
		1	3.75		-26,-76,-40	No label found	-25, -69, -39	L cerebellum, posterior lobe, inferior semi-lunar lobe
		1	3.6		-24,-88,-34	No label found	-23, -81, -34	L cerebellum, posterior lobe, pyramis
		1	3.56		-30,-68,-44	No label found	-28, -61, -42	L cerebellum, posterior lobe, cerebellar tonsil
		1	2.6		-40,-66,-28	No label found	-38, -61, -27	L cerebellum, posterior lobe, tuber
		1	2.37		-36,-74,-34	No label found	-34, -68, -33	L cerebellum, posterior lobe, pyramis
FaceShape VSM	1IU IV<placebo	1	3.78	0.046	-42, -58, -40	No label found	-40, -52, -37	L cerebellum, posterior lobe, cerebellar tonsil

Event	Conditions	Cluster	Z	P	MNI coordinates (x,y,z)	Harvard-Oxford Cortical Structural Atlas localisation (MNI)	Talairach coordinates (x,y,z)	Talairach Client localisation (single point)
		1	3.63		-26, -76, -40	No label found	-25, -69, -39	L cerebellum, posterior lobe, inferior semi-lunar lobe
		1	3.53		-24, -88, -34	No label found	-23, -81, -34	L cerebellum, posterior lobe, pyramis
		1	3.35		-32, -66, -44	No label found	-30, -59, -41	L cerebellum, posterior lobe, cerebellar tonsil
		1	3.27		-26, -84, -26	L FFG, LOC	-25, -78, -27	L cerebellum, posterior lobe, tuber
		1	2.53		-48, -56, -28	L IT, temporal occipital FFC	-45, -51, -27	L cerebellum, posterior lobe, pyramis
Happy>Angry ESM	1IU IV<placebo	1	3.54	0.007	-4, 50, -4	No label found	-5, -48, -4	L cerebellum (anterior), culmen
		1	3.47		14, -58, -12	R lingual gyrus	12, -55, -12	R cerebellum (posterior), declive
		1	3.18		4, -56, 0	R lingual gyrus	3, -54, -1	R cerebellum (anterior), culmen
		1	3.03		4, -44, -10	Brainstem	3, -42, -9	R cerebellum (anterior), cerebellar lingual
		1	3.01		-4, -56, -10	No label found	-5, -53, -10	L cerebellum (anterior), culmen
		1	2.99		6, -66, -12	R lingual gyrus	5, -62, -12	R cerebellum (posterior), declive

*Harvard-Oxford Cortical Structural Atlas does not include labelling of the cerebellum