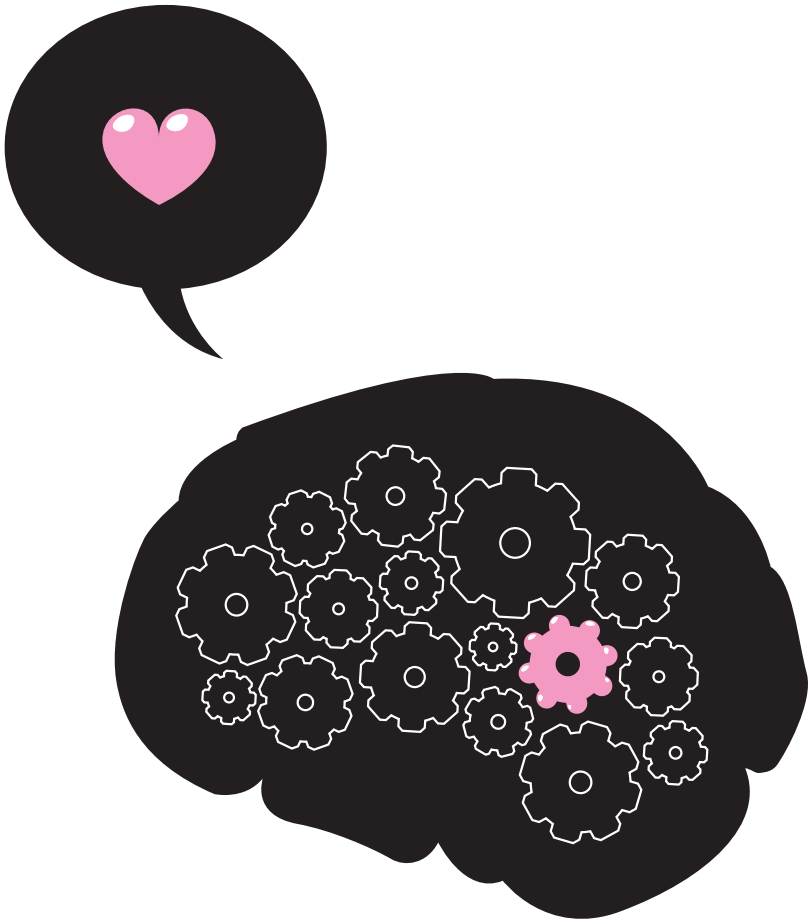

THE SOFT MACHINE

The soft machine: oxytocin and bonding

Vincent Tijms
Supervisor: Dr. Wim Ghijsen
Co-Assessor: Prof. dr. Peter Burbach
For the Master's Programme in Brain and Cognitive Sciences, University of Amsterdam
Track Cognitive Neuroscience

August 30, 2010

Abstract

Research during the past decade has shown that the neuropeptide oxytocin influences a puzzling variety of social behaviour in humans, in line with its role in other mammals. This paper explores the mechanisms underlying these phenomena by comparing the role of oxytocin in bonding in various species. Animal research shows a vital role for the compound in pair-bonding and parent-offspring bonding, with oxytocin promoting both the formation of social memories and subsequent other-oriented behaviour. Species-specific variations in behaviour depend on oxytocin receptor distribution and dynamic regulation of the oxytocin gene. In humans, this bonding system may have grown to include communal bonding, a prerequisite for complex societies that depend on trust and mutual sympathy. Current research suggests that pharmacological use of the peptide in treatment of social anxiety, autism and dysfunctional relationships can be effective.

Contents

Mens

Mens is een zachte machine,
een buigbaar zuiltje met gaatjes,
propvol tengere draadjes
en slangetjes die dienen
voor niets dan tederheid
en om warmer te zijn dan lucht.
Och, hij heeft ademzucht
en hart-arbeid.

Heeft hij een welvig lijfje,
hier en daar wat vetjes,
dan vindt hij iets niet netjes
en noemt zichzelf een wijfje;

bovenin zijn haarkleedje
draait hij dan vaak springveren.
Daar kan hij niet mee leren;
ze dansen alleen een beetje.

Het leren gebeurt in een kastje;
je mag dat niet openmaken,
wel teder, teder aanraken,
maar de rest van het zotte bastje
blijft ingepakt en bewaard,
want als het zich bepoedert,
ontwatert of ontvoedert,
ontroert, ontstemt, onthaart,
dan kruipt het een hokje in.
Een deurtje gaat op slot,
en het loopt niet naar buiten tot
het kleertjes heeft, kalmte, en zin.

Maar soms voelt het zich zoet;
het bekje prevelt: "trouwen",
het gladde buikje moet
een klein machientje bouwen.

God behoede de mens
en geve hem een zoen:
er is verder niets met hem te doen.
Streel zijn zoete pens,
want mens is een zachte machine,
een ingewikkeld liefje.
Verzilver zijn statiefje,
leid hem in een vitrine,
doe bij hem een lichtje aan.

Loop zachtjes om hem heen en
ga elders om hem wenen,
maar laat hem staan.

Leo Vroman (1915 -)

Introduction

Man is a soft machine, Dutch poet Leo Vroman wrote in 1961, in allusion to the naturalistic 18^{th}-century treatise *L'homme machine* by Julien Offray de La Mettrie. De La Mettrie, a French physician and natural philosopher, had argued the workings of man to be mechanistic and subject to the same laws as the cogwheels and springs of man-made machinery. Vroman poetically agreed with de La Mettrie's materialist position, but added to his view that while man is a machine, he is a soft machine, defined by his search for love and tenderness.

Human affiliation is indeed striking. In many species, herd or flocking behaviour is merely a protective strategy, a way to have the dice of predator attacks roll in your favour. Although bonding to kin and other individuals does exist in a variety of species, the human capacity for community sense may be unrivalled. Humans bond with those around them and tend the mourn the loss of their community members. Partner bonds and parent-offspring bonds are especially strong, perhaps because of the anomalous helplessness of the human neonate, who requires parental care for years into its existence[1], but more ideal affiliations such as patriotism are also known to be powerful motivators of human behaviour.

In fact humans are, to borrow from Baruch de Spinoza, social animals to such a great extent that impairments of interaction with others can be detrimental to their health, with the risk factor of social deprivation competing with those of smoking and obesity [116]. Other remarkably human traits, such as language, music, political institutions and complex economies can be argued to depend strongly on human sociality, thus rendering it a prime mover in human cultural development.

Of course, as all biological traits, sociality lies on an evolutionary continuum. This is why sociality has been researched not only by psychologists, sociologists and anthropologists, but also by the biological sciences, in the belief that some of the origins of human sociality can be found through interactions seen in other species. The most obvious candidates for such research were of course primates, whose behaviour has been studied by primatologists in excruciating detail, revealing hierarchies based on individuality, partner bonding, economical activity and perhaps even the first faint shimmerings of morality.

One might think such animal research translates poorly to actual humans. After all, human sociality is a function of complex metacognitive abilities and advanced reasoning skills. Empathy, trust and other human traits are bound to depend on cognition not found in other denizens of the animal kingdom. However, a 2005 paper by economist Michael Kosfeld put this view in doubt. Titled *Oxytocin increases trust in humans*, it describes how a nasal spray containing the peptide oxytocin specifically alters the willingness of people to engage in transactions with others, a willingness that could not be explained by an increase in risk seeking by itself. Subsequent research implicated oxytocin in a variety of human skills related to sociality, including face recognition, generosity and empathy. Oxytocin seemed to be the oil of the soft machine.

The peptide is not exclusively found in humans. In fact, it is released endogenously in all mammals, and small variations of the molecule are found in pre-mammalian species as well, from earthworms to fish. In all these species, oxytocin-like molecules serve a "social" or reproductive role, be it through facilitating vocalizations or egg-laying behaviour. The fact that socio-cognitive traits in humans are apparently sensitive to the peptide, too, suggests

[1] This evolutionary reasoning is often employed, but of course hard to test. Biological anthropologist Helen Fisher examined the claim by looking at the distribution of divorce rates in several cultures. Interestingly enough, her data show a peak after some four years of marriage [81]. Evolutionary psychologists have argued this to correspond to the period of human pregnancy and infant helplessness. This so-called *provisioning model* is discussed in reference [275].

a highly conserved system underlies sociality. The translation from animal research might not be as far-fetched as it intuitively seems.

How could a single compound mediate such diverse behaviours, which can all be categorized in the somewhat fuzzy, elusive concept of sociality? This question serves as the starting point of this thesis. Sociality is a blanket term covering many aspects of interactions and tying this to a single molecular substrate may sound stretched to even the most reductionist of scientists. To explore how a "social molecule" could even exist, this pluriformity itself should be studied, at both the cellular and behavioural level.

Comparative biology offers a great opportunity to do so. To a large part, the mechanisms of oxytocin can only be delineated by invasive research that needs to be done in non-human animals, for obvious ethical reasons. Also, different species can possess different behavioural repertoires. Comparative biology could show how changes in social machinery correspond to changes in behaviour.

This thesis will focus on the role of oxytocin in bonding, which is a process shared by many species, albeit in different forms, making it ideal for cross-species comparison. Bonding is also interesting as it encompasses two separable processes of sociality: the receptive part (recognition of the other) and the expressive part (initiating specific, other-oriented behaviour). Furthermore, disrupted bonding lies at the heart of a variety of psychiatric conditions, so that any addition to its understanding is welcome in clinical practice.

Elucidating the role of oxytocin in the social machinery of the brain is largely an exploratory exercise, but along the way some directed questions will be addressed. The first is whether oxytocin is indeed conserved as a *prosocial peptide* across mammalian species and whether its mode and sites of release have changed throughout evolutionary history. The second is question is whether social behaviour that is idiosyncratic to a given species can be explained by differences in the oxytocin system.

A third question regards the way in which oxytocin could exert its social role. Does it act on brain regions that are specialized for social cognition? Or does it change the activity of neural circuits that are engaged in less specialized processing? This is the social version of the modularity question found throughout cognitive science and evolutionary psychology: is region X *domain-specific* (i.e., specialized) for cognitive process Y or does it play a role in other processes as well?

Fourthly, the matter of clinical relevance is investigated. Can conditions like autism, social phobia and attachment disorders be understood through (malfunction of) the oxytocin system? And can addiction, which in a way is an intense bonding to some substance of abuse, be described in the same way as interpersonal bonding? Finally, the thesis will consider Vroman's claim: is man really a soft machine, driven to seek affiliation?

The thesis consists of two parts. Part I starts with an introduction to oxytocin. As this peptide takes center stage, it is important to consider the ways in which it is released and how it acts on the central nervous system. Subsequently, animal studies on the role of oxytocin in social recognition and bonding are discussed. This leads to a suggested framework in which the function of oxytocin can be conceptualized. In part II of the thesis, data on oxytocin's role in humans will be discussed and interpreted in light of this conceptualization. The thesis will be summarized by revisiting the aforementioned questions.

Before the exploration of oxytocin starts, it is important to add a caveat. The neuropeptide is *involved* in a wide range of social processes, but this is done in concert with numerous other compounds. These compounds are not the focus of this thesis, but that does not mean oxytocin holds a privileged chemical position among all the molecular substrates of psychology. The rich behavioural tapestry that is seen in both human and non-human animals is woven by an interplay of chemicals, of which relative concentrations and sites of action are dynamically modulated to shape behavioural states. Describing the role of oxytocin can only give a coarse view of the workings of the soft machine.

Theses

1. A core oxytocin system, associated with social behaviour, is conserved across species.
2. Species-specific sociality can be understood through oxytocin action.
3. Oxytocin signals sociality and acts only on general-purpose circuits.
4. Autism, social phobia and attachment disorders can be conceptualized as oxytocin system disorders.
5. Addiction is a love.
6. Man is a soft machine.

Part I

Animals

"Let satirists then laugh their fill at human affairs, let theologians rail, and let misanthropes praise to their utmost the life of untutored rusticity, let them heap contempt on men and praises on beasts; when all is said, they will find that men can provide for their wants much more easily by mutual help, and that only by uniting their forces can they escape from the dangers that on every side beset them: not to say how much more excellent and worthy of our knowledge it is, to study the actions of men than the actions of beasts."

–Baruch de Spinoza, Ethics IV: XXXV

Chapter 1

Oxytocin

The story of oxytocin begins more than a century ago, when folk medicine used extracts from farm animal uteri to induce labour in pregnant women. In 1909, Sir Henry Dale discovered that the responsible active compound could be isolated from the posterior pituitary gland [53] and he dubbed it oxytocin, from the ancient Greek ὁξύς τόκος, meaning "rapid birth", without anyone understanding the mechanism or even the chemical identity of the compound. This changed in the 1950s, when American chemist Vincent du Vigneaud not only sequenced [69], but also synthesized [68] oxytocin, thereby creating the first synthetic peptide. He showed that his synthetic oxytocin shared a number of physical and chemical properties with the natural compound, and also proved to be just as effective in contracting uteri and stimulating milk ejection in test animals. This discovery brought du Vigneaud the 1955 Nobel Prize in chemistry.

1.1 Oxytocin

1.1.1 Structure and release

The amino acid sequence du Vignaud determined is shown in figure 1.1. Oxytocin is a nonapeptide with a sulphur bridge between cysteine residues, rendering a cyclic structure of six amino acids with an α-amidated tail of three more amino acids. Nonapeptides with a basic residue at the number 8 position (lysine or arginine) are counted as part of the vasopressin family, while those with a neutral amino acid are counted as part of the oxytocin family. In humans (and most mammals for that matter), only oxytocin and arginine vasopressin are neuropeptides of interest, but it is worth noting that variants are found in other life forms. Table 1.1 shows oxytocin, vasopressin and a selection of homologues, illustrating how the basic chemical structure of the compounds has remained relatively unchanged throughout biological evolution. What is more, even in primitive species such as the earthworm *Eisenia foetida* the oxytocin homolog is involved in reproductive behaviour (in this case to induce egg-laying [195]), pointing to conserved functionality as well. In mammals, oxytocin is mostly expressed within magnocellular cells in the paraventricular and supraoptic nuclei of the hypothalamus, from where it is transported in secretory vesicles along axon terminals to the pituitary gland, which releases it into the bloodstream. As all neuropeptides, there is no reuptake mechanism for synaptically released oxytocin: it exerts its action on specialized receptors and is enzymatically degraded, only to be replenished by *de novo* synthesis. Parvocellular neurons in the paraventricular nucleus also express oxytocin and target regions in the central nervous system. In the rat, such oxytocin fibers were shown to reach the dorsomedial hypothalamic nucleus, dorsal and ventral hippocampus, subiculum, entorhinal cortex, medial and lateral septal nuclei, (mostly central) amygdala, the olfactory bulbs, substantia nigra, locus coeruleus, the raphe nuclei, nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve [34], [215]. Oxytocin release is triggered via serotonergic activation [128], but also noradrenergic, dopaminergic and glutamatergic projections [139].

From the pituitary gland, oxytocin is released into the bloodstream, but it does not readily cross the blood-brain barrier, keeping central and pe-

Table 1.1: Oxytocin and related peptides. Residues in italics differ from those in oxytocin, basic residues at position 8 are shown boldface and signify membership of the vasopressin family, as opposed to the oxytocin family. Note that this same position is a large contributor to molecular diversity. The last column indicates in what animals the respective peptide is found. Adapted from reference [93].

Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	$Gly(NH)_2$	Placentals
Mesotocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	*Ile*	$Gly(NH)_2$	Salmonids, marsupials
Isotocin	Cys	Tyr	Ile	*Ser*	Asn	Cys	Pro	*Ile*	$Gly(NH)_2$	Osteichtyes
Cephalotocin	Cys	Tyr	*Phe*	*Arg*	Asn	Cys	Pro	*Ile*	$Gly(NH)_2$	Molluscs (*Octopus vulgaris*)
Vasotocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	***Arg***	$Gly(NH)_2$	Nonmammalian vertebrates
Annetocin	Cys	*Phe*	*Val*	*Arg*	Asn	Cys	Pro	*Thr*	$Gly(NH)_2$	Annelids (*Eisenia foetida*)
Vasopressin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	***Arg***	$Gly(NH)_2$	Mammals
Lysipressin	Cys	Tyr	*Phe*	Gln	Asn	Cys	Pro	***Lys***	$Gly(NH)_2$	Pig, some marsupials
Phenypressin	Cys	*Phe*	*Phe*	Gln	Asn	Cys	Pro	***Arg***	$Gly(NH)_2$	Marsupials

ripheral concentrations uncoupled. However, active transport of the peptide may exist [177]. Peripheral actions include the uterine contractions and milk ejections mentioned earlier[1]. Measurements from the cerebrospinal fluid - in which oxytocin contents are measured that likely derive from limbic, brainstem and spinal cord neurons - typically show oxytocin concentrations of 10-50 pM. A study measuring cerebrospinal fluid oxytocin in human females via lumbar puncture found basal oxytocin levels in the 5-11 pM range [61]. These concentrations can be much higher locally, though: high-frequency signaling by oxytocin neurons has been shown to lead to oxytocin concentrations of above 10 nM [152].

Not all oxytocin signalling is synaptic: magnocellular neurons also show dendritic and even some somatic release of oxytocin into the extracellular fluid of the supraoptic nucleus. Volume transmission following dendritic release can complement the focal oxytocin release at the synaptic terminals, allowing for oxytocin signalling at multiple time scales [153]. Dendritic release of oxytocin has been shown to increase under positive feedback via autoreceptors, leading to peptide concentrations in the ventricular space that could easily impact periventricular neurons. This point will be elaborated in section 5.1.1.

[1]Although an holistic view of the body grasps the interwoven nature of physiology best, this thesis merely focuses on central effects, for the sake of brevity. An excellent review of oxytocin action in the peripheral system is given by Gimpl [93].

1.1.2 Oxytocin gene expression and regulation

Consistent with the idea that the arginine vasopressin and oxytocin families arose through duplication of an ancestral gene, their genes are situated on the same chromosomal locus and are transcribed in opposite directions [230]. Within hypothalamic neurons, oxytocin is expressed from the *OXT* gene as a prepropeptide[2]. This prepropeptide consists of three exons, of which the first encodes for oxytocin, a translocator signal, a tripeptide processing signal (glysine-lycine-arginine) and the first nine residues of carrier protein neurophysin I, while the other two exons code for the rest. Oxytocin is initially extended at its C-terminal by three amino acids. Processing of prepropeptide occurs within secretory vesicles that are transported to their target region and leads to a oxytocin-neurophysin I dimer, which dissociates before release from the neuron [213].

Oxytocin expression is regulated by a multitude of factors. Polyadenylation of its mRNA, which has been argued to occur during pregnancy and lactation [284], may be one of these: increasing the length of the polyadenylate tail stabilizes the mRNA and can increase translational efficiency. Another possible source of regulation comes from thyroid and estrogen modulation. The hexanucleotide sequence AGGTCA and variations thereof are present near the oxytocin gene in both rats and

[2]Within this thesis, genes will be denoted in italics, while their product will be displayed in standard typeface. Genes written in upper-case refer to the human alleles, while lowercase genes refer to animal ones.

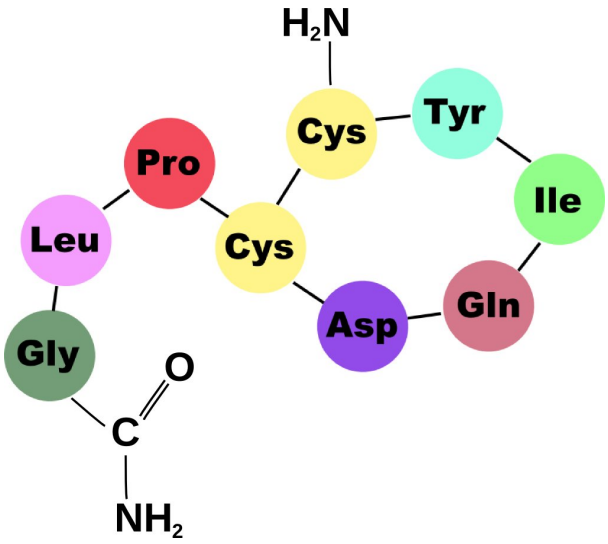

Figure 1.1: The nonapeptide oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly) has a ring structure due to an internal sulfide bridge between cysteine residues. The related arginine vasopressin differs by only two amino acids: phenylalanine (Phe) instead of isoleucine (Ile) and arginine (Arg) instead of leucine (Leu). The latter substition provides vasopressin with a basic tail, making it primarily a ligand for vasopressin receptors. Vasopressin also binds to oxytocin receptors with some affinity, while the reverse does not hold (see section 1.2).

humans and is capable of binding both thyroid and estrogen receptors, which can act as transcription factors. The thyroid hormone triiodothyronine was shown to increase oxytocin levels in the pituitary gland and plasma *in vivo* and upregulate hypothalamic oxytocin mRNA synthesis [1], in competition with the estrogen estradiol benzoate [60]. While this does not mean the same regulation takes place under natural circumstances, or even that thyroids act through the AGGTCA sequence, it does illustrate the control endocrine factors potentially have on oxytocin expression. Another important regulator of oxytocin expression is mediated by the aforementioned autoreceptors in hypothalamic regions, that allow for positive feedback of oxytocin release.

1.2 Oxytocin Receptors

1.2.1 Genetic regulation of oxytocin receptor expression

Oxytocin receptor genes have been identified in a number of species, including rat [224], sheep [216], mice [151], rhesus monkeys [226] and humans [144]. In humans, the oxytocin receptor gene *OXTR* is possibly under control of promoter regions sensitive to the transcription factors SP1, nuclear factor interleukin-6, GATA-binding factor 1, c-Myb and activator protein 1 and 2, among others. It is also flanked by three half-palindromic estrogen response element (ERE) sequences, which might collectively function in response to estrogen. Another mechanism of regulation is epigenetic: reduced methylation of the oxytocin receptor gene has been associated with its increased expression.

Mouse and rat oxytocin receptor genes seem to be regulated somewhat differently. In the mouse, the gene is potentially under control of a classical ERE and a few interleukin-response elements. The rat gene shows promoter regions sensitive to cAMP, nuclear factor interleukin-6, activator proteins 1 to 4 and also contains a classical ERE. The multitude of potential gene regulators allows fine-tuned dynamic control over receptor expression, differentiated over cell type and location and hormonal state of the organism. Also, as these few examples show, regulation differs across species, which may be related to the different behavioural roles the oxytocin system as a whole plays in different animals: different gene regulation potentially translates into regionally differentiated expression.

1.2.2 Molecular biology of the oxytocin receptor

The only oxytocin receptor known to date is a 389 amino acid long G-protein coupled receptor consisting of seven α-helical transmembrane domains. It belongs to the rhodopsin family and its main downstream effects are thought to be mediated through phospholipase C, mobilizing Ca^{2+} and phosphatidyl inositol. The three-dimensional structure of the oxytocin receptor has not been determined empirically, but its primary sequence shows moderate homology to the vasopressin 1 and 2 receptors (50% and 40%, respectively). However,

where the vasopressin receptors show high selectivity for vasopressin over oxytocin, the oxytocin receptor binds its preferred ligand with only a 10-fold higher affinity than it does arginine vasopressin [143], as it is mostly sensitive to the cyclic part of the peptide.

Ligand affinity of the oxytocin receptor has been shown to increase under allosteric modulation by cholesterol, which can make the dissociation constant K_D drop 100-fold and stabilizes the receptor [92]. Because cholesterol distribution differs across cell types (or even membrane location) this interaction adds another level of regulation and complexity to the biological role of the receptor. The divalent cations Mn^{2+} and Mg^{2+} play similar roles to cholesterol [93].

1.2.3 Neural distribution

In contrast to the neural sites of oxytocin expression, the oxytocin receptor distribution varies wildly across species [93]. It should be noted, however, that distributions are often measured using autoradiography and that receptor densities in apparently "empty" areas may simply be below the detection threshold. This is important to realize because oxytocin receptor density does not equate oxytocin signalling: high receptor density in an area with low oxytocin concentrations may lead to quantitatively similar signalling as low receptor density in a high concentration region. With that caveat in mind, it is still wholly reasonable to consider cells in which energy is dedicated to the synthesis, trafficking and maintenance of oxytocin receptors to rely on oxytocin for their functional roles.

Alternative methods, such as mRNA hybridization techniques, have higher sensitivity. However, even results using these techniques should be interpreted with care: firstly, hybridization shows the site of receptor expression, which can be remote from its membrane position. Secondly, receptor distribution may not only vary across species, but also within them. For example, oxytocin binding sites are found in infant rat cingulate cortex using autoradiography, but not in adult rats [261], suggesting receptor presence is a function of neurodevelopment. In a similar vein, gonadal steroids were shown to affect autoradiographical binding [125], which is in line with the genetic regulation pathways sketched above. Endocrine-dependent receptor expression may influence the role of the oxytocin system as a function of organism state (e.g., pregnancy) or gender. Sexual dimorphism has been shown in the oxytocin system of a number of species, with females preferentially expressing the receptor [150].

Differential localization of oxytocin receptors - be it across or within species - allows the peptide to exert different functions in different organism or organism states. One might imagine oxytocinergic effects on memory-related structures such as the hippocampus being categorically different from oxytocin action on the reward-related striatum.

1.3 Neurophysiological effects of oxytocin

The oxytocin receptor is coupled to the $G_{q/11}$ protein, meaning that oxytocin binding to the receptor results in activation of phospholipase C, which hydrolyzes phosphatidylinositol-(4,5)-bisphosphate to diacylglycerol and inositoltriphosphate. This is a common biomolecular pathway, that ultimately results in intracellular Ca^{2+} release and increased protein phosphorylation through protein kinase C. Both the efficacy and the cellular effects of this pathway depend on the state of the neuron, as every step of the cascade can theoretically serve as a site of regulation and Ca^{2+} release will influence gene expression or membrane excitability in some neurons, while having no such effect in others. This versatility of oxytocin also becomes apparent at the circuit level, where it may activate synapses of different strengths and signs. A full appreciation of the working of oxytocin therefore does not only encompass its sites of action, but also its downstream effects. Such effects have been elucidated for a number of regions.

1.3.1 Amygdala and bed nucleus of the stria terminalis

As will become apparent in subsequent chapters, one particularly important neural site for the behavioural role of oxytocin is the amygdala. Research by neuroscientist Joseph LeDoux [155], has shown that the amygdala plays a vital role in fear-

conditioned learning. This learning has been argued to take place through synaptic modification within the lateral nucleus of the amygdala. The lateral nucleus projects to the central nucleus which in turn projects to brainstem and hypothalamus, regulators of the physiological expression of fear. Using anterograde tracing, four subdivisions of the central nucleus were elucidated [127], dubbed the medial, lateral, intermediate and capsular subnuclei. The medial subnucleus forms the main output to brainstem regions.

Local injections of vasopressin and oxytocin in rat amygdala showed anxiogenic and anxiolytic effects, respectively, as measured by behavioural and stress response [219]. A similar dichotomy was found with regard to the receptor distributions within the central nucleus: autoradiography showed oxytocin receptors are primarily found in the lateral and capsular subnuclei, while vasopressin receptors are mostly present in the medial subnucleus [268]. This division continues into the bed nucleus of the stria terminalis, part of what is known as the extended amygdala together with the central and medial amygdaloid nuclei. Indeed, oxytocin and vasopressin were found to excite distinct populations in the central nucleus of the rat amygdala [117], one of which was activated by vasopressin but inhibited by oxytocin, while the other was unresponsive to vasopressin and excitable by oxytocin.

Using a clever combination of extracellular single-cell recordings, selective antagonists, cell labelling, tracing studies and patch-clamp recordings, Ron Stoop and colleagues at the University of Lausanne convincingly argued that the circuitry underlying these effects consists of oxytocin-responsive γ-aminobutyric acid (GABA) cells in the lateral and capsular subnuclei projecting to the medial subnucleus, where they inhibit neurons directly excited by vasopressin through action at vasopressin 1a (V1a) receptors (see figure 1.2) [117]. Indeed, activation of the lateral and capsular neurons decreased the probability of eliciting a post-synaptic potential from the medial nucleus. It was therefore proposed that, while vasopressin increases effective throughput from lateral nucleus to brainstem, oxytocin attenuates this effect. In this way, the two neuropeptides act as a chemical gain for the physiological expression of the fear response.

As mentioned, the separation of lateral/capsular

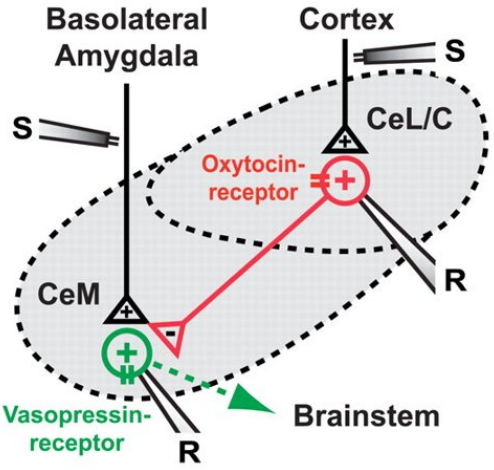

Figure 1.2: Simplified model of how oxytocin and vasopressin affect processing in the central nucleus of the amygdala (CeA). Input from the (baso)lateral amygdala excites the medial central nucleus (CeM), which projects to brainstem to elicit the fear response. CeM activity is positively modulated by vasopressin. The lateral and capsular central nuclei (CeL/CeC) GABAergically inhibit CeM. This can be due to excitation from the cortex, modulated by oxytocin. S and R are stimulating and recording electrodes used in the experimental setup. Taken from reference [117].

oxytocin receptors and medial vasopressin receptor is found throughout the extended amygdala. However, converging evidence points to functional differentiation within the extended amygdala, with the medial subnucleus of the central amygdala processing fear, while the bed nucleus of the stria terminalis is more involved in anxiety[3]. Just as the medial central nucleus, the bed nucleus of the stria terminalis receives input from the (baso)lateral part of amygdala. It has been suggested anxiety follows once negative feedback inhibits the medial central nucleus, but not the bed nucleus of the stria terminalis (see figure 1.3). Before discussing how oxytocin could influence the anxiety response, a special emphasis should be placed on the often con-

[3]In the terminology of Michael Davis, a neuroscientist investigating the extended amygdala, these two phenomena can be called *phasic fear* and *sustained fear*. For a recent review on the operationalisations of and experimental findings on these concepts, see reference [55].

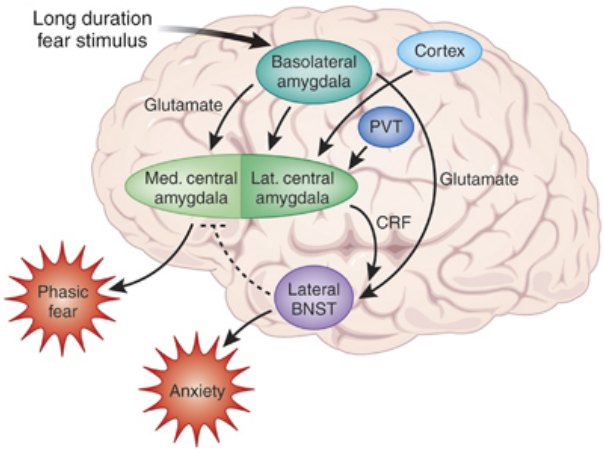

Figure 1.3: Schematic representation of the fear and anxiety circuit, as suggested by Davis [55]. Basolateral amygdala (BLA) projects strongly to the medial central nucleus and the lateral bed nucleus of the stria terminalis (lBNST), and weakly to the lateral central nucleus. The latter projects strongly to lBNST via corticotropin-release factor (CRF), which possibly enhances synaptic transmission from BLA terminals. Inhibition from the lBNST and/or the lateral central nucleus to the medial central nucleus decreases the direct fear response and instead leads to the more sustained form of fear that is anxiety. Cortex and paraventricular thalamus (PVT) are also shown as inputs to the lateral central nucleus. Figure taken from reference [55].

fusing nomenclature used in describing the many compartments found in the limbic system. Where the medial nucleus of the amygdala projects to the medial bed nucleus of the stria terminalis, the lateral portion of this latter region receives input from the *central* nucleus of the amygdala. As mentioned, the central nucleus is subdivided in a medial and a lateral/capsular part. The lateral bed nucleus of the stria terminalis has a similar subdivision, but here they are called the central lateral nucleus and the capsular lateral nucleus, respectively. These capsular and central nuclei seem to have a similar dichotomy of oxytocin and vasopressin receptors as does the central amygdaloid nucleus, but no research has been performed to see whether this holds functionally as well. However, GABAergic projections from the capsular bed nucleus of the stria terminalis towards the central bed nucleus of the stria terminalis have been found [268]. It is therefore plausible that oxytocin can attenuate anxiety in the same way as it can attenuate fear.

Neurons in the medial nucleus of the amygdala - and the medial bed nucleus of the stria terminalis - also show oxytocin binding. These nuclei have strong reciprocal connections with hypothalamic nuclei related to reproduction [268]. Also, the medial amygdaloid nucleus serves as a hub for the olfactory and vomeronasal pathways, two chemical detection paths that are discussed in more detail in section 2.2. The medial nuclei have been shown to be steroid-sensitive and sexually dimorphic, and oxytocin administration into the medial bed nucleus of the stria terminalis has been implicated in the milk-ejection reflex in rats and pair-bonding in prairie voles (see section 3.2 for more on the latter). In the case of the amygdala, the medial nucleus showed greater sensitivity to oxytocin than the central nucleus, and less desensitization [254].

1.3.2 Hippocampus

In the 1960s, research by David de Wied and others at the Dutch Rudolf Magnus Institute showed vasopressin and oxytocin effects on memory [57]. In later years, it became clear the two peptides had distinct effects on memory: where vasopressin enhanced long-term avoidance behaviour, oxytocin attenuated such learning. Oxytocin was also shown to influence drug tolerance and intake in rats [58].

Although these effects can be explained through action at the extended amygdala and striatum, they also initiated research on oxytocin and memory in the hippocampus. This research found that inhibitory neurons in rat CA1 were directly excited by the nonapeptides, while pyramidal cells were unresponsive or indirectly inhibited [210]. Oxytocin perfusion of mouse hippocampal slices was shown to improve long term potentiation, similar to that found in hippocampus of multiparous mice. Moreover, intracerebroventricular administration of oxytocin to virgin mice improved their spatial memory [258]. Oxytocin administration in the hippocampus has also been shown to reduce the behavioural response to stress [43].

1.3.3 Olfactory bulbs

Oxytocin in the main olfactory bulb of male rats was shown to act on oxytocin receptors and increase local noradrenaline levels, either directly through activation of noradrenergic cells or indirectly by suppressing inhibitory GABAergic interneurons [64]. The noradrenaline increase has been argued to disinhibit negative feedback on the olfactory system's mitral cells, which are odour-sensitive (see section 2.2). During this time of released inhibition, activated mitral cells can strengthen their synapses to downstream regions and increase positive feedback autoreceptor expression, thus forming a synaptic memory of odour input that is contingent on noradrenaline presence [227]. *In vitro* work using slices from the accessory olfactory bulb corroborated this view by showing *N*-methyl-*D*-aspartate (NMDA) receptor-dependent long term potentiation could be enhanced by oxytocin in a dose-dependent manner [76].

1.4 Summary

An evolutionarily old peptide, oxytocin is expressed in hypothalamic nuclei in a highly regulated fashion and released axonally, somatically and dendritically. The compound affects cells in both the peripheral and central system, which explains why it is involved in diverse phenomena, ranging from uterine contractions to olfactory learning. In some cases, oxytocin acts in concert with its molecular sibling vasopressin. For example, oxytocinergic attenuation of the fear response is opposed by vasopressinergic promotion of this same response.

Oxytocin acts on the oxytocin receptor, which is also dynamically controlled. The oxytocin receptor is differentially distributed in neural regions across and within species, being sensitive to differences in endocrine state. For a number of regions, the biochemical and circuit effects of oxytocin have been elucidated.

As will become clear in the following chapters, these effects are best not considered in isolation. In fact, oxytocin plays its role in regulating animal behaviour mostly through affecting neural regions that are highly interwoven.

Chapter 2

Social recognition

The recognition of conspecifics is of great importance to many species. Identifying potential mates, offspring or dominant members of the colony can be vital to survival and reproduction. In the context of bonding, a mere recognition of age, gender, receptive status and other general properties of others is not enough. The recognition must be more specific, it must be *individual recognition*. The distinction between individual recognition and more generic social recognition is not always clear, as individuals can sometimes be identified by (a combination of) generic properties. As a consequence, the literature on social recognition does not use the vocabulary in a consistent fashion.

In this thesis, individual recognition is operationally defined as the capacity to discriminate between animals that are of equal gender, age, social standing and species/strain. This is an admittedly arbitrary list, but it covers the typical confounds within social recognition research. As they are often not (all) controlled for, many of the studies cited in this chapter will only deal with social recognition. This will prove to provide a firm basis for understanding findings on individual recognition and social bonding, though, which takes center stage in chapter 3.

For social encounters, most rodents rely heavily on their noses: if a rat is confronted with a novel conspecific that does not provoke aggression or sexual behaviour, it will naturally investigate the other through anogenital and head sniffing (see figure 2.1), often accompanied by close following. This will last for some two minutes, depending on the strain used. It was found that this investigation time correlates negatively with the novelty of the conspecific [257], prompting researchers to use it as a measure of social memory. In rats, removing a conspecific for 30 minutes and then returning him leads to a drop in investigation time of about 50%, while exposure to a new conspecific, matched on age and strain, leads to renewed investigation. Over time, three main paradigms were developed that capitalized on this recognition-dependent investigation time.

Figure 2.1: Anogenital sniffing is an important part of social interaction in mice.

2.1 Three paradigms

These three paradigms are usually named social recognition, habituation-dishabituation and social discrimination and have been used (sometimes in minor variations) across species.

Social recognition is the most basic of the three paradigms. In this setup, the subject animal is confronted with a stimulus conspecific (the target) for some time, allowing for olfactory and close investi-

gation, after which the conspecific is removed. A predetermined amount of time passes and the animal is then confronted with either the same individual target, or a novel one. As mentioned, a typical response to an identical target in the rat would be a 50% drop in investigation time, but a novel conspecific should lead to the full, initial investigation time. Manipulation of either the subject, the target or the time between exposures allows for exploration of this type of social memory.

Habituation-dishabituation extends the social recognition paradigm by repeatedly exposing the subject animal to an identical target during a relatively short number of trials. This will robustly show a decrease in investigation time with each subsequent trial. To assure this lack of interest is not due to motivational factors, the habituation is followed by a dishabituation stage, in which a new conspecific is introduced. The typical response is a renewed interest upon this final exposure - if not, motivation or some other factor has changed throughout the experiment.

Social discrimination also builds on the social recognition paradigm. However, instead of exposure to a new target or reexposure to a familiar target, the animal is confronted with both simultaneously. The typical response would be for the animal to be biased towards investigating the new conspecific.

Although these three paradigms sound simple enough to perform, in practice there are many variables to consider, depending on the species under investigation. In many rodents, exposing a male to a novel target would lead to fighting (in the case of a male target) or sexual behaviour (in the case of a female target). In both cases no real measurement of recognition time can be performed. Using an ovariectomized female target removes the problem of sexual behaviour [78], but does lower baseline investigation time [189]. If using male targets, juvenile conspecifics do not evoke aggression in many rodent species, but as this is often the result of disinterest one should be careful to distinguish poor social memory from poor motivation. Also, as mentioned before, targets should be matched on age, gender and strain if one wishes to measure individual recognition with certainty, instead of generic social recognition. It has also been suggested that social recognition studies could measure self-odour recognition in at least some rodent species. This argument stems from the fact that mice, for example, are known to deposit odours on conspecifics during investigation. The argument then is that investigation ends once the animal is sure its odour has been left behind. It might be then, that some experimental manipulation interferes with odour deposition or recognition, instead of social recognition. This critique is often solved by physically separating subject and target animal, at the expense of true close investigation opportunities.

Another variable that has proven to be of great importance is the housing used for both subject and target animals. Group-housing, as opposed to single-housing, can significantly improve social recognition skills in male rats [148]. In contrast, the target animals to which the subject is exposed should be singly-housed, to prevent them from smelling alike due to cross-contamination of urinary proteins [40]. To help ensure that lab ecology does not affect research outcome, litter mates are best used as control subjects.

Finally, the paradigms as decribed have been developed mostly using rats, but not every species shows the assumed behavioural responses. For example, using spiny mice for social memory tasks would require a slight modification of the setup: they are biased towards familiar, not novel conspecifics [173]. As a general guideline, experimental settings that investigate social behaviour should be informed by ecological findings from field studies and social variables (housing, rearing) should be reported. Unfortunately, as of now, strict protocols for social memory studies and reports have not been developed, meaning different studies should only be compared with the greatest of care.

2.2 The smell of rodents

2.2.1 Two chemosensory systems

As mentioned above, rats respond to novel conspecifics by head and anogenital sniffing, as part of investigative behaviour. While the use of visual, tactile and auditory cues can rarely be wholly excluded, smell has been shown to be a dominant sense for recognition across many species of rodents and other mammals. The release of compounds, be they by-products of metabolism or especially synthesized for the purpose, can inform animals and

steer their behaviour.

The detection of these chemical cues is mostly performed by two sets of sensory neurons, one of which is present in the main olfactory epithelium (MOE), while the other is located within the vomeronasal organ (VNO). Both structures lie in the nasal cavity, but where the MOE is lined at its posterior side and relatively easy to access for inhaled compounds (especially the more volatile ones), the VNO lies in a capsule that opens in the base of the cavity and senses molecules that are actively pumped into its fluid-filled lumen (see figure 2.2). This difference led researchers to originally believe the two systems picked up distinct types of signals [232]. The MOE was argued to pick up volatile compounds for analysis, where the VNO would pick up proteins and other biological chemicals used for signalling (so-called semiochemicals). This latter system would drive endocrine and behavioural changes, while the former would detect odourants.

This view did not hold. Extracellular single neuron recordings performed in mouse main olfactory bulb (MOB) - a region receiving MOE, but not VNO projections - showed it was responsive to methylthiomethanethiol, a volatile attractor pheromone found in the urine of male mice [52], illustrating how semiochemicals could be detected by the MOE. Soon after, using studies blocking signalling cascades unique to MOE chemosensory neurons, it was shown even the large, non-volatile major histocompatibility (MHC) protein can be detected by the MOE of male mice, which mediates the protein's role in social preference tasks [246]. The VNO also detects the MHC protein, though. In female mice it was shown to induce the Bruce effect - the phenomenon whereby a pregnant female spontaneously aborts upon investigation of a novel male conspecific [157].

These findings show both chemosensory systems can play roles in social recognition and that overlap exists in the compounds they can detect. So how are they different? Aside from the differences that do exist in selectivity (the MOE shows low selectivity over a wide range of concentrations [70], while the VNO chemoreceptors are specifically tuned [158]) and that do make them specialized for certain types of sensing, the two organs employ different biochemical signalling cascades upon detection of chemicals (for a review on signalling in VNO, MOE and other olfactory subsystems, see [188]). Moreover, they show distinct connectivity to downstream brain regions.

2.2.2 Connectivity of the MOE and VNO

The sensory neurons of the MOE have apical dendrites along the surface of the epithelium, and a basal axon projecting to the dendrites of mitral cells in the main olfactory bulb (MOB). The VNO axons project dorsally along the septum and ultimately synapse with the apical dendrites of mitral cells in the accessory olfactory bulb (AOB). In both cases, the synapses are located in glomeruli (see figure 2.3) but the MOB projects to the piriform cortex, entorhinal cortex, olfactory tubercule, anterior olfactory nucleus and lateral amygdala, while the mitral cells in the AOB skip these structures and instead project to the bed nucleus of the stria terminalis, the nucleus of the accessory olfactory tract, the medial amygdaloid nucleus and the posteromedial amygdaloid cortical nucleus. MOB also projects to several limbic areas, but these are mostly distinct from the vomeronasal ones: the nucleus of the olfactory tract and the anterior cortical nucleus [72]. Figure 2.2 shows the two pathways.

The medial and posteromedial amygdala, which receive AOB input, project to the medial preoptic area and the ventromedial hypothalamus, two hypothalamic nuclei. The MOB also has indirect connections to hypothalamus - the anterior cortical nucleus is in turn connected to the medial preoptic area [72]. Although the main input to medial amygdala comes from the AOB, the MOB also projects to this region [176], making it one of the convergence points of the VNO and MOE pathways. Intrinsic connections among the amygdalar subnuclei likely add to this convergence. The ultimate projection to hypothalamic regions can cause endocrine state changes, influencing both gene expression and behaviour.

Besides these different connections, the mappings of the VNO and MOE sensory neurons on the mitral cells also differ. The diverse olfactory receptors of the MOE seem to map chemotopically on the glomeruli [185] (i.e., identical olfactory receptors map to the same glomeruli and receptors sensitive to similar compounds map to glomeruli that are topograpically close to each other), with mitral cells

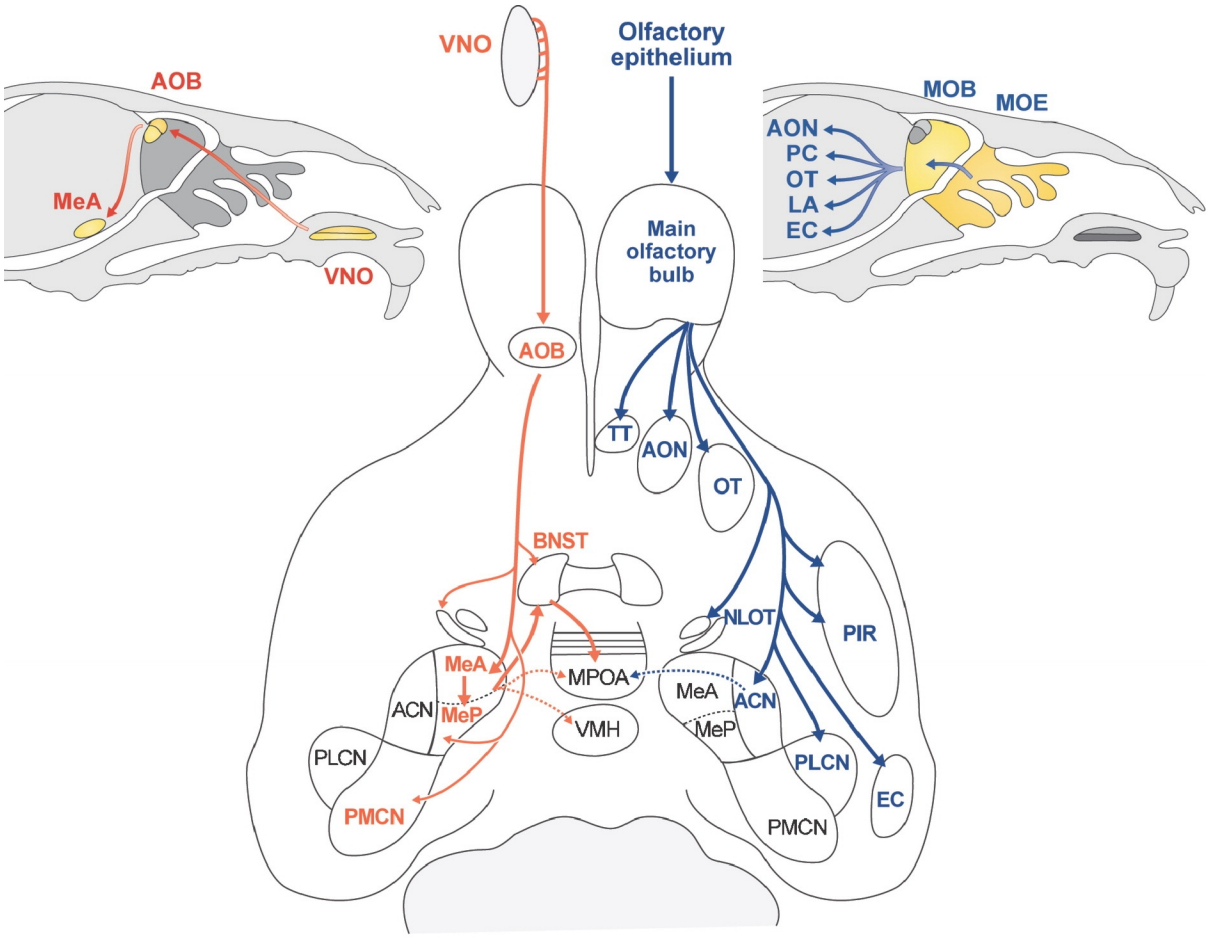

Figure 2.2: The vomeronasal (VNO) and main olfactory epithelium (MOE) pathways. The VNO send chemosensory input to the accessory olfactory bulb (AOB), which projects to the medial amygdala (MeA/MeP), the posteromedial cortical amygdaloid nucleus (PMCN) and the bed nucleus of the stria terminalis (BNST). Note the projection from the anterior medial amygdala to the BNST. The MOE connects to the tenia tecta (TT), the anterior olfactory nucleus (AON), the olfactory tubercle (OT), the piriform cortex (PIR), the entorhinal cortex (EC), the nucleus of the lateral olfactory tract (NLOT) and the anterior cortical nucleus (ACN). Both pathways converge on the hypothalamic medial preoptic area (MPOA), while the VNO pathway also reaches the ventromedial hypothalamus (VMH). Only main connections are shown, see text for more. Taken from reference [72].

sending out their dendrites to a single glomerulus. The mapping of the VNO sensory neurons occurs in a more complex fashion. VNO neurons with identical receptor types - one of which is specific to MHC detection - project to distinct glomeruli in the AOB [25], and AOB mitral cell dendrites reach multiple glomeruli [251], potentially allowing them to receive input from different sensory neuron receptor types. It has been argued that the topographical distribution of AOB glomeruli displays some clustering corresponding to receptor types, but that glomeruli innervated by other types of VNO sensory neurons are intermingled in between [270], allowing for integration of signals. The possibility of AOB glomeruli being behaviourally specific has also been raised [71]. In an electrophysiological examination of the

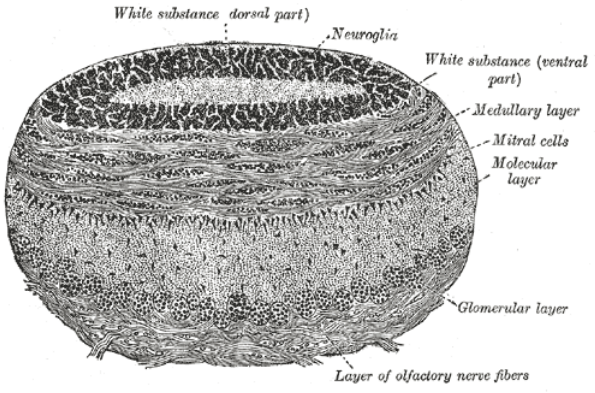

Figure 2.3: Glomeruli are structures in the olfactory bulb where axons of chemosensory neurons synapse with the dendrites of mitral cells. Surrounding glial cells give glomeruli their distinctive spherical shape. Besides the chemosensory-mitral synapses, glomeruli contain intrinsic neurons, allowing for local information processing. In the MOB, identical chemosensory receptor types converge on the same glomerulus, with neighbouring glomeruli forming a chemotopic map. In the AOB, glomeruli are biased to a receptor type, but have other inputs in between. Also, while MOB glomeruli have dedicated downstream mitral cells, the dendrites of AOB mitral cells reach multiple glomeruli. In both the AOB and MOB, mitral cells are inhibited by periglomerular and granular interneurons. Figure taken from [96].

AOB in mice, the specific receptive fields of its mitral cells became clear: some cells displayed a preference for strain, others for sex but many were sensitive to particular combinations of strain and sex [170]. Pending further research into the processing within the AOB, these findings have led to the proposal that it differs from the MOB by its amount of early processing [72]. While relatively straightfoward connections send chemosensory information through the MOB to downstream regions for higher-level cognitive processing, the AOB lends itself to combination of chemical cues, allowing it to integrate or discriminate compounds in a blend.

In conclusion, the two main chemosensory systems are not completely distinct in their ability to detect chemical cues with relevance to social behaviour, but differ in the type of neural processing that follows. The MOE relays detected chemicals to higher cortical areas, while compounds sensed by VNO neurons activate exclusive limbic areas, possibly after early processing, Both pathways lead to the hypothalamus, allowing them to influence behaviour through hormonal state changes. Other convergent loci of the pathways exist however, potentially allowing for their integration [209].

2.3 The neural systems of social recognition

In the early 1990s, it was shown that injection of oxytocin into the rat medial preoptic area increased its social recognition capabilities [206]. In female rats, recognition of juveniles in a social discrimination task was unaffected by intracerebroventricular administration of oxytocin, but was impaired after administration of an oxytocin receptor antagonist [75], suggesting endogenous oxytocin was necessary for social recognition, but improved it only up to a fixed level. In as far as animals use the VNO and MOE pathways to gather social information, any sociocognitive processes happen downstream of them. Research of the last decades has shed light on the contributions of both pathways to social recognition.

2.3.1 The MOE-MOB

The contribution of the MOE pathway to social recognition was investigated by destroying the MOE of spiny mice (*Acomys cahirinus*) using nasal irrigation with a $ZnSO_4$ solution, thus rendering them temporarily anosmic[1] (without smell). This was followed by a social discrimination task: while spiny mice prefer conspecifics whose odour is familiar to them, this preference is lost upon anosmia [173]. A similar effect was shown for rat recognition of juvenile conspecifics [207].

In sheep[2] (*Ovis aries*), severing the vomeronasal

[1]Irrigating the nasal cavity with aqueous $ZnSO_4$ causes complete (but temporary) deafferentiation of the mouse MOB, while keeping VNO-AOB axons intact. Suprisingly, the method is not fully effective in all species, which is why general olfactory testing of $ZnSO_4$-treated animals is recommendable. For a methodological study into the effect of intranasal $ZnSO_4$ on mice, see reference [175].

[2]For sheep, recognition of the young is especially important, as they live in herds and are seasonal breeders. In

nerve does not interfere with maternal selectivity, but irrigating the nose with $ZnSO_4$ solution does [161], albeit only in primiparous ewes. A similar study into the maternal behaviour of primiparous Wistar rats revealed no significant effects for either intervention, but did not take non-specific parental care into account: not all rodents take exclusive care for their own offspring [241], which abolishes dependence on individual recognition. Interestingly, while common laboratory animals such as the Wistar rat indeed display adoptive behaviour toward alien pups, they do prefer their own young if given the choice, a preference that has long been known to depend on an intact MOB [22]. The problem with this finding, however, is that it is based on olfactory bulbectomy, which is known to cause non-sensory effects such as hyperactivity [159], that could also explain the shift in maternal behaviour.

Seen from the other side of the mother-offspring relationship, neonate rodents have a remarkable capacity of olfactory learning during their first 10 postnatal days. This learning is facilitated by stroking, which likely reflects the grooming pups normally receive from their mothers. Studies have shown this tactile memory enhancement depends on noradrenergic innervation of the MOB from the locus coeruleus [211]. This is stimulated by acetylcholine administration in the latter region up to postnatal day 10, at which time increases in inhibitory α2-noradrenergic autoreceptors in locus coeruleus decrease innervation of the MOB [186].

In ewes, comparable mechanisms occur in the MOB. While mitral cells in the region are normally only responsive to food odour, after a birth increased numbers are responsive to lamb odour, of which some are selective to the subject's own lamb [142]. As in rodent pups, such olfactory learning is dependent on noradrenergic input [160]. As described in section 1.3.3, the importance of noradrenaline lies in its ability to disinhibit mitral cells, leading to selective odour memory. The glutamate release from mitral cells is enhanced through nitric oxide signalling from their respective granular cells, ensuring proper potentiation. Indeed, blocking nitric oxide synthase diminishes both the glutamate increase and the selectivity of mitral cells [140]. It has been shown the learning coincides not only with glutamate, but also with GABA release in the MOB. Accordingly, the competitive $GABA_A$ receptor antagonist bicuculline interferes with lamb recognition if administered to the region [137]. This is likely because bicuculline releases mitral cells from granular control non-specifically, occluding the selectively potentiated mitral synapses.

Also similar to the rat pups, somatosensory activation aids establishment of olfactory memory in (hormone-primed) ewes, as shown through genital stimulation studies [205]. Such stimulation is thought to increase noradrenaline projection from locus coeruleus to the MOB and is coincident upon parturition under natural circumstances. Oxytocin, upregulated at birth by definition, can serve a similar function as genital stimulation, also by increasing noradrenaline levels in the MOB [139]. In fact, in estrus and proestrus rats genital stimulation has been shown to induce MOB oxytocin release and improve performance on a juvenile social recognition test [154]. Crucially, this study showed oxytocin receptor antagonists in the MOB eliminate the effect of vaginocervical stimulation. The results were shown to depend on intact vagus and pelvic nerves and noradrenaline release in the MOB, in line with earlier research on male rats [64].

Neurophysiologist Matthieu Keller decided to investigate what regions downstream of the sheep MOB were activated. Using immunohistochemistry, he investigated expression of the protein c-Fos, an indirect marker for neural activity. He found increased levels of c-Fos in the entorhinal cortex, piriform cortex, orbitofrontal cortex, medial frontal cortex and cortical amygdaloid nucleus in ewes exposed to their lambs' odours [133]. This expression pattern was not seen in anosmic ewes, so it can be concluded that it resulted from chemical detection by the MOE. Whether these effects were specific to social processing cannot be said, as no control with non-social odours was performed. In a similar vein, a study on social recognition in mice revealed c-Fos in piriform cortex, cortical amygdala, and the lateral septum, but also only offered socially relevant input [78]. The lack of expression found in entorhinal-perirhinal cortex in these mice is remarkable, as this region has been associated with specific recognition and formation of short-term memory olfactory memory in a num-

other words, if an ewe has lambs, others in the herd do as well. Add to this that sheep are *precocial*, meaning the lambs walk around and suckle their mothers after a few hours, and it becomes clear that sheep that are incapable of recognizing their young are at risk of weaning those of others.

ber of species [194], [13], [201] (see section 2.3.4 for more information).

The c-Fos found in orbitofrontal cortex probably reflects outcome or valence processing of the olfactory cue[3]. The activation of medial frontal cortex was suggested by the authors to reflect long-term memory storage [133]. The cortical amygdaloid nucleus, which is reciprocally connected with the medial olfactory bulb, likely integrates olfactory cues.

2.3.2 The VNO-AOB

One would expect the VNO pathway to play a great role in social and individual recognition since it is a detector of semiochemicals. What better role for these signalling molecules to play than informing on identity? Indeed, social recognition as in gender, status and other "state" discrimination, strongly depends on an intact VNO. Male mice with a homozygous *trp2* deficiency (a gene coding an ion channel expressed solely in VNO) fail to discriminate between genders and will attempt to copulate with male and female conspecifics alike, instead of approaching males with aggression and only attempting to mate with females [162]. Also, the VNO shows receptors that are optimal for MHC discrimination [157].

The case for true individual recognition is much weaker, however, with the VNO playing a replaceable role at best. This is illustrated by a study from the 1990s in which vomerectomized rats were put in a social recognition paradigm, using juvenile target conspecifics [28]. Figure 2.4 shows the results of this task. As can be seen, VNO ablation leads to a short-term deficit in task performance, which later returns to normal, suggesting other cue processing (possibly the MOE system described above) takes over. Interestingly, while vasopressin antagonist administration to the bed nucleus of the stria terminalis interferes with social recognition in intact rats, it does not influence the task performance of vomerectomized rats, which is in line with the AOB pathway outlined in section 2.2: the AOB, but not the MOB, is strongly connected to the bed nucleus of the stria terminalis.

[3]The orbitofrontal cortex appears to calculate *expected reward* from its multimodal input and has therefore been argued to relect a neural currency in decision-making. For a recent mini-review on orbitofrontal function, see reference [233].

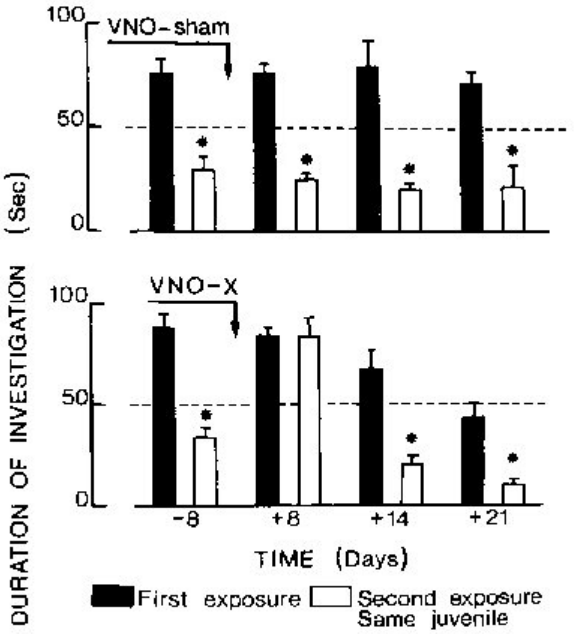

Figure 2.4: Social recognition as measured by investigation time in a series of trials. The top figure shows rats with sham vomerectomy stayed at their pre-surgery investigation time, while the lower one shows vomerectomized rats have a dip in their recognition skills after surgery, as shown by lack of investigation time decrease. This deficit goes away with time. Taken from reference [28].

The MOE pathway cannot always take over individual recognition roles. Briefly alluded to in section 2.2, the Bruce effect is the phenomenon whereby a female mouse returns to estrus if she encounters a novel male conspecific before embryo implantation has occurred [33]. This effect has been shown to follow VNO detection of MHC class I peptides. The MHC is a protein that is involved in separating self from other at the level of the cell, and is used accordingly by other animals at the behavioural level, which can presumably categorize different MHC proteins due to the very specific subsets of peptides to which it is bound.

If a female is exposed to MHC molecules originating from males with which she has not mated, the Bruce effect can occur [157] [256]. The required memory of the previous mate has been suggested to reside in the AOB: MHC peptides (and possibly other semiochemicals) originating from this previ-

ous mate fail to lead to VNO-mediated excitation of corticomedial amygdala and downstream hypothalamic regions, which interferes with the pregnancy block. In contrast, semiochemicals originating from other male mice do lead to activity in the downstream areas, ultimately preventing implantation of developing embryos (for review, see [106]).

How does such learning in the VNO-AOB pathway take place? Administration of lidocaine, a use-dependent Na^+ channel blocker, to the corticomedial amygdala did not interfere with memory formation for the Bruce effect, but injecting it into the AOB did [129]. As mentioned, mitral cells in the AOB receive input from multiple glomeruli and are under inhibitory control of granular interneurons. These granular cells GABAergically provide the mitral cells with negative feedback, but this control is diminished if noradrenaline from locus coeruleus is released in the AOB during mating. The noradrenaline inhibits the granular cells, causing the mitral cells to fire more. Those mitral cells that are activated by incoming VNO input strengthen their synapses to the granular cells.

Once the effect of noradrenaline subsides, the mitral cells corresponding to the VNO input from the earlier mate receive stronger negative feedback from their granular partners. If the female investigates this male, his semiochemicals are filtered out at the level of the mitral cells, so that downstream regions are unaffected. Males with different chemical signatures, however, will excite different mitral cells and are more likely to activate the downstream hypothalamic nuclei and associated Bruce effect. Knock-out $Oxt^{-/-}$ mice fail to display the Bruce effect like wildtype do [273]. Instead, they abort even if reexposed to their previous mate. This can be understood by assuming oxytocin has a similar role in the AOB as it does in the MOB, enhancing local noradrenaline release.

The fact that the VNO mediates the Bruce effect fits not only with the direct limbic connection of the VNO-AOB pathway, but also with the type of molecules the VNO is particularly suited for. The Bruce effect is initiated on close inspection, giving the female a chance to avoid it if more distal cues of the novel conspecific (e.g. odourants or visual appearance) do not signal a higher fitness.

2.3.3 Medial nucleus of the amygdala

The medial nucleus of the amygdala is a convergence point for the MOE and VNO pathway that is sensitive to hormonal state due to a multitude of steroid receptors. This makes it an interesting target for research on social recognition and it has indeed been shown to play a vital role.

The strongest findings are the result of the development of a mouse oxytocin knock-out model. Male $Oxt^{-/-}$ mice performed as well as $Oxt^{+/+}$ mice on tasks involving olfactory recognition of non-social cues, but not on a habituation-dishabituation task using ovariectomized female targets, even though the females behaved similarly towards both mutant and wildtype mice [79]. Identical results were obtained using intact male and female target mice. Spatial memory of the $Oxt^{-/-}$ mice, as measured per Morris water maze and Y-maze tasks, was considered to be intact.

Site-specific oxytocin injections in the medial amygdala of $Oxt^{-/-}$ mice prior to target exposure rescued their social memory deficit, while similar administration of an oxytocin receptor antagonist in wildtype mice led to them mimicking the mutant behaviour [78]. These effects were not seen after injection into the MOB, despite the aforementioned finding in sheep. In contrast to wildtype male mice, male $Oxt^{-/-}$ mice also revealed a lack of neural activity in medial preoptic area, bed nucleus of the stria terminalis and medial amygdala as measured per c-Fos immunoreactivity after a 90 minute exposure to a novel female. The opposite genotypic effect was seen in somatosensory cortex, CA1, CA3 and dentate gyrus of hippocampus.

Another study showed the role of the medial amygdala extends to female mice, as interfering with oxytocin receptor mRNA translation in that area, through the use of anti-sense oligodeoxynucleotides, limited their performance in a habituation-dishabituation task involving other female mice [41]. Infusion of lidocaine in either the cortical or medial amygdala of sheep resulted in a deficit in offspring selectivity [135], that was argued to reflect poor social learning, instead of impaired social memory retrieval or odour perception.

The role of the medial amygdala is interesting, as it is a convergence point of the VNO and MOE pathways, both through direct connections (see sec-

tion 2.2) and intra-amygdalar projections [176]. In fact, research on Syrian hamsters (*Mesocricetus auratus*) has suggested that the medial amygdala serves as the point where vomeronasal input becomes associated with olfactory input [179]. Such linkages could cause olfactory input to drive responses initially brought about by vomeronasal input.

2.3.4 Hippocampal areas

Through perirhinal and entorhinal cortex, MOB projects to hippocampus. The perirhinal and entrorhinal cortex, but not the hippocampus were shown to be vital to sex-related individual recognition in male Syrian hamsters [201]. Both the entorhinal cortex and the ventral subiculum proved to be responsive to individual-specific odour cues [200]. Infusion of voltage-gated Na^+ channel blocker tetracaine in the region inhibits lamb recognition in ewes, but only in the short term (4 hours after memory formation). In the longer run, the MOB, but not the entorhinal cortex remained vital [227]. Rats also showed an early dependence on entorhinal cortex in olfactory learning [131]. Apparently, the entorhinal cortex is important in memory formation, while ultimate consolidation takes place in other regions, such as the MOB.

The role of the hippocampus proper in social recognition is controversial. Chemical lesioning of the hippocampus in mice impaired performance on a juvenile social recognition test [148], but a comparable study in rats found only a slight increase in investigation time [12], independent of housing[4] [248]. Neurobiologists Howard Eichenbaum and Aras Petrulis have suggested social memory becomes hippocampus-dependent only after an extended period [201], but proper paradigms to investigate such long-term encoding in rodents are lacking.

2.3.5 Hypothalamus

Hypothalamus, the ultimate projection of the VNO and MOE pathways, is systematically found to be involved in social recognition. The expected role of this region is however more related to the state and behavioural changes that follow recognition than to cue processing itself. Indeed, immunohistochemistry on anosmic ewes that could not form olfactory memories but did show maternal behaviour (e.g. through visual processing) showed equal c-Fos levels in medial preoptic area and the paraventricular and supraoptic nuclei as did ewes without olfactory deficits [133], while interfering with activity in the paraventricular nucleus impaired maternal behaviour [51].

Given the role of hypothalamus in expressing oxytocin endogenously, however, uncoupling the region from social recognition is too simplistic. It is more probable that hypothalamus plays a dual role: the endocrine changes it mediates in response to social information - part of which is oxytocin release - may both cause prosocial behaviour as well as facilitate upstream processing of social cues.

2.3.6 Other sensory modalities

Besides chemical cues, input in other modalities can also inform animals on the properties of others. Visual, auditory and tactile input are all - theoretically at least - capable of signalling information relevant to individual recognition. The obvious example are primates, which are much more dependent on visual than chemical cues, but even the intensely sniffing rodent might be using non-chemical input to guide its social interactions. The use of visual information is especially important in sheep [137] which, as shown in section 2.3.1, have contributed as model organisms to the knowledge on social recognition. Put in a Y-maze, where they were shown a facial picture along the arm in which they could find the depicted individual, some breeds of sheep were shown to prefer sheep faces to human faces. In an auditory version of this social discrimination task, sheep preferred vocalizations from their brethren over human vocalizations [138]. The influence of chemical cues through the maze was not accounted for directly, but as less clear preferences of the sheep existed when using pictures displayed in profile, it is likely that they were using the visual cues.

How do the other modalities tie in with the olfactory-driven neural systems described in section 2.2? It has become clear that face-selective neurons exist within the inferotemporal (IT) cortex [137], [100] and superior temporal sulcus [262], but how this high-level feature processing is achieved

[4]Socially housed animals can show longer retention of social memory in the paradigms used.

remains elusive. In humans, a dedicated face-processing area, dubbed the *fusiform face area*[5] has even been suggested [130]. An obvious place for such neurons to project to would be the amygdala, as this is also the hub that receives input from both the VNO and MOE pathways. Indeed, both IT cortex and superior temporal regions project to lateral and basal nuclei of the amygdala, which are sparsely connected to central amygdala [155]. Connections to medial amygdala have not been identified. The visual pathway therefore is more likely to share a common node with the MOE than with the VNO pathway, although the latter cannot be ruled out as long as the internal circuitry of the amygdala has not been elucidated. Auditory and somatosensory pathways also converge on the lateral and basal nuclei after initial processing, and so are likely to take a similar route [176].

It should be noted that backprojections exist from the basal nucleus of the amygdala to virtually all parts of the ventral visual processing stream, including primary visual cortex. This means that some of the feature extraction - such as emotional salience - can originate from the amygdala, either directly or through its interactions with hippocampal regions. Such backprojections were not found in the MOE pathway, but the connection from the MOB to the entorhinal cortex may serve a comparable purpose, although probably not through interaction with hippocampus [201]. Also, subcortical visual pathways have been suggested to exist independently of cortical processing [193], namely through the superior colliculus and pulvinar nucleus of the dorsal thalamus. This route could explain fast modulation of visual processing to aid feature extraction, via connections to the lateral nucleus of the amygdala.

2.4 Summary

Animal studies on social recognition have elucidated a distributed network involving hypothalamus, amygdala and cortical regions. In the cases that were discussed, recognition was based on chemosensory input, which follows a cortical route (the MOE-MOB pathway) or a mostly subcortical route (the VNO-AOB pathway). Convergence in medial amygdala may underlie some association of the two information flows, but most recognition was shown to rely on the MOE-MOB pathway, in which case memories are likely stored through interactions of entorhinal cortex and MOB. Mating, grooming and related behaviours help encoding of such memories, through temporary disinhibition of sensory-cortical synapses in the MOB. This disinhibition is at least partly mediated by oxytocin.

At the same time, oxytocin enhances social recognition by action at the medial nucleus of the amygdala. The mechanisms of this enhancement are unknown, but the effects are dramatic, as illustrated using oxytocin knock-out mice, which essentially suffer social-specific amnesia. The role of the more traditional substrate of memory, hippocampus, remains unclear. It has been proposed the region is involved in long-term encoding of social memories, but this has not yet been demonstrated.

Oxytocin is intertwined through all the pathways in an upstream type of way. Released from hypothalamus, the neural structure that forms the goal of social processing pathways, it interacts with the social recognition function of medial amygdala, the social processing in the olfactory bulb and modulates hippocampus, perirhinal cortex and entorhinal cortex in at least some species. It is also picked up by hypothalamus itself, increasing oxytocin release through a positive feedback loop.

In species less dependent on chemosensory input, information from other modalities may also act on amygdala, through reciprocal connections with the basal and lateral subnuclei. It is currently not known whether oxytocin in visually oriented species, such as primates, acts on visual cortical areas to enhance memory storage. However, it has been shown that oxytocin modulates activity in amygdala and parts of the striatum in such species. As chapter 3 will show, this latter finding may have great behavioural consequences.

[5] The region might not be face-selective. Instead, it may be the locus of visual feature integration, with face-selectivity deriving from the particular expertise humans have for processing faces.

Chapter 3

Pair-bonding and parent-offspring bonding

As chapter 2 already showed, the recognition of a conspecific can be intimately tied to behaviour, such as maternal grooming or the Bruce effect. Perhaps the most dramatic case of individual-oriented behaviour is bonding, whether it occurs between a parent and its offspring or between sexual partners. Bonding does not only require recognition, but also a valuation of the other that makes it worth any investment of resources. In human relations, such bonding is called love, but animal research likes to avoid this term and the subjective state it implies. Instead, animals display *social bonding* which is called *pair-bonding* if it is between animals that stay together after mating and *parent-offspring bonding* if it refers to the selective care-taking of offspring.

As will become clear in this chapter, oxytocin and vasopressin play a large role in both types of bonding. The two peptides are not only important in the processes of recognition discussed in the previous chapter (*receptive sociality*) but also in modulation of the social behaviour that follows on recognition (*expressive sociality*). Bonding processes provide a clear demonstration of such behaviour.

Figure 3.1: The prairie vole (*Microtus ochrogaster*) often leads a monogamous family life and displays a preference for side-by-side sitting with conspecifics.

3.1 Measuring bonds

To investigate the bonds between animals, a special type of social discrimination task was developed, in which the preference for a conspecific is measured. In a typical example of such a social preference task, the subject animal is placed in a three-chambered environment in which it has access to two conspecifics, that are kept in separate cages. The proportion of time it allots to being with these conspecifics (or by itself) provides a measure of bonding. The social preference task in this way can be seen as a hybrid of a social discrimination and a classic place preference task.

For pair-bonding, a number of special species have risen to the status of model organism. Voles are small, easily bred rodents that display a wide variety of affiliative behaviours. The prairie vole (*Microtus ochrogaster*, see figure 3.1) and the pine vole (*Microtus pinetorum*) are both monogamous

rodents, while the closely related montane vole (*Microtus montanus*) and meadow vole (*Microtus pennsylvanicus*) are promiscuous[1]. The prairie and pine voles live with extended families, while the montane and meadow vole are solitary creatures[2].

Because monogamous and promiscuous vole species differ in so little but their sociality, they have offered an excellent window through which to investigate the biological roots of pair-bonding. A number of measures have been devised to assess the degree of pair-bonding. In the social preference test, monogamous voles express pair-bonding by lying side-by-side with their preferred conspecific. Even they, however, only robustly show pair-bonding in laboratory settings if 24 hours of cohabitation with mating is allowed. Shorter periods are usually only employed if some manipulation that might increase pair-bonding is under investigation. Other measures quantify biparental care or selective aggression against intruders.

Parent-offspring bonding can be studied in a wide range of species and is operationally defined in two main ways. The first way is similar to that used in pair-bonding: a preference test between pups or, seen from the other side of the divide, between one of the parents and a matched control. Pups have been found to be rewarding to maternal rats [171], and any bias towards the own pup could be seen as a measure of bonding. The second way is a quantification and qualification of the care the parent provides. This is a rather indirect measure of bonding and is susceptible to confounds that impoverish care non-specifically. However, it has proven to be useful. The form of the care is heavily dependent on the behaviour of the species under investigation, but in mammals at least includes providing warmth and, in the case of maternal care, access to the nipple.

It should be emphasized that such measures of parent-offspring bonding are always weaker than those obtained in pair-bonding. In the partner preference test, a male prairie vole could in principle be tempted to mate or seek contact with a novel conspecific. Such mating would not be costly to his evolutionary fitness. However, if given the choice between caring for her own offspring or that of another, there is a real cost involved in spending resources on the wrong neonate. This is why measures of preference or care are hard to disentangle from clearly selfish motives, that solely rely on recognition. In the same way, if a mother rat provides one pup in her litter with especially good care, this may not reflect bonding, but instead point to mere recognition of the particular pup being well-adapted to ecological circumstances.

It is important to emphasize that all operationalized measures of pair-bonding and parent-offspring bonding are proxies for the bond itself, which is not measurable. Indeed, usage of the term may be somewhat of an anthropomorphical misnomer, reflecting the subjective state felt by parentally or romantically bound humans.

3.2 Pair-bonding

The monogamous nature of the male prairie vole becomes apparent after it has mated, when it shows an increase in aggression towards other males and a preference for its mate, a phenomenon called *mate guarding*. It has been known for some time that administering arginine vasopressin can induce these behaviours, while a V1a receptor antagonist blocks them [278]. Oxytocin does not have this effect in male prairie voles, but does enhance social preference in female prairie voles [50]. In contrast, montane or meadow voles do not display such prosocial behaviour towards mates, regardless of neuropeptide administration.

Could the nonapeptide effects be caused by improved recognition? That is, could the monogamous voles have a capacity for recognition that the promiscuous voles lack, and that depends on oxytocin or vasopressin? Or do the effects of oxytocin and vasopressin rely on neural substrates beyond the recognition system discussed in chapter 2? Studies investigating the oxytocin and vasopressin receptor distribution in the two types of voles suggest the latter is the case (see figure 3.2).

Prairie voles show relatively high amounts of V1a

[1] In the literature, *M. montanus* and *M. pennsylvanicus* are often called polygamous. This is not proper usage of the term, as it implies multiple bonds, while the voles are unlikely to have any at all. The voles might instead be called *agamic*, but this thesis will use the more colloquial term *promiscuous* to describe their aselective sexuality.

[2] These are generalizations. It has been estimated about 40% of the male prairie voles prefer a wandering mating strategy above pair-bonding. Still, with the amount of promiscuous voles that bond to their mate being negligible, this is enough to consider them a monogamous species.

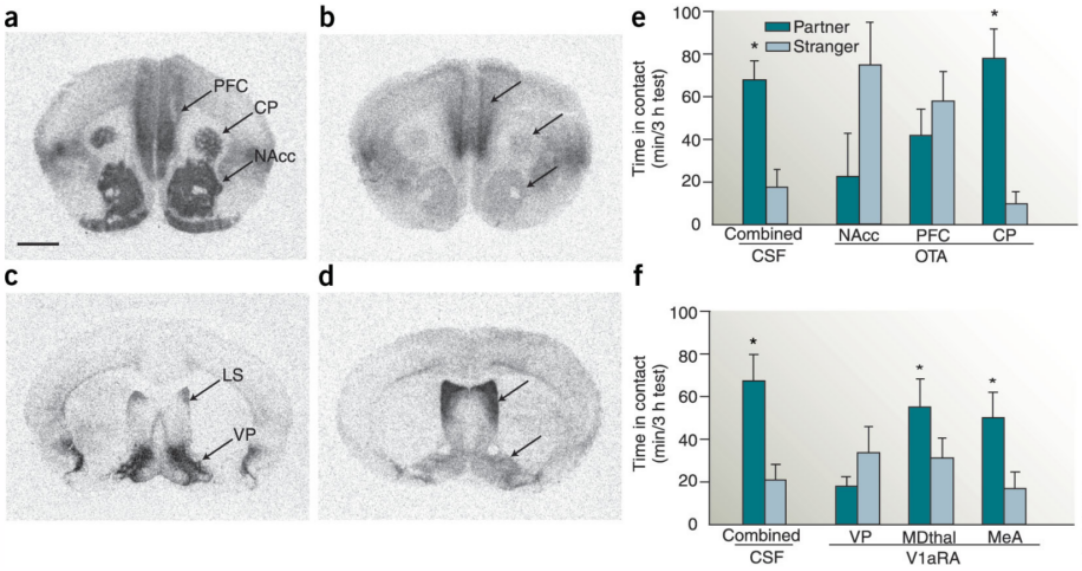

Figure 3.2: Pair-bonding associated receptor distribution. Monogamous prairie voles (a) have increased oxytocin receptor binding in the nucleus accumbens (NAcc) and caudate putamen (CP) compared to promiscuous montane voles (b), but both species have oxytocin receptors in the prefrontal cortex (PFC). Male prairie voles (c) show higher vasopressin receptor V1a binding potential in the ventral pallidum (VP) than montane voles (d). (e) A selective oxytocin receptor antagonist (OTA) infused bilaterally into the NAcc or PFC,(but not CP) blocks partner-preference formation in female prairie voles. (f) Infusion of a selective V1aR antagonist (V1aRA) into the VP, but not into the mediodorsal thalamus (MDthal) or medial amygdala (MeA), prevents mating-induced partner preference formation in male prairie voles. Scale bar, 1 mm. Taken from reference [280].

receptors in ventral pallidum, medial amygdala, cingulate cortex and mediodorsal thalamus [123], and oxytocin receptors in nucleus accumbens and caudate putamen [124], as compared to the montane and meadow voles. The regions that can be most clearly separated from the social recognition system are the nucleus accumbens and the ventral pallidum, both of which are commonly associated with reward-related learning and behaviour. This hinted at a plausible substrate for the social preference. Through activation of the "reward circuitry", vasopressin and oxytocin might be reinforcing contact with a specific individual.

Led by this hypothesis, the roles of the ventral pallidum and nucleus accumbens in pair-bonding were investigated. The contribution of the former region to monogamy in male prairie voles was shown through injection of V1a receptor antagonists, which blocked pair-bonding [165]. Infusion into medial amygdala or mediodorsal thalamus had no such effect. Overexpressing V1a receptors in the ventral forebrain using viral-vector-mediated gene transfer facilitated partner preference behaviour in male rats after mere overnight cohabitation [204], while overexpression of oxytocin receptors in the nucleus accumbens did the same for female prairie voles [222]. To understand these effects, a closer look at the role of the reward system in pair-bonding is warranted.

3.2.1 Love as a commodity: the dopamine system and pair-bonding

The mesolimbic dopamine "reward" system predates the rise of monogamy. As a system that reinforces approach behaviour - for example, to nutrient sources - it is perhaps not surprising social bonding co-opted the system. A central role in the reward system is played by the nucleus accum-

bens. The nucleus accumbens consists of a core

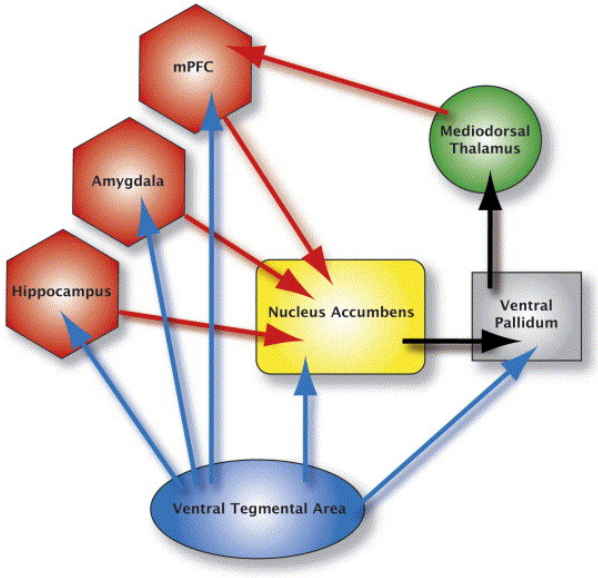

Figure 3.3: Simplified scheme of the mesolimbic reward system and its associated regions. The blue connections are dopaminergic, while the red ones are glutamatergic and the black ones GABAergic. Taken from reference [203].

and a shell, of which the differentiated functions have been elucidated mostly through addiction research. The (medial) shell of the nucleus accumbens is considered to promote motivation for reward, while the core is important for consolidation of stimulus-outcome relations and action selection [122]. To put this in terms of core and shell connectivity: the shell has strong reciprocal connections with hypothalamus and so is ideally suited to receive and modulate physiological state information. The core, in contrast, mostly receives amygdalar and hippocampal inputs, which associates it more with memory [172].

The nucleus accumbens is under both direct and indirect control of the ventral tegmental area (see figure 3.3). Addiction research has shown that glutamate receptor blocking in the ventral tegmental area leads to positive reinforcement through dopamine release in the nucleus accumbens and this turned out to be relevant to pair bonding as well: administering NBQX[3] to the ventral tegmental area leads to partner preference formation in male prairie voles without mating [48], probably by acting on non-dopaminergic inhibitory cells, which leads to increased dopamine release in nucleus accumbens. The indirect control of the ventral tegmental area is mediated through the medial prefrontal cortex, where dopamine modulates glutamatergic projection to nucleus accumbens, ultimately affecting dopamine release.

The ventral pallidum is also considered to be an important node in the reward network. Besides the input shown in figure 3.3, it receives gustatory information. Its projection to the medial thalamus serves as a relay towards prefrontal and insular regions. The ventral pallidum is generally associated with the hedonic properties of reward processing [245]

Besides through the effect of NBQX administration to the ventral tegmental area, the role of nucleus accumbens in pair-bonding has also been illustrated by direct pharmacological action. Infusion of haloperidol (a non-specific dopamine receptor antagonist) in nucleus accumbens impaired mating-induced pair-bonding in male prairie voles. In contrast, apomorphine (a non-specific dopamine receptor agonist) induced partner preference without mating being required [5]. At the subnucleic level, the agonist effect was shown to occur if applied to the part of the shell rostral to the corpus callosum genu (the 'rostral shell'). Application to the rostral core or caudal shell was not effective [6]. The difference between the rostral and caudal shell has been argued to lie in positively and negatively motivated behaviour, respectively [214]. As mating induces extracellular dopaminergic transmission to nucleus accumbens, it is plausible that the apomorphine action mimicked a natural process [5].

3.2.2 The D_2 receptor in nucleus accumbens underlies pair-bonding formation

So how does dopamine induce pair-bonding through the nucleus accumbens? Dopamine acts on a family of G-protein coupled receptors, which can be categorized as D_1-like and D_2-like. Activation of D_1-like receptors - which are the D_1 and D_5 subtypes - leads to a biochemical cascade in which

[3]NBQX is a sulfonamide that blocks α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors.

cAMP concentrations are increased through activation of adenylate cyclases, in order to activate protein kinase A. In contrast, the D_2, D_3 and D_4 receptors (the D_2-like receptors) inhibit adenylate cyclases and lead to lowered cAMP concentrations, causing a relatively lower activity of protein kinase A.

The D_2-like receptors are vital to pair-bond formation. The rostral shell effects mentioned in the previous section were obtained using ligands selective for D_2-like receptors [6]. Administration of Rp-cAMPS, a ligand that blocks the protein kinase A activation sites and thereby mimicks D_2 effects, also induces pair-bonding without mating in male prairie voles. Indeed, D_1-like mimicking through the use of Sp-cAMPS, a ligand that stabilizes the active state of protein kinase A, *inhibits* pair-bonding effects, pointing to an "anti-bonding" role for the D_1-like receptor [7]. Infusion of D_1 agonist SKF 38393 into nucleus accumbens diminishes the ability of quinpirole-induced D_2 activation to initiate pair-bonding and it even blocks mating-induced bonding in male prairie voles [6]. In female prairie voles, the dependence of pair-bonding on D_2-like receptors is similar, but inhibition of partner preference formation by D_1-like activation has not been shown [272].

Once mating-induced pair-bonding is initiated, D_1-like receptor binding potential in nucleus accumbens rostral core and shell increases in male prairie voles [6]. This increase was found only after two weeks, meaning it does not directly correspond with partner preference formation (which occurs within 24 hours), but instead reflects longer term neural reorganization. The most immediate use of increased D_1-like binding potential is probably that it interferes with the formation of new bonds, thus keeping monogamy in place. It has been shown to also underlie aspects of mate guarding: in male pair-bonded prairie voles, administration of the D_1-like antagonist SCH 23390 reduced their selective aggression, while the D_2-like antagonist eticlopride had no such effect [6]. Interestingly, SCH 23390 administration to the nucleus accumbens of male meadow voles significantly increased their affiliative behaviour, even though it did not lead to partner preference formation. This suggests some aspects of social behaviour are mediated by nucleus accumbens dopamine even in promiscuous vole species.

Do the changes in D_1-like binding potential also affect other reward and motivation related behaviour? Drugs of abuse are known to act on dopamine receptors in the nucleus accumbens and a recent study indeed showed amphetamine treatment attenuates the pair-bonding potential of prairie voles, through activation of D1-like, and not D_2-like receptors, an effect that could be counteracted by SCH 23390 administration [167]. mRNA labeling even pointed to upregulation of D_1-like receptors as a consequence of amphetamine use. Amphetamine can hardly be called a natural incentive, however, and it may be that natural reinforcers do not share dopaminergic synapses with the pair-bonding system.

3.2.3 Dopamine-oxytocin interactions in nucleus accumbens

While accumbar D_2-like receptor activation facilitates pair-bonding in prairie voles, co-infusing quinpirole - a D_2 agonist -with an oxytocin receptor blocker in female prairie vole nucleus accumbens abolishes this effect. The reverse also holds true: oxytocin administered into the nucleus accumbens facilitates pair-bonding, except if a D_2-like (but not D_1-like) antagonist is co-administered [166]. These findings point to a combined role of oxytocin receptors and D_2-like receptors in eliciting pair-bonding, which fits with the aforementioned finding that monogamous voles show oxytocin receptor expression in nucleus accumbens, while promiscuous voles do not [124].

The interaction of dopamine and oxytocin makes sense given the fact that both compounds are released upon mating [221]. It can also explain why pair-bonding does not occur in many species of rodents, even though they do release dopamine with mating. Also, the role of oxytocin in social recognition (see chapter 2) makes it ideally suited to couple individual recognition with reinforcement. The manner in which dopamine and oxytocin do so is less easy to intuit: if the D_2-like agonist and oxytocin act on a "shared" neuron, their biochemical effects are likely similar and additive (e.g., oxytocin receptor activation could inhibit protein kinase A through a similar mechanism as the D_2-like receptors) but if their action is segregated at the cellular level this does not need to be the case. As of now, the details of dopamine-oxytocin interactions in nu-

cleus accumbens are not known.

Taking findings from other species into account, it is known that oxytocin and dopamine release can be coupled to complex chemical feedback loops. Apomorphine, the non-selective dopamine receptor agonist mentioned earlier, was shown to downregulate oxytocin in male rat hypothalamus (at high doses) and increase its presence in hippocampus, probably through D_2-like receptors [178]. Hippocampal oxytocin then, could GABAergically inhibit glutamatergic projections from hippocampus to the nucleus accumbens, thereby decreasing dopamine release in this region [229]. At the same time, oxytocin has also been shown to *increase* dopamine levels in female rats [202]. These findings illustrate the complex molecular interplay between dopamine and oxytocin. In all likelihood, this interaction is not limited to oxytocin and dopamine, but also involves other hormones and neurotransmitters. The effects of such dynamics are hard to predict and will strongly depend on species-specific receptor expression.

3.2.4 The pair-bonding circuit

The litmus test for understanding the pair-bonding system in voles would be inducing monogamy in promiscuous voles. As mentioned, V1a receptor antagonists interfere with male prairie vole bond formation, while overexpressing the receptors in the ventral forebrain enhances it. Could the same be done in promiscuous males? To find out, the V1a receptor gene *Avpr1a* was overexpressed in the ventral pallidum of male meadow voles. The meadow voles with artificially elevated vasopressin binding showed increased pair-bonding after mating, except if pretreated with a D_2-like antagonist [164]. An attempt to induce monogamy by oxytocin receptor overexpression in female meadow vole nucleus accumbens shell did not succeed, however [222].

Why are male meadow voles easier to convert to monogamy than females? An answer might lie in the reward circuit as sketched in figure 3.3. The ventral pallidum is sometimes considered the *limbic final common pathway*[4], as it takes actions of the rest of the circuit into account, without strongly affecting them. Interfering at this level is relatively downstream of motivational processing, where regulation is weak. At the level of the nucleus accumbens, however, dopamine release is regulated by both the medial prefrontal cortex and the ventral tegmental area. Receptor binding properties in these regions vary across promiscuous and monogamous voles, so even a genetically altered nucleus accumbens is under differential regulatory control. Indeed, female prairie voles show higher oxytocin receptor binding in the medial prefrontal cortex, as well as higher D_2 -like receptor binding [242]. Female meadow voles with overexpressed oxytocin receptors in the nucleus accumbens may therefore still lack the necessary inputs to reinforce social contact. This lack of input may also stem from species-related differences in nucleus accumbens oxytocin expression during mating. While vasopressin may be released in male meadow vole ventral pallidum, oxytocinergic innervation of female meadow vole nucleus accumbens may be sparse.

The use of distinct neuropeptide systems and neural loci in male and female prairie voles likely reflects their different behavioural programmes in response to bonding. For example, in prairie voles, c-Fos-immunoreactive staining showed increased neural activity in the anterior hypothalamus in pair-bonding males which displayed mate guarding [94] and this was shown to be dependent on vasopressin [95]. Those behavioural effects are additions to the core bonding system, which is summarized in figure 3.4, showing the roles of vasopressin, oxytocin and dopamine in the pair-bonding process.

As discussed in the previous sections, mating-induced oxytocin adds to the effect of D_2-like dopamine receptors in nucleus accumbens, probably to reinforce the respective sexual contact, that is recognized through the social recognition system. Besides the reinforcing effect of striatal activation, oxytocin may also directly modulate social learning in olfactory bulb and medial amygdala, among other regions (see section 2.3). Oxytocin is also released into prefrontal cortex, where it might interact with dopamine in the same way as it does in nucleus accumbens. The higher numbers of D_2-like and oxytocin receptors in prairie vole medial prefrontal cortex may enhance glutamatergic transmission to nucleus accumbens. Nucleus accumbens is in turn connected to the ventral pallidum, which is mostly subject to vasopressin and projects to mo-

[4] This common pathway refers to an integration of hedonic and motivational stimuli, possibly reflecting *reward*. It is not the only way for the "reward system" to influence cognition and behaviour, however.

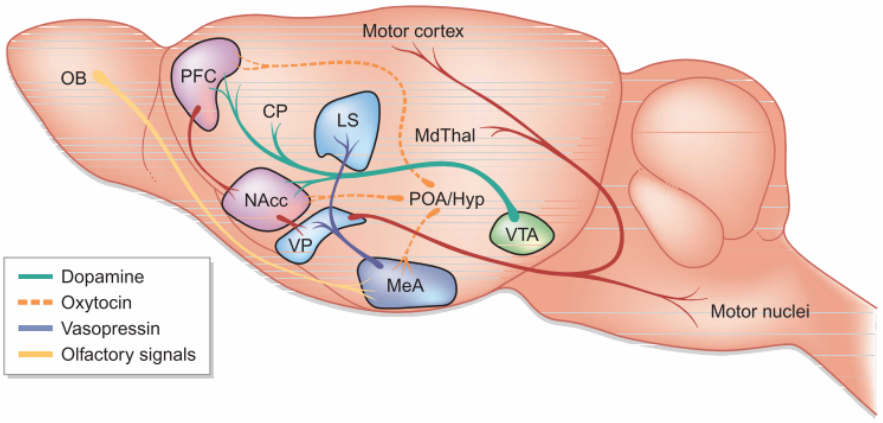

Figure 3.4: Simplified scheme of the pair-bonding system. Seen in the saggital plane, the prairie vole brain is thought to mediate pair-bonding via ventral tegmental area (VTA) activation upon mating, leading to dopaminergic projections to prefrontal cortex (PFC) and nucleus accumbens (NAcc). Mating-induced oxytocin release from preoptic area/hypothalamus (POA/Hyp) modulates olfactory learning in the olfactory bulb (OB) and medial amygdala (MeA) as described in chapter 2, while adding to the reinforcing effects of dopamine in the NAcc. Oxytocin is also released in PFC, which is connected glutamatergically to NAcc. NAcc projects to ventral pallidum (VP) which is subject to modulatory effects of vasopressin, just as the lateral septum. VP projects to motor nuclei, motor cortex and mediodorsal thalamus (MdThal). Taken from reference [280].

tor regions.

3.3 Parent-offspring bonding

In late pregnancy, estradiol levels in rats get boosted, upregulating estrogen receptors in neurons in the medial preoptic area of the hypothalamus. This, as explained in chapter 1, is one of the ways in which oxytocin is regulated. Indeed, together with prolactin, oxytocin is vital for initiation of maternal behaviour in the rat. In 1979, Cort Pedersen showed intracerebroventricular administration of oxytocin to virgin rats can even render them mother-like [198], illustrating the harmonious role the peptide plays: stimulating uterine contractions and milk ejections in the peripheral system, it promotes the accompanied behaviour through central action.

In many rodents, this is a quite sudden change. Virgins tend to be either indifferent or infanticidal if confronted with pups. Upon parturition, however, a set of behaviours including nest building, pup retrieval, licking and grooming, protecting and nursing is set into motion. This radical change is not only mediated by oxytocin - research following the Pedersen study found some strains need additional estrogen priming or even anosmia as well. However, while not sufficient, oxytocin is necessary for induction of rat maternal behaviour, as illustrated through experiments using oxytocin receptor antagonists postparturiously [265]. Interestingly, once maternal behaviour is induced in rats, oxytocin is not strictly necessary for its maintenance. Also, $Oxt^{-/-}$ mice did show maternal behaviour, but quantitatively decreased [199]. A large proportion of pup-licking and kyphotic nursing[5] remains sensitive to oxytocin, however [197].

Does oxytocin help forge mother-offspring bonds, in the same way as it bonds prairie voles? It has been shown the peptide influences the quantity of licking and grooming (LG), a rough measure that was developed to quantify rat maternal care. Oxytocin binding sites in the medial preoptic area, paraventricular nucleus, bed nucleus of the stria terminalis, lateral septum and amygdala correlate positively with LG [83], [37]. Moreover, administration

[5]From Greek κύφος, meaning humpback, kyphotic nursing is nursing in an arched-back posture.

of an oxytocin receptor antagonist was shown to remove care differences between high LG and low LG mothers. Insofar as LG is a proxy for maternal bonding, oxytocin seems to play a vital role.

Interestingly, high LG is also correlated with oxytocin and vasopressin receptor levels in the offspring. Female rats show increased oxytocin receptor binding in the central nucleus of the amygdala and the bed nucleus of the stria terminalis if they have received higher levels of maternal care, while male rats displayed increased V1a receptor binding in the amygdala in those cases [84]. In mice, a higher weaning age increases the social interactivity of offspring and also causes sexual dimorphism of oxytocin receptor expression in ventromedial hypothalamus: females show increased receptor binding, while males show decreased receptor binding in the region [47]. These adaptations may partly explain why pup-licking and kyphotic nursing behaviour by dams are inherited from caretaking mothers, even by foster pups [82].

The motivation of rat mothers is reminiscent of prairie vole bonding: maternal care is regulated by projections from the medial preoptic area to the ventral tegmental area. Extracellular dopamine in the nucleus accumbens - induced by nursing under natural circumstances - is necessary for the display of maternal behaviour, as shown by the disruptive effect of locally administered SCH 23390. As eticlopride infusion into the nucleus accumbens was without effect, the dopaminergic induction of maternal care relies on D_1-like receptors in nucleus accumbens [191]. This finding is particularly interesting given the dependence of prairie vole pair-bonding on D_2-like receptors, which have a downstream effect opposite to D_1-like receptors. In prairie voles, D_1-like receptors are upregulated *after* pair-bonding, assumedly to prevent new bonds from forming (see section 3.2.2).

The extracellular dopamine was suggested to act on the accumbens shell, where higher concentrations of the monoamine are found in high LG than in low LG mothers. This concentration difference was dissolved by GBR 12909[6] administration, as was the LG difference [38]. The action of dopamine in nucleus accumbens was shown to depend on oxytocin release in the ventral tegmental area via direct connections from the medial preoptic area [236], but no oxytocin effect in nucleus accumbens itself has been found in relation to parent-offspring bonding. Infusion of oxytocin receptor antagonist in the ventral tegmental area abolished the LG-associated dopamine levels in nucleus accumbens.

Data from studies that focus on pup preference by maternal rats show similar effects. It has been shown maternal rats prefer access to pups over access to cocaine in some conditions [174] and a set-up in which maternal rats bar-press to get access to either pups or Fruit Loops has a certain outcome: the mothers prefer the pups. This effect is gone, however, after lesioning the lateral nucleus of the amygdala, the medial preoptic area or the nucleus accumbens [156]. The necessity of the first region can be explained using the findings from chapter 2: input, either olfactory or visual, may have been impaired through the lesioning. The latter regions can be interpreted in light of the effects mentioned above: oxytocinergic innervation from the medial preoptic area is required to make pups rewarding, by releasing dopamine in nucleus accumbens shell. In this context, it should be noted that maternal responses extinguished over time after lesioning nucleus accumbens, likely reflecting weaker reinforcement due to a damaged reward system.

One difficulty with these studies is that rats are hardly selective in their maternal behaviour. In fact, the pups delivered in the bar-pressing task were foster pups, which are still highly rewarding to rats, possibly due to the rewarding effect their suckling has. From an evolutionary point of view, this makes some sense. Rats generally do not nest with others, so they do not have to fear spending resources on the offspring of others. This is not the case for all species: as mentioned before, sheep are seasonal breeders and herd animals. A selective pressure exists to have them provide more exclusive care to their offspring, which fits better with an intuitive definition of bonding.

So what is the difference between mother-offspring bonding in rats and in sheep? In contrast to postparturious rats, ewes are not immediately selective towards neonate lambs. Around parturition, they become attracted to amniotic fluid and will start licking any newborn within range. This phase is important for regulation of the onset of sheep maternal behaviour, but also drives social learning in both mother and neonate. After 30

[6] GBR 12909, or vanoxerine, is a piperazine derivative that acts as a selective dopamine reuptake inhibitor, increasing the period of synaptic availability of the monoamine.

minutes to two hours, most ewes are selective [134]. The subsequent suckling phase further aids social learning in the lambs, which recognize their mother after some 12 to 24 hours. These learning processes are mainly olfactory and are driven by the mechanisms discussed in chapter 2, although auditory and visual cues also play a role.

The maternal response of sheep is mediated by the hypothalamic nuclei [133],[135], but there have been no reports connecting their activity to the ventral tegmental area or the nucleus accumbens. Similarly, no reward system effects have been reported for the neonate. However, at the behavioural level lamb suckling is reminiscent of a reinforced behaviour [190], suggesting future research might uncover a similar system as that found in rats - or pair-bonding prairie voles.

Figure 3.5: Squirrel monkeys (*Saimiri sciureus*) quickly form social hierarchies. Oxytocin differentially affects behaviour of dominant and subordinate male squirrel monkeys. Photo courtesy of Paul Foch-Gatrell.

3.4 Primate studies

Besides rats, voles, mice and sheep, bonding and its associated behaviour has also been investigated in other species. Most relevant to translation of animal research to humans, oxytocin has been shown to play a role in primates. Intracerebroventricular infusion of oxytocin in male, pair-housed squirrel monkeys (*Saimiri sciureus*, see figure 3.5) increased aggressive and sexual behaviour in the dominant male, upon exposure to a female conspecific [276]. This effect could be blocked by a selective oxytocin receptor antagonist. Subordinate monkeys, characterized by lower plasma levels of testosterone, only showed increased grooming, approach and marking behaviour. The difference between dominant and subordinate monkey responses may be caused by a yet unfound dependence of oxytocin receptor distribution on social status, but could also be the effect of different behavioural translations of an identical oxytocin signal. For example, if oxytocin would promote approach behaviour, the contingent position in the social hierarchy could determine whether this approach would manifest itself as grooming or aggression.

In marmosets (*Callithrix penicillata*), an occassionally pair-bonding primate species, intranasal oxytocin administration was shown to increase huddling with the partner, while application of L-368,899[7] interfered with food sharing [244]. Intranasally applied oxytocin also enhanced marmoset partner preference formation. A pilot study suggested that L-368,899 decreases the interest of nulliparous female rhesus monkey (*Macaca mulatta*) in infants and sexual behaviour [29]. In two species of macaques, differing by their sociality, the oxytocin content of their cerebrospinal fluid was determined [220]. The pigtail macaque (*Macaca nemestrina*), a strongly hierarchical species not unlike the rhesus monkey, was found to exhibit lower central oxytocin levels than did the bonnet macaque (*Macaca radiata*), a more gregarious species. To provide this finding with meaning: central oxytocin levels in the related rhesus monkey (but not plasma oxytocin) were associated with affiliative behaviour [277]. In fact, in a weaker version of the maternal separation paradigm[8] that Harry Harlow used in the late 1950s and early 1960s [109], nursery-reared rhesus monkeys were found to show lower central oxytocin levels than mother-reared conspecifics, in line with the former group's sociobehavioural deficits.

[7] A non-peptide oxytocin receptor antagonist, developed for binding to uterine oxytocin receptors. L-368,899 passes the blood-brain barrier after intravenous administration.

[8] The experiments performed by Harry Harlow, in which monkeys were isolated until they displayed huge social deficits and behavioural deviance, are now deemed highly unethical. According to Gene Sackett, a doctoral student of Harlow, the cruelty of the experiments gave rise to the animal liberation movement [27].

These findings indicate that oxytocin plays a prosocial role in primates as well, but are not instructive about the neural regions that are involved. A study using positron emission tomography (PET) and structural magnetic resonance imaging (MRI) explored this question in pair-bonded coppery titi monkeys (*Callicebus cupreus*) and found reductions in baseline glucose uptake in the nucleus accumbens, ventral pallidum, medial nucleus of the amygdala, supraoptic nucleus of the hypothalamus, medial preoptic area and lateral septum as a function of bonding status [11]. While such reductions are not easily interpreted, the listed areas do overlap with those found in the prairie vole studies.

3.5 Summary

As the olfactory bulbs did in social learning, the mesolimbic reward system is integrated in the oxytocin system in pair-bonding and - at least in the case of rats - in mother-offspring bonding. In female prairie voles, oxytocin released at mating interacts with dopamine in the nucleus accumbens to reinforce social contact, forming a preference for a specific partner. The pair-bonding role of oxytocin seems to be largely replaced by vasopressin in male prairie voles, in which it also regulates mate guarding, the selective aggression seen after pair-bonding.

Similarly, oxytocin build-up during pregnancy and release at parturition promotes a reinforcement-like bonding between rat mothers and pups. Oxytocin may not play a direct role in nucleus accumbens in those cases, but it does enhance dopaminergic transmission to the region from the ventral tegmental area. Interestingly, while the dopaminergic part of pair-bonding in prairie voles was shown to depend on D_2-like receptors, the D_1 receptor was implicated in rat mother-offspring bonding. In prairie voles, replacing D_2-like with D_1-like receptors is considered to solidify the partner bond.

The findings on the role of nucleus accumbens dopamine in rat mother-offspring bonding have not been extended to the more selective sheep, even though the latter species displays more selective offspring preference. Nucleus accumbens, together with other brain regions found in the pair-bonding circuit of monogamous voles, has been associated with partnership in the coppery titi monkey, suggesting the vole findings can be translated to primates. Oxytocin effects in primates have been demonstrated for squirrel monkeys, rhesus monkeys and marmosets.

Interlude

Chapter 2 and 3 sketched the neural systems underlying recognition and bonding. Despite the many unknowns that continue to exist in the science of these phenomena, a picture of the neurobiology of sociality is emerging, in which the neuropeptide oxytocin plays a large role, together with its molecular brother vasopressin.

The role of oxytocin seems to be a signalling role: its release coincides with sensory input that can be argued to be social in nature most of the times. The most clear example of this is tactile stimulation, either through parturition, mating or suckling, that leads to hypothalamic release of oxytocin. The content of the signal might be a binary notification of social context, or even of positive social context.

Upon release, oxytocin is picked up by neuronal circuits that are involved with non-social processing as well. Odour learning in the olfactory bulb or reward processing in the striatum occurs in a variety of non-social settings, but these processes apparently get a chemical boost if exposed to oxytocin release. Naturally, regulation of which regions are responsive to oxytocin occurs via specialized receptors: this is why monogamous voles show increased oxytocin receptor expression in nucleus accumbens and ventral pallidum, as compared to related promiscuous voles.

Oxytocin does not only act as a cognitive enhancer, aimed at facilitating learning in social contexts. As most neurohypophyseal peptides, it also induces state changes, such as parental behaviour. An open question is whether such state changes continue to depend on somatosensory-induced oxytocin release, or whether recognition by itself can become a trigger for hypothalamus to upregulate the neuropeptide. Given the extensive connectivity and multi-layered regulation of oxytocin gene expression, it is not implausible that, for example, the synaptic changes in the olfactory bulb that were prompted by oxytocin during social learning, later serve to increase oxytocin levels.

Such a driving force of oxytocin release could provide a missing link between the animal behaviour discussed in previous chapter and social recognition in humans. Humans are able to learn individual identity without tactile stimulation and this capacity can be enhanced through oxytocin administration, as will be discussed in chapter 4. Pair-bonding, although often accompanied by a plethora of tactile inputs that are reserved for potential mates, can also occur in the absence of such inputs, instead relying on visual or verbal cues. If this is mediated by oxytocin in humans, how is it achieved? The next chapters will investigate how the findings from animal research can be translated to humans and to what extent such translation can inspire applications.

Part II

Humans

"How, indeed, can we suppose it possible in any one, who wears a human heart, that if there be subjected to his censure, one character or system of conduct, which is beneficial, and another, which is pernicious, to his species or community, he will not so much as give a cool preference to the former, or ascribe to it the smallest merit or regard? Let us suppose such a person ever so selfish; let private interest have ingrossed ever so much his attention; yet in instances, where that is not concerned, he must unavoidably feel some propensity to the good of mankind, and make it an object of choice, if every thing else be equal. Would any man, who is walking along, tread as willingly on another's gouty toes, whom he has no quarrel with, as on the hard flint and pavement?"

–David Hume, An Enquiry Concerning The Principles of Morals, 5:II

Chapter 4

Humans and oxytocin

Given the role of oxytocin in animal sociality and the impressive display of social behaviour in humans, it is only natural to investigate whether the neuropeptide underlies recognition, bonding and other social functions in our species. This does not only deepen the understanding we have of ourselves, but may also allow for improvement of the lives of those who are impaired at social interactions.

4.1 Oxytocin and neuroeconomics

Years before Michael Kosfeld published the paper mentioned in the introduction to this thesis, the effect of intranasal administration of oxytocin in humans was investigated. As mentioned in section 1.3.2, rodent research had shown effects of the compound on memory, and studies in the 1980s explored whether this was also the case in humans [80]. The recent surge of interest, however, is mainly inspired by the series of economical experiments of the 2005 Kosfeld paper [149]. As a behavioural science, economics is interested in human choice behaviour and during the 20^{th} century the field devised a number of experimental paradigms, mostly inspired by game theory, that test how human decision-making is influenced by experimental manipulations.

4.1.1 Dictators, ultimata and trust

The paradigm used by Kosfeld was the *trust game* (see 4.1), in which two players anonymously interact and either play the role of investor or trustee.

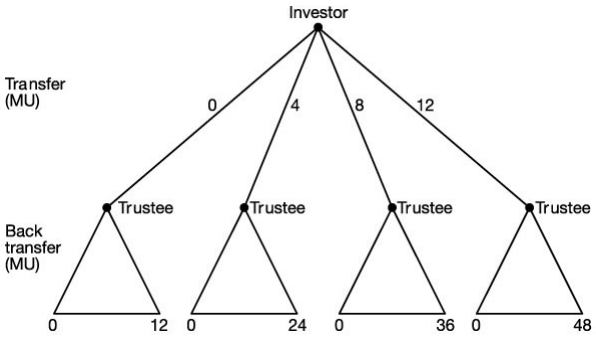

Figure 4.1: The players in the trust game both received 12 monetary units (MUs). The investor was given the choice to put MUs in the care of the trustee, in whose hands the amount of invested MUs triple. The trustee then, is given the choice to repay the investor with any amount of MUs in her possession. For example, if the investor transfers 4 MUs to the trustee, this amount is tripled and the trustee has 12 MUs in addition to the 12 initial MUs. She can then choose to back-transfer any amount from 0 to 24 MUs to the investor. Taken from reference [149].

The investor is confronted with the choice of trusting the trustee with an alloted sum of points: if she does so, the amount of points triple, but are in possession of the trustee. The trustee can then decide how to distribute the points he owns. As an incentive to play the game, Kosfeld used real monetary stakes: a flat fee of 80 Swiss francs and an additional payment of 0.4 Swiss francs times the points won in the game.

If the trustee would be rational in the classical economical sense of the word (i.e., seeking to max-

imize personal payoff), he would keep the points if trusted, as the game is not played iteratively, but only once. Then, if the investor were rational and assumed trustee rationality, she would never trust him, knowing full well he would not return any points. The classical economical assumptions do not hold completely: people do invest their alloted points and trustees do share the profit they reap. However, an aversion exists against such trusting that goes beyond risk-aversion: humans are seemingly more willing to risk losing by chance than by intent of another [30].

Oxytocin modifies the willingness to trust such intentional others, as measured by the increased transfer of points from a group treated intranasally with oxytocin, as compared to a placebo group. To check whether the effect was specific for social interactions, a control experiment was performed with a non-intentional (i.e., random chance) trustee, simulating an "investment project". This gave results reflecting decision-making under risk, but was not sensitive to oxytocin administration (see figure 4.2). Similarly, trustee behaviour in the two-player version of the experiment was also not influenced by the administration of oxytocin. In other words, while oxytocin influenced initiation of the partnership, it had no impact on reciprocity in this experiment.

This latter point warrants a further exploration. The investor and the trustee are confronted with distinct schemes of reasoning. The investor has to reason about the intent of her partner. She can choose to trust him, but from that point on she has no control on the outcome. The trustee, in contrast, can only act in full control of the outcome and does not have to make any inferences about the beliefs and motivations of the other. Oxytocin might influence such reasoning about intentions selectively, thus being only of importance to the decision-making of the investor, in the two-player version of the game. An interesting modification of the trust game would therefore be to play it iteratively. This changes not only the game theoretical underpinnings of the paradigm, but also forces the trustee to employ social reasoning, as reciprocity has consequences for future social interactions. Indeed, repeated trust games lead to behavioural changes [145], but how they are influenced by oxytocin has not been looked into yet.

What has been shown is that plasma oxytocin levels of trustees increase if the investor places her trust in him and that this does not happen if the offer is made through a random draw [281]. Whether plasma levels of oxytocin can be considered a proxy for central oxytocin release is unknown - the compound does not cross the blood-barrier easily and peripheral and central release are potentially segregated (see section 1.1.1). However, the plasma levels did correspond to a behavioural measure according to the authors: trustees with high oxytocin levels tended to transfer back higher sums of money. This effect remains if correcting for the size of the "trust signal" given out by the investor, which was considered to be proportional to the amount of money invested. Surprisingly, the plasma oxytocin levels of trusting investors was not found to be raised, which contrasts with the Kosfeld research. Although this discrepancy may result from peripheral and central oxytocin being uncoupled, it is also possible the trust-inducing effect measured by Kosfeld does not reflect (or is much bigger than) endogenous oxytocin function.

What if trust is betrayed? In a variation of the trust game, the brain activity of the investor was examined using functional magnetic resonance imaging (fMRI) [20]. The trust game was slightly modified and allowed the investor to trust the trustee with a discrete amount of points of his choosing, which was then tripled. The trustee also started out with a number of points and, if trusted by the investor, could choose to payback only by equalizing the point amount. Again, a non-social interaction was used as a control task and both oxytocin and placebo-treated investors were investigated. Multiple trials were played, but every trustee was only encountered once by the subject and non-social trials were randomly intermitted in a balanced fashion. Only halfway across the session did investors receive feedback of the trustee responses from the first half of the experiment, splitting the session in a *prefeedback* and *postfeedback* phase. Subjects were fully instructed about the game and experimental setup.

In the experiment, subjects receiving intranasal placebo spray tended to show decreased trust in the postfeedback social trials, but not during the non-social trials. Investors receiving oxytocin did not show such behavioural adaptation for either type of trial. Moreover, they reached their decision to invest quicker. Interestingly, oxytocin did not en-

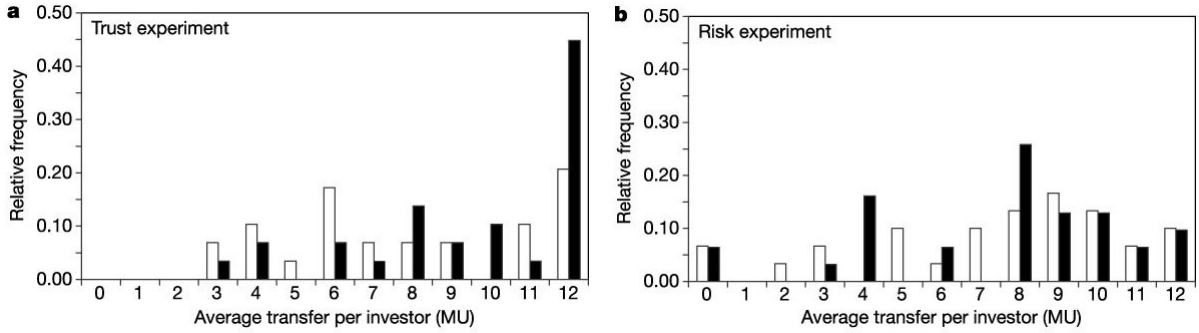

Figure 4.2: Each investor performed four trials (using different trustees). Results shown are the average monetary units (MUs) transferred by the investor (horizontal axis) versus the relative frequency of the result (vertical axis). Filled bars represent the oxytocin-treated group and empty bars placebo. Figure **a** shows the trust experiment, in which human trustees were used ($n = 58$) and figure **b** shows the non-human "investment project", which reflects decision-making under risk ($n = 61$). A pronounced effect of oxytocin can be seen for the trust experiment, but not for the risk experiment. Taken from reference [149].

hance trust in the prefeedback phase, which was argued by the authors to be caused by the experimental setup: according to them, subjects were incentivized to explore trustee strategies during the prefeedback phase, but this incentive was lowered by oxytocin as it caused a loss of fear of betrayal, occluding the trust increase. It is important to note that the reinforcement rate within the experiment was the same for both groups, so the lower exploration of the oxytocin-treated subjects did not translate in decreased opportunities for learning.

During the non-social trials, oxytocin and placebo groups showed no difference in neural activation, but during the social trials differences were seen in the amygdala, midbrain and striatum (see figure 4.3), with amygdala and striatum showing increased activation in placebo as compared to the oxytocin group. As discussed in section 1.3.1, oxytocin excites inhibitory cells in amygdala to attenuate fear response expression in rats, which may fit with the postulated lower fear of betrayal in oxytocin-treated subjects. However, research exploring oxytocin binding found none in human amygdala [168] and the activity change in amygdala may also be driven by other regions. Lesion studies have shown amygdala damage to lead to trusting behaviour in a facial processing task [3] and another study has shown oxytocin attenuates human amygdala activity in affect processing and uncouples the amygdala from the brainstem, where the physiological expression of fear takes place [180]. The lower striatal activation was argued to reflect a lower propensity to learn from feedback in the oxytocin group, as is also witnessed if investors are under the impression they are dealing with a morally "good" trustee [59].

The trust game is not the only neuroeconomic game played under oxytocin administration. In the dictator game, which simply comes down to one player having the choice of handing a portion of an endowed sum to another player, oxytocin was shown not to have any effect on transactions, while in a variation called the ultimatum game it does. In this latter game, the potential recipient has the choice whether or not to accept the offer made by the endowed player. If he accepts, the distribution of money follows the offer. If he does not accept, neither person gets any money. As with the trust game, classical economics makes predictions that do no fit with actual human behaviour. Under the assumption of rationality, the potential recipient should accept any non-zero offer, as they all exceed the alternative in which he gets nothing. If offered *nothing*, refusal and acceptance should be valued equally. The player making the offer should maximize his profit as well, which would come down to him offering the smallest possible amount, thus

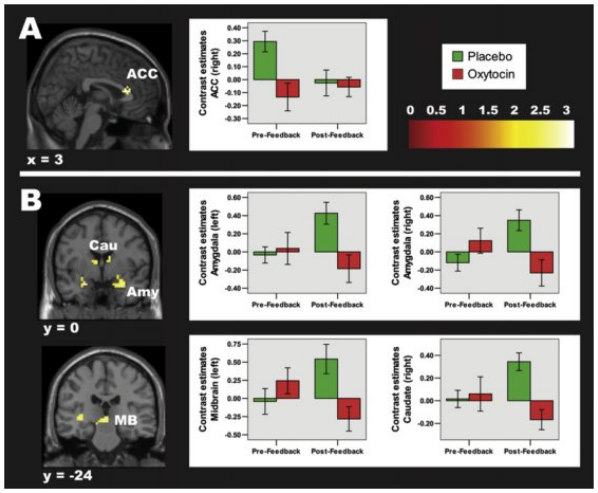

Figure 4.3: BOLD response for contrast placebo > oxytocin in the modified trust game is shown in the slices at $p < 0.01$., although significance was assessed at $p < 0.005$. Bar plots show the contrast between the non-social risk and the social trust condition (trust > risk) in *a priori* defined ROIs for the oxytocin and the placebo treated subjects. **A.** The prefeedback phase and **B.** the postfeedback phase. Taken from reference [20].

ensuring acceptance of the recipient player[1].

As the ultimatum game was first played in the 1980s, however, it became clear humans violate the assumptions of classical economics. The acceptance of offers depends on the amount that was given to the offerer, his prior record and other manipulations [249]. A typical offer is about 50% of the endowed amount and offers of less than 20% are usually rejected by the potential recipient [228]. As mentioned, oxytocin can also manipulate outcome: by operationalizing generosity as offering more money to the recipient than would be necessary on average, Paul Zak and colleagues showed such generosity to be boosted 80% over placebo by oxytocin in the ultimatum game [282]. As offerers in the dictator game remained unaffected by oxytocin, the compound did not merely affect altruism. In line with the trust game results, it seems to work only in the circumstance where reciprocation existed, that is where the choice involves *mind-reading*[2] or empathy to predict the behaviour of the other player. It is important to note, however, that subject identities were masked and that no real assessment of the other could therefore be made. Any expectations of the behaviour of others had to be based on subjects' general social knowledge.

4.1.2 Social decision-making in the brain

Can neuroscience corroborate the idea of increased mind-reading or empathy through oxytocin? Unfortunately, no neuroimaging data exists of humans playing the ultimatum game under influence of oxytocin and the imaging data obtained during the more classical version of the task has solely focused on how fairness is processed in the recipient player [228], [104]. Pending direct empirical evidence, the effect of oxytocin on the brain during an ultimatum game is only open to speculation.

Oxytocin might attenuate amygdala responses, as it does in the "social" part of the trust game. Such a view would hint oxytocin acts by reducing social anxiety, but this seems paradoxical: oxytocin-administered subjects are more generous, meaning they donate more than is necessary for acceptance of the offer. This is risk-*avoiding* behaviour, which does not logically follow from lowered anxiety. One might argue that amygdala attenuation would lead to the subjects being less anxious about letting go of resources, but then oxytocin should act on social and non-social situations equally. If decreased amygdala activity is found in oxytocin-treated ultimatum game offerers, it is unlikely to be the whole story.

4.2 Oxytocin and social learning

With oxytocin seemingly influencing such abstract phenomena as human trust, generosity and empathy, it is interesting to look whether social recognition and learning is under influence of the peptide.

[1] This does not hold if the minimum fraction f the endowed player is allowed to offer is more than 50% of the endowed sum x. This is because the expected gain after offering nothing is $0.5x$, which in that case is more than the expected gain $(1-f)x$ if making the minimum allowed offer.

[2] Mind-reading is a term used in cognitive science to denote the phenomenon whereby humans infer beliefs, intentions and experiences from the behavioural states of others.

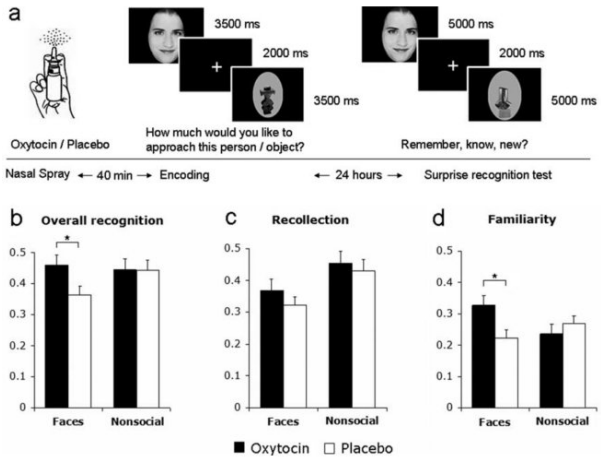

Figure 4.4: **a.** After intranasal application of oxytocin/placebo, pictures of faces and objects/landscapes/houses were shown which were rated for approachability. A subsequent surprise test assessed recollection, familiarity and forgetting. **c.** Oxytocin did not influence recollection ($p < 0.05$) **b,d.** Oxytocin enhances recognition ($F_{(1,39)} = 4.90$; $p < 0.05$) and familiarity ($F_{(1,39)} = 14.32$; $p < 0.001$) for faces, but not nonsocial stimuli. $*p < 0.05$ for pair-wise comparisons, error bars signify standard error from the mean. Figure taken from reference [217].

These more receptive forms of social cognition interact robustly with oxytocin in animals (see chapter 2) and they are relatively easy to test in laboratory settings. Using intranasal oxytocin administration, it was argued the compound selectively improves memory for happy faces in males [103]. Men that were shown pictures of direct gaze faces, houses, landscapes and objects while instructed to rate the stimulus' "approachability" showed improved retrieval of only the faces under influence of oxytocin, albeit solely as a sense of familiarity (see figure 4.4) [217]. Both these tasks applied oxytocin pre-task: a post-task administration of oxytocin after facial stimuli encoding suprisingly showed improved recognition of neutral/angry, instead of happy faces [231].

How does oxytocin exert this effect? One suggestion is that the modulation of amygdala, as found in the trust game and animal studies, allows for a better investigation of faces. This idea is suggested by the lack of eye-directed gaze in amygdala-lesioned subjects [247] and the observation that oxytocin administration can increase gaze directed at the eye region [102]. The subsequent increase in emotionally relevant input may aid social cognition in the processes described above, be they mind-reading or recognition. Alternatively, decreasing amygdala reactivity to aversive scenes and negative faces - as was shown to happen under oxytocin administration [146] - may render faces less intimidating and thereby decrease memory imprinting. This would explain the lack of encoding of neutral/angry faces under oxytocin administration, but not the fact that happy faces consolidate less in memory using post-task oxytocin. One very speculative explanation for that effect is that oxytocin biases the subject towards encoding of *new* positive social stimuli which interfere with consolidation of the happy faces, but not the negatively valenced faces shown during the task.

While decreased amygdala activity may explain a number of observations regarding oxytocin, it is certainly not the whole story. A recent study explored the effects of oxytocin in affective face processing in females using fMRI [67], while controlling for gaze using eye-tracking. It was found oxytocin gaze-independently increases BOLD response in fusiform gyrus (in the case of fearful or happy faces, as compared to neutral faces), medial and superior temporal cortex (in the case of fearful faces as compared to neutral faces), inferior frontal gyrus (if angry or happy faces are presented, as compared to neutral faces) and ventrolateral prefrontal cortex (if angry faces are presented, as compared to neutral faces). In this particular experiment, the amygdala did not even seem to be influenced by oxytocin, which contrasts with the fact that BOLD response in the region decreased with oxytocin in affective facial processing in males [65]. This difference may be explained by task instructions: while the male subjects were asked to identify the gender of depicted faces, the females were asked to rate the faces for emotional arousal. Any increased involvement of amygdala in this latter case may have occluded oxytocinergic effects.

The regions mentioned in the female study results are all part of a proposed facial processing circuit, alluded to in section 2.3.6. Figure 4.5 shows a scheme of this circuit. Although it should be noted oxytocin receptors were only found in auto-

nomic and limbic regions of the human brain [168], it is possible the peptide acts on these other areas directly, too. Alternatively, their activation can be the consequence of altered activity in upstream regions. The fusiform gyrus, associated with face recognition, takes center stage in the circuit and is connected to the amygdala. Superior temporal gyrus has been associated with facial features subject to change (i.e., expressive parts), while inferior frontal gyrus has been associated with imitation and the "mirror neuron system". A thorough discussion of this latter system is beyond the scope of this text, so suffice it to say that it has been associated with empathetic or mind-reading skills[3]. Interaction of such regions with oxytocin could improve the feature extraction and cognitive processing that is required to identify individuals or their affective state.

A recent enquiry by Hurlemann and colleagues into the effect of oxytocin on reinforcement learning did show involvement of amygdala in facial affect processing. In this study, participants had to learn an item-category association [120]. The items were three-digit numbers and the categories were named 'A' and 'B', so no prior knowledge or expectation was likely to intervene. While learning the associations, subjects received feedback on their choices through either a coloured dot or an expressive face (see figure 4.6). Besides healthy subjects, monozygotic twins were used who suffered from Urbach-Wiethe (UW) disease[4] and had bilateral calcification of their amygdalae. While healthy subjects learned faster from social feedback, the UW twins did not. Still, they could properly attribute affective states to happy and angry faces. Similar results were obtained by giving healthy subjects a single dose of the β-noradrenergic antagonist propranolol [182], which was shown to attenuate amygdala activity using fMRI [121].

The UW twins also did not show improved feedback learning in the social trials under influence of oxytocin, although healthy subjects did (see figure 4.6). As their lesion was shown to be specific to amygdala, this suggests the region to be necessary for the cognitive enhancement effects of oxytocin in this context. Moreover, the UW twins scored poorly on the "Multifaceted Empathy Test" (MET), a test developed by German psychologist Isabel Dziobek to measure experiential empathy [74], as opposed to the more cognition-driven mind-reading that might guide the decision-making tasks mentioned in section 4.1[5]. Oxytocin was shown to improve MET scores in healthy subjects, but not in the UW twins.

This leads to a view in which cognitive appraisal of facial affective states can occur without amygdala contribution, but learning from such appraisal is impaired. It may be that the amygdala adds a motivational component to facial affect processing that is required for reinforcement learning. Emotional understanding of the facial expressions may need to complement cognitive understanding, perhaps in the same simulative manner as proposed for (nonconscious) "feeling" in decision-making by Antonio Damasio, in his somatic marker hypothesis [24][6]. The amygdala is often only regarded in its well-described role as the processing unit of fear, but it is also associated with pleasurable states (for review, see reference [21]). In the context of face processing, valence has been suggested to be processed by distinct amygdalar subnuclei [88], with oxytocin enhancing negative affect processing through inhibition and positive affect processing

[3]Briefly put, mirror neurons are cells that respond to both the observation and the performance of a specific action. This finding led to hypotheses on the neural basis of imitation and action attribution. In fact, mirror neurons have been put forward as explaining the rise of language [8], social skills [192] and art [85]. The controversy about these claims mostly focuses on the fact that mirror neuron existence has only been demonstrated in pigtail macaques, which score poorly on all of these skills. At the same time, the proposed human mirror neuron system has been argued to consist of dissociable populations of action and performance sensitive neurons [62].

[4]A rare recessive genetic disorder, possibly caused by aberrant cell differentiation and proliferation. In the brain, it can manifest itself as bilateral symmetrical calcification in the medial temporal lobes.

[5]Literally speaking, empathy presupposes such experience. The word entered the English language as the translation of the German *Einfühlung*, which was coined by the 19^{th}-century physician and philosopher Rudolf Lotze to describe the phenomenon of experiencing the subjective states of others in pieces of fiction.

[6]Antonio Damasio, a cognitive neuroscientist investigating the functions and mechanisms of emotion, proposed that decision-making is guided by rapid simulations of the affective state associated with expected outcomes. He beliefs prior experience has labeled outcomes with "somatic markers" of relevant physiological states. Human choice, he argues, can be understood through algorithms using these markers to calculate which are the most pleasureable physiological states within reach.

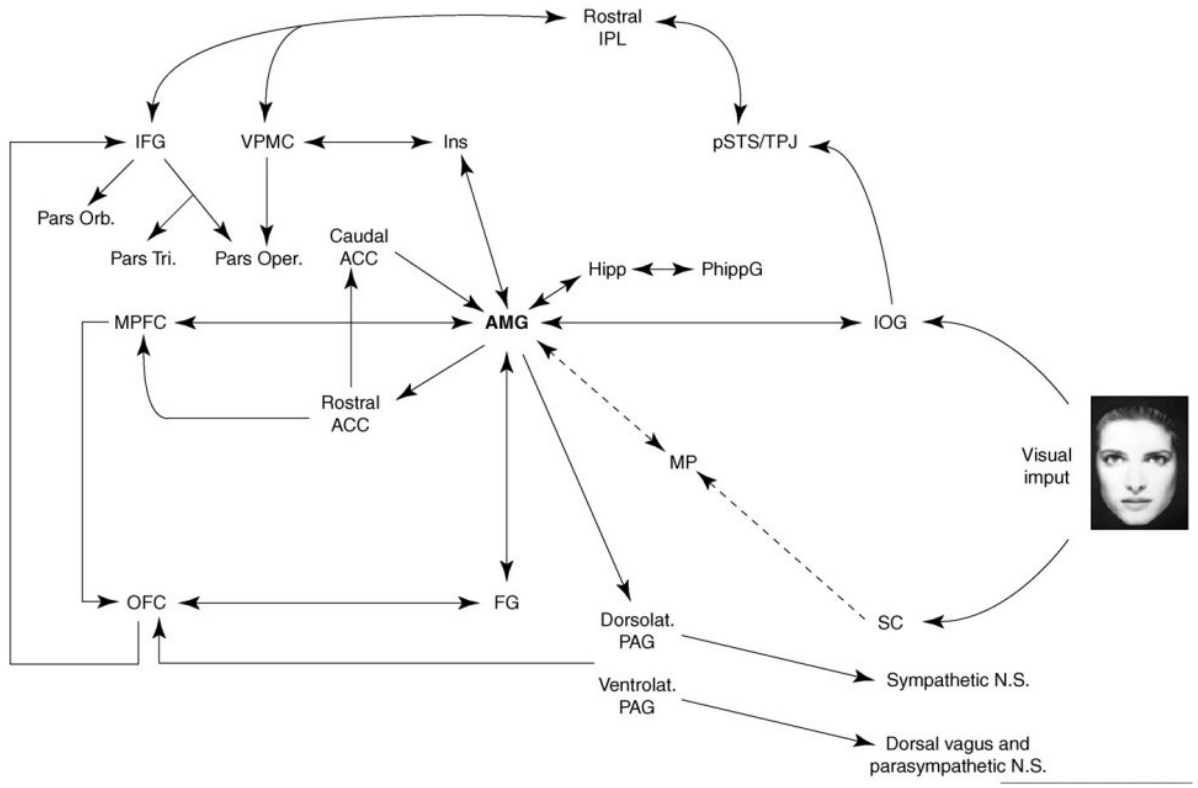

Figure 4.5: A scheme of the proposed face processing circuit. As in the olfactory system sketched in chapter 2, the amygdala (AMG) plays a central role. Connected regions are the anterior cingulate cortex (ACC), fusiform gyrus (FG), hippocampus (Hipp), inferior frontal gyrus (IFG), inferior occipital gyrus (IOG), insula (Ins), medial pulvinar nucleus of the thalamus (MP), medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), periaqueductal gray (PAG), pars opercularis (Pars Oper), pars orbitalis (Pars Orb), pars triangularis (Pars Tri), parahippocampal gyrus (PhippG), posterior superior temporal sulcus (pSTS), rostral inferior parietal lobule (Rostral IPL), superior colliculus (SC), temporoparietal junction (TPJ) and ventral premotor cortex (VPMC). Figure taken from reference [240]

through activation of the measured BOLD signal. A simulative view of empathy could also explain the finding that oxytocin did not affect empathizing with a loved one in pain [239]: such empathy would depend on insula, the region mediating disgust and physical pain and that region may not be susceptible to oxytocin effects.

Of course, arguing that the amygdala is necessary for true empathetic responses to happy and angry faces does not argue in favour of it being sufficient. The regions that were pinpointed in the study that explored facial affect processing in females, or wholly different regions altogether may be required for empathetic processing as well. Given the motivational significance required in social reinforcement learning, one might expect ventral striatal modulation to occur or, in line with demonstrated oxytocin binding sites in humans, the ventral pallidum [168].

4.3 Oxytocin and autism

Empathy, facial affect processing and other social behaviours may come natural to most (the so-called *neurotypic* humans), but in a small portion of humanity these skills are underdeveloped. Autism, a condition characterized by compulsive-like behaviour (such as stereotypies in both interests and

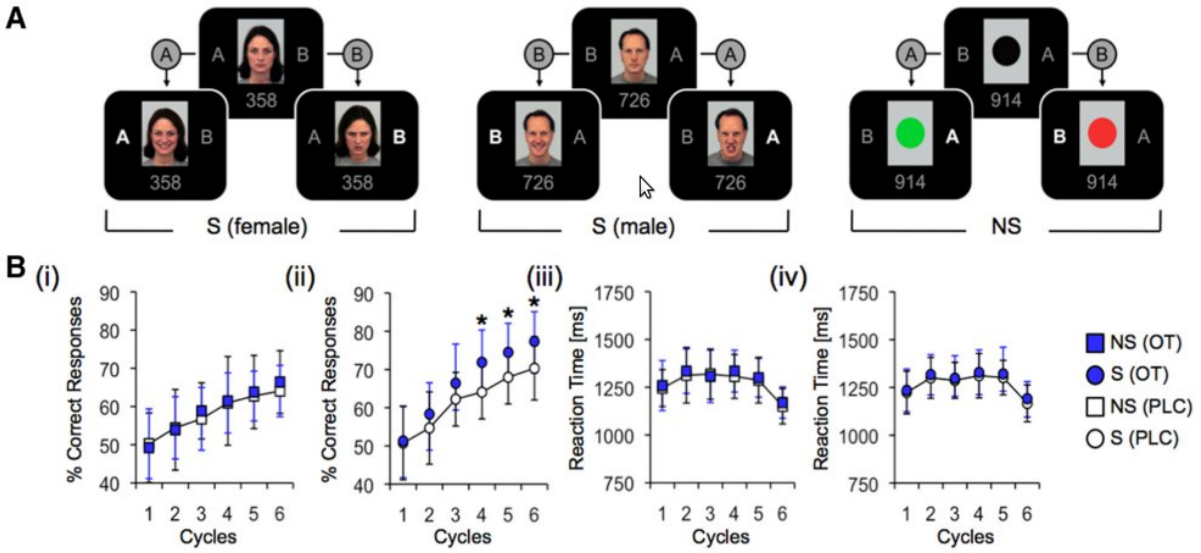

Figure 4.6: **a.** Subjects were shown a three-digit number and a neutral face (social trials) or a black dot (non-social trials). They categorized the number as either 'A' or 'B'. The subjects received immediate feedback: an angry/happy face in the social trials or a red/green dot in the non-social trials signaled incorrect/correct performance, respectively. **b.** Oxytocin enhanced performance on the social trials as compared to placebo (ii and iv) but not on the non-social trials (i and iii). Figure taken from reference [120].

behaviour, and repetitive speech and movements), often accompanied by mental retardation, is known to affect social skills, albeit to varying degrees. Related conditions placed on the autism spectrum, such as high-functioning autism and Asperger's syndrome, do not include mental retardation, but do contain the social and compulsive aspects. Early conceptualization of the condition was framed in terms of an attachment system gone awry, but this view has now been largely abandoned. However, research on the attachment system in autists is still ongoing (see reference [225] for a recent review).

Perhaps the most well-known of later views is the *theory of mind hypothesis* of autism, proposed by Simon Baron-Cohen and colleagues in 1985, which states that autism follows from poorly developed metacognitive traits such as empathy and mind-reading [14]. This account is supported by false belief experiments (see box 1). Despite its success in explaining social deficits, the theory of mind hypothesis proved ill-suited as an explanation for the compulsive-like aspects of the condition. This lack has been met by postulation of an additional malfunction of the executive control system by Elisabeth Hill [113]. Baron-Cohen extended his theory of mind account by considering it part of an "extreme male brain" [16], later integrated in the *empathizing-systemizing theory* [17]. The non-social factors of autism, he argues, can be explained by considering autists to score high in the "masculine "systemizing personality dimension, which represents the drive to build or analyze. At the same time, the social deficits stem from a lack of "feminine" empathic skills.

Given these conceptualizations, it is interesting to tie in oxytocin to autism. Being upregulated by estrogen, oxytocin is expressed more strongly in females in at least some species (see section 1.2.3). This fits Baron-Cohen's male brain view and, more importantly, could explain the higher incidence of autism in males than in females. Given the behavioural findings discussed in the previous section, oxytocin may be endogenously involved in empathy and the disturbed attachment that led to the early formulations of the condition can be seen in light of the bonding role of oxytocin.

Empirical evidence strengthens this view somewhat. It was found that intravenous oxytocin infusion reduces repetitive behaviours in adult autists [115] and intranasal administration improves performance of neurotypic subjects on a "Reading the Mind in the Eyes Test" (RMET) [66], a test that measures the subjects' proficiency in telling emotional states from photographs depicting eye-centered facial fragments. Eye contact is known to be impaired in autism and has been suggested to depend on the amygdala, as damage to this region reduces it [247]. As mentioned, oxytocin was shown to increase gaze directed at the eye region in neurotypic humans [102].

Surprisingly, neuroimaging studies on an RMET-like task showed lower amygdala activation in autists as compared to matched controls [15]. If oxytocin exerts its role in the RMET by increasing BOLD activation, this would stand in stark contrast to its attenuating role in the neuroeconomical experiments discussed in section 4.1. This may be the result of the subnucleic differentiation in the amygdala: the aberrant BOLD activation seen in autists may be not be caused by the same structure as the one relevant for the neuroeconomic studies. It may also be that the lower activity in autists mainly reflects reduced metabolism of *inhibitory* neurons, so that the decreased amygdala BOLD fMRI signal could still correspond to increases in fear-related throughput, which could be inhibited by oxytocin. Alternatively, the direct target of oxytocin in RMET-like tasks may not be the amygdala. It has been argued autism severity and face processing deficits are related to decreased connectivity between fusiform face area and amygdala [147], so that the lowered amygdalar activity in autists can also be increased by oxytocin-induced coupling increases between these two regions. Future neuroimaging work on the role of oxytocin in RMET performance will undoubtedly elucidate this matter.

Besides the results on the RMET, oxytocin also improved performance of subjects diagnosed with autism or Asperger's disorder on an affective speech processing task [114], in which they were instructed to appraise the emotional, prosodic aspect of speech with neutral content (e.g., "The game ended at 4 o' clock") and judge the speaker's emotional state. During infusion of either oxytocin or placebo, their performance was monitored. After some 2.5 weeks, they returned to perform the task while given the alternative treatment. While in both cases treatment initially improved performance to a comparable degree, the retention of speech processing capacity over the delay was significantly larger in the oxytocin group.

Testing Theory of Mind
In a false belief test, children are tested for their ability to understand others can have notions about the world that are wrong. In a typical setup, called the *'Sally-Anne task'*, they are shown two dolls, Sally and Anne, the former of whom has a basket, while the latter has a box. Sally puts a marble in her basket and leaves for a walk. Anne then, puts the marble in her box. When Sally returns, the child is asked where she will look for her marble. Neurotypic children the age of four readily pass this test by saying she will look in the basket, but children with autism spectrum disorder do not. Adults with high-functioning autism or Asperger's syndrome pass false belief tasks, but implicit measurements using eye-tracking can still reveal a perceptual bias towards, in the example given, the box [235].

Box 1: False Belief Test

A recent study explored whether the social deficits found in high-functioning autists or people with Asperger's syndrome could also be influenced by intranasal, as opposed to intravenous oxytocin administration [4]. It was tested whether performance in a virtual ball tossing game was affected by oxytocin. In this computer game, the subject joins three computer-controlled characters in a series of ball throws. If the subject receives the ball, he can choose to toss it to one of the other players. By setting players as bad (i.e., excluding the subject by not tossing the ball to him), neutral (i.e., tossing the ball to the other players randomly) or good (i.e., preferring the subject), an artificial social situation is created. Humans typically do not like being excluded and will prefer to toss the ball to good players, while avoiding bad players. People with Asperger's syndrome or autism do not show such preferences but, after placebo-controlled intranasal oxytocin administration, they do. Also, they rate good players as being trustworthy. Intranasal oxytocin administration was also shown to increase the eye-directed gaze of high-functioning autists and people with Asperger's syndrome in a face processing task and raise plasma oxytocin lev-

els within 10 minutes. Notably, oxytocin responsiveness in the ball tossing game did not predict responsiveness in the face processing task, suggesting dissociable effects in a heterogeneous subject pool.

It is striking that oxytocin impacts both the repetitive and the social characteristics of autism, providing a molecular bridge between two facets of the condition that proved so hard to connect by psychological theorists. Can autism then, at least in part, be seen as an oxytocin deficit? Given the role of the peptide in social recognition and bonding and the aforementioned results, such a view does not seem far-fetched. Indeed, oxytocin has been found in lowered levels in autist plasma [184], but as mentioned in section 1.1.1, plasma and central oxytocin levels do not necessarily correlate. Does the anomalous plasma content reflect a general deficit of the oxytocin system?

In the neurotypic population, social deficits have been related to single nucleotide polymorphisms (SNPs) of the *OXTR* gene. The rs53576 (A/G) polymorphism has been associated with attachment [45], empathy as measured by RMET performance [218] and positive affect [169]. A recent study investigated the relation between this polymorphism, structural and functional coupling in the brain[7], and score on the Tridimensional Personality Questionnaire (TPQ), a behavioural index of prosocial temperament [260]. Carriers of rs53576A showed gray matter decreases in hypothalamus and increases in (right) amygdala. A stronger structural coupling of the hypothalamus with both dorsal anterior cingular gyrus and amygdala was found. During an emotional face processing task, homozygous rs53576A carriers showed the lowest amygdala BOLD activation, while homozygous rs53576G carriers showed the highest BOLD response. The A carriers also showed increased functional connectivity between hypothalamus and amygdala. Notably, the A allele load was predictive of lower TPQ score, as were the A allele gray matter deviations: an inverse correlation between amygdala volume and TPQ score was found and males (but not females) showed a TPQ score proportional to hypothalamus volume.

The rs53576 polymorphism has been associated with autism as well, but not consistently. In the Chinese Han population, two SNPs (one of them rs53576A) and a related haplotype were associated with autism [279]. The rs53576 polymorphism could not be associated with autism in a study investigating a U.S. Caucasian population [126], while the allelic effects of the other polymorphism were the reverse of those found in the Han population. A more recent investigation of this latter polymorphism in Slovak autistic boys could not corroborate either of the findings, while also not finding a genotype-phenotype association for polymorphisms in the *OXT* gene [132]. A more extensive study investigating 18 SNPs in Irish, Portuguese and British populations also found no associations that could be replicated across groups [252], although it should be noted genetically and phenotypically heterogeneous subject pools were used.

These genetic findings do not argue strongly in favour of direct mutation of the oxytocin system in autists, but it is fully possible they display alterations that regulate oxytocin or oxytocin receptor expression, either during neurodevelopment or constitutively. Alternatively, systems under influence of oxytocin may be affected. None of the oxytocin or oxytocin receptor transcription factors discussed in section 1.2.1 have been reported to relate to autism, but preliminary data has pointed to hypermethylation of DNA regions associated with *OXTR* regulation [98]. Another possibility would be that not oxytocin gene expression, but post-translational processing of oxytocin is affected in autists, as suggested by a study that found increased plasma levels of the C-terminal extended forms of oxytocin [97] (see section 1.1 for more on oxytocin synthesis).

Regardless of the research into the root causes of autism, the question remains whether oxytocin can be used to alleviate the social cognition difficulties autists face. The enhancing effects described above for autists, as well as the boost oxytocin gave neurotypic humans in facial memory and performance on a social reinforcement task (see section 4.2) suggests this to be the case. None of the studies looked into the long-term effects of oxytocin, instead assuming oxytocin effects are acute and provide a window in which the behavioural effects exist. This may seem plausible given the relatively quick en-

[7] Structural coupling can be determined by looking at the covariation of gray matter in different regions as determined by voxel-based morphometry. Functional coupling is assessed by the correlation between regional BOLD responses.

zymatic degradation of peptides, but the possibility remains oxytocin application leads to long-term modification of the nervous system.

If the desired behavioural effects of intranasal oxytocin administration prove transient, the window it provides may be lengthened by chemical derivation of the peptide, ensuring a longer half-life of the compound, but it remains to be seen how such pharmacological engineering would influence the compound's effects. Alternatively, any acute effect of oxytocin can be exploited in therapy: if it facilitates *learning* in social context, it is not far-fetched to suggest consolidated memory to survive after oxytocin is metabolized. Administering oxytocin during real-life social reinforcement may, for example, enhance autists' learning of social rules, which they can then apply outside of oxytocin treatment. In a similar vein, oxytocin treatment could be integrated in social skills training programmes, which are therapies directed at improving social behaviour in children with high-functioning autism or Asperger's syndrome (for a review on the current efficacy of such programmes, see reference [212]).

It is tempting to consider that the less secure attachment sometimes found in autistic children [225] can also be treated with oxytocin. After all, as discussed in chapter 3, oxytocin plays an important role in animal (parent-offspring) bonding phenomena. Indeed, some of these animal findings can be extended to humans as well, as considered in section 4.4. Enhancing bonding in autistic children through oxytocin, however, would be made difficult by the fact that diagnostic tools are generally poor for children below two years of age, while the critical period of attachment formation is widely considered to lie in the first few years of life. In the absence of improved diagnostics, it is therefore impractical to improve parent-infant bonding in autists with oxytocin, even if it that can be shown to be effective.

4.4 Oxytocin and bonding

In animals, oxytocin was shown to influence pair-bonding not only through social recognition, but also by directly acting on the mesolimbic dopamine pathway (see section 3.2 for more information). Like a drug of abuse, oxytocin acts as an agonist at D_2-like receptors and reinforces contact with other individuals. It plays a similar role in mother-offspring bonding, where it promotes dopamine action on D_1-like receptors in the nucleus accumbens of mothers. Hints at a similar role for oxytocin in humans come from the effects of 3,4-methylenedioxymethamphetamine (MDMA) on human behaviour. Also known as ecstasy, MDMA is known to cause prosocial, love-like behaviour towards others, which coincides with peripheral oxytocin release [73]. In an animal model of MDMA-induced sociality, the oxytocin receptor antagonist tocinoic acid was shown to block the effect [255].

Does this mean oxytocin plays a role in interhuman bonding, as it does in the non-human mammals discussed in chapter 3? And if so, does it also relate to love, the subjective state associated with human bonding? Investigation of plasma levels of oxytocin in humans in a relationship led to an association with relationship stress [253]. This finding could not be extended to general stressors, so that it was speculated oxytocin release signifies a homeostatic drive towards establishing or improving bonds. However, another set of enquiries found peripheral oxytocin to correlate positively with reported relationship quality in one case [99], but not in another [163]. Also, plasma oxytocin level shifts, in response to cortisol administration, were found to correspond with emotionally open relationships [259]. Why these correlations are so paradoxical, is currently not clear.

Genetic analyses on human bonding and oxytocin have been sparse, but some results exist. Attachment style, a two-dimensional measurement defined by scores on *attachment anxiety* and *attachment avoidance*, was found to relate to SNPs in the *OXTR* gene [45]. More specifically, the *OXTR* gene haplotype rs53576GG was found to be overrepresented in unipolar depression patients who scored high in the dimension attachment anxiety and moderately in the dimension attachment avoidance. Interestingly, anxious attachment has also been associated with polymorphisms of the *5HT2A* and *DRD2* genes [91]. The 5-HT_{2A} serotonin receptor has been argued to play a part in oxytocinergic release [264] and the D_2-like dopamine receptors are, as discussed in section 3.2.2, relevant for the dopamine-oxytocin interactions that underlie the pair-bonding role of the neuropeptide in the prairie

vole reward system.

An investigation of the oxytocin-neurophysin I gene *OXT* itself among subjects diagnosed with adult separation anxiety disorder (ASAD), a condition characterized by attachment anxiety, found no correlating polymorphisms [46]. The *OXTR* gene was associated with early pregnancy and parenting in females, which was argued to serve as a proxy for pair-bonding [208]. However, a plethora of other mechanisms could explain this correlation, such as increased risk-taking or increased fertility. An *AVPR1A* polymorphism was linked to pair-bonding in a slightly more direct and convincing manner: the polymorphism is associated with marital difficulties [271]. Section 3.2.4 discussed how overexpression of the *Avpr1a* gene renders male meadow voles monogamous. Interestingly enough, the human polymorphism was also associated with increased amygdalar activity in a facial processing task [181].

Neuroimaging studies in the human bonding process itself are hindered by the fact that it is unpractical to follow the process from its initial steps onwards. Also, testing for an active role of oxytocin in human bonding would require manipulations that are considered unethical. This is why neurobiological insight in human bonding and love is mostly limited to mapping the brain regions responsive to the loved other. Attempts have been made to correlate activations and deactivations in brain regions to bonding-related personality metrics, but these studies have unfortunately fallen prey to remarkably high "voodoo correlations"[8]. As such correlations are often the consequence of a non-independence error, it is impossible to draw any meaningful conclusions from them [269]. Still, the activations and deactivations are in themselves interesting to note.

4.4.1 Romantic bonding

Increased BOLD response was seen in the caudate nucleus and the ventral tegmental area of early-stage romantic lovers, as they viewed a photograph of their loved one versus a photograph of an acquaintance [9]. This fits with the involvement of the "reward system" in prairie vole bonding (see section 3.2.4). Deactivation of amygdala was also shown, which is again is in line with animal research and with oxytocinergic effects demonstrated in humans, as discussed in the previous sections.

Another study exploring human relationships used a similar setup, but investigated people in later stages of love. This study found BOLD activations in medial insula, (ventral) anterior cingulate cortex, caudate nucleus and putamen, while BOLD deactivations were reported for right prefrontal cortex, intraparietal sulcus, posterior cingulate cortex, superior gyrus and amygdala (see figure 4.7) [18]. Again, the activations fit with the "reward circuitry" - in fact the same circuit is activated with cocaine-related euphoria [32]. The deactivated regions are interesting as again the amygdala shows up, but also cortical regions of which deactivations have been argued to correspond to happiness (right prefrontal cortex and the regions in parietal and temporal cortices) [90].

Can oxytocin be involved in these effects? As mentioned, genetics makes a stronger case for vasopressin to be involved in bonding than oxytocin. Still, oxytocin binding sites have been demonstrated in the ventral pallidum [168] - which could modulate the reward system, as shown in figure 3.3. Similarly to mating-induced oxytocin release in rodents, sexual stimulation in humans increases peripheral oxytocin levels [35]. The compound has also been argued to maintain affiliation in bonded human couples, as intranasally applied oxytocin increased positive verbal and nonverbal behaviour during conflict [63]. In a recognition task that is difficult to interpret, male subjects who received intranasal oxytocin administration showed faster, selective recognition of sex and relationship related words emerging from a gradually disappearing mask [263]. This result is suggestive of an

[8] To appreciate how an upper limit of plausible correlations is established, consider the psychometric finding that the correlation R'_{AB} between two measureables A and B depends on their true correlation R_{AB} and their respective reliabilities ρ_A and ρ_B in the following manner:

$$R'_{AB} = R_{AB} * \sqrt{\rho_A \rho_B}$$

Given typical psychometric reliability scores of 0.80 and neuroimaging scores of 0.70, even a perfect R_{AB} should lead to an R'_{AB} of 0.75. Still, correlations in the .80-.90 range have been reported in the last decade. This was argued to be caused by *double dipping*, i.e., selecting voxels that correlate well to a measure of interest and then investigating only *their* variation with the measure. This method can effectively put Gaussian noise in a high correlation with any desired measure. For a thorough discussion of double dipping in fMRI, see reference [269].

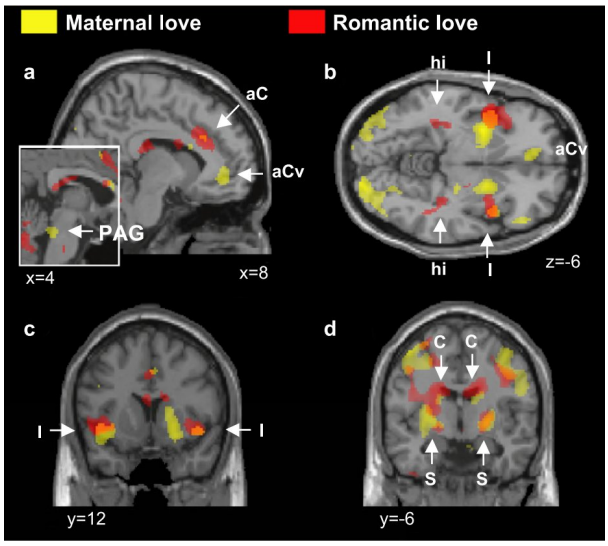

Figure 4.7: Overlap between neural regions activated by maternal love and romantic love. Maternal love activity (contrast: own child versus acquainted child) is shown in yellow along with romantic love activity (contrast: partner versus friends) shown in red. For illustration, a–c were thresholded at $P < 0.01$, and d with $P < 0.05$, to reveal overlapping activity in the caudate nucleus. aC = anterior cingulate cortex, aCv = ventral anterior cingulate cortex, C = caudate nucleus, hi = hippocampus, I = insula, PAG = periaqueductal gray, S = striatum. Taken from reference [19].

oxytocin-induced priming effect, readying subjects to process any information that is relevant to bonding. Inasfar as this interpretation holds true, it is an argument connecting the peptide to human affiliation.

Given the intimate and unpredictable nature of pair-bonding, it is difficult to truly study early stage romantic bonding in humans. However, another form of bonding shows great overlap with romantic love and might lend itself more easily for manipulation. Mother-offspring bonding, or maternal love, shares the neural substrates of romantic love, except for hypothalamic regions (see figure 4.7). These latter regions likely mediate the erotic components of romantic love, which may be related to their role in homosexuality [250].

4.4.2 Parent-child bonding

Like romantic love, maternal love was shown to involve oxytocin in animal studies. In contrast to romantic bonding, maternal bonding can be followed from a very early stage. Psychologists at the Israelian Bar-Ilan University measured plasma oxytocin levels in pregnant females during their first and third trimester and one month after giving birth [77]. They found these levels to be steady within an individual, but varying across individuals. Females with higher levels of peripheral oxytocin were argued to score higher in tests indexing attachment, to display more maternal behaviour (e.g., touching, gazing at the infant) and to check their newborn more often. The *OXTR* r53576 polymorphism mentioned in section 4.3 was associated with maternal care: carriers of the A allele in a Dutch population were found to display less *maternal sensitivity*, a measure that is determined by the way in which a mother aids her child in problem-solving [10].

The effect of maternal care on infants was illustrated by a study investigating previously neglected children, who had been living with foster parents for about three years [86]. These children, who had received poor care during the early stages of their lives, displayed the same base peripheral oxytocin levels as found in control children from family-reared environments, measured using high performance liquid chromatography analysis of urine samples. However, unlike the control children, regularly timed physical contact (e.g., tickling, counting fingers) did not lead to an increase of oxytocin levels in the previously neglected children, suggesting the early-life experience had altered their oxytocin systems. Similarly, adult women who had experienced an abusive childhood displayed lowered cerebrospinal fluid levels of oxytocin [110].

These findings are strongly reminiscent of those obtained in the animal literature. Does maternal love also involve the same neural substrates as those found in rodents? The neuroimaging studies done on maternal love so far have offered some potential brain regions. As mentioned, they have yielded similar activations as found in romantic love. Showing mothers pictures of their own and other infants with whom they were familiar showed differential BOLD activation of the medial insula and cingulate gyrus, much like seen in romantic lovers [19].

Lateral orbitofrontal cortex and lateral prefrontal cortex also showed increased BOLD signal in the mothers, as did occipital cortex and lateral fusiform cortex. Bilateral striatum, posteroventral thalamus and periaqueductal gray also showed increased activation, of which only striatum was overlapping with romantic lovers (see figure 4.7 for more information). Deactivations were found bilaterally in middle prefrontal cortex, the superior temporal sulcus, paracingulate cortex and temporal poles, as well as posterior cingulate gyrus, medial cuneus and amygdala. The ventral tegmental area activation seen in romantic love was not clearly present in maternal love and the anterior cingulate cortex showed a smaller BOLD response increase than was found in romantic love.

The overlap with regions associated with romantic love is all the more interesting as some of them correspond with human oxytocin and vasopressin binding sites, as shown in figure 4.8. This is not direct proof for oxytocin (or vasopressin) involvement in either type of love, but it does add to the plausibility of their involvement.

4.4.3 Group bonding

Besides pair-bonding and parent-offspring bonding, a third type of attachment exists that is of utmost importance to understanding humans. This attachment is the bonding to the group - a form of identity that has been extensively studied by social psychologists and sociologists. Group bonding has recently been argued to be under modulatory influence of oxytocin, in an investigation by Dutch psychologist Carsten de Dreu [56]. This is interesting not only as a direct finding of oxytocin mediating bonding in humans, but also as an example of oxytocin promoting non-affiliative behaviour.

De Dreu investigated *parochial altruism*, the tendency of members of a group to self-sacrifice to add to the welfare of a group ("in-group love") and to aggress against competing groups ("out-group hate"). To examine whether oxytocin influenced these stances, two experiments were performed on male humans. In the first experiment, each subject received €10 and for each euro was given the choice whether to keep it (gaining €1), donate it with multiplication to the three-person group to which he was randomly assigned (adding €0.50 to each member of the group, including himself) or add it to a between-group pool (not only adding €0.50 to each group member including himself, but also deducting €0.50 from each group member of a "rivalling" three-person group). Donation to the group was considered to reflect in-group love, while addition

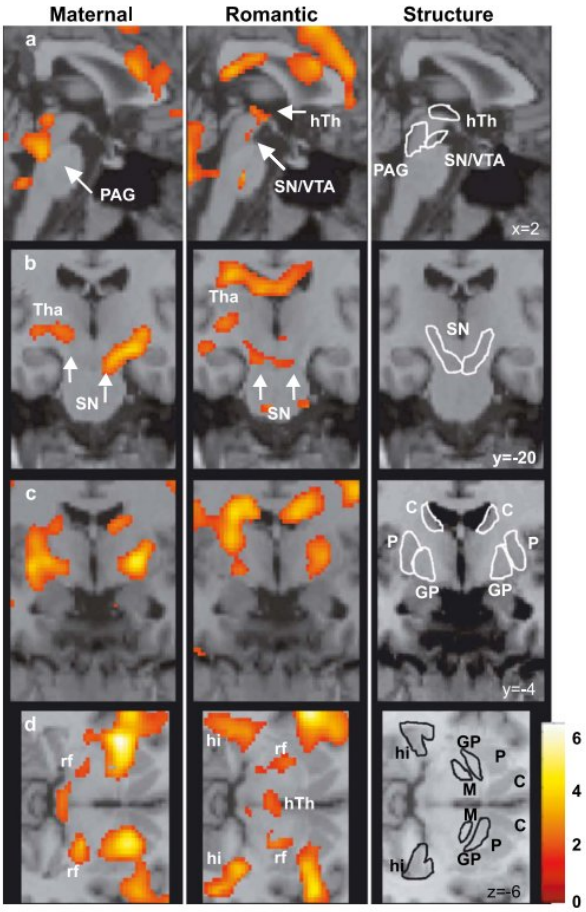

Figure 4.8: Romantic and maternal love BOLD responses, as compared to oxytocin and vasopressin binding sites found in humans [168]. Abbreviations are (oxytocin binding sites in bold): C = caudate nucleus; **GP = globus pallidus**; hi = hippocampus; **hTh = hypothalamus**; P = putamen; PAG = periaqueductal gray; **M = nucleus of Meyndert**; rf = retrorubal fields/intralaminar/subthalamic nuclei; SN = substantia nigra; Tha = lateral thalamus; VTA = ventral tegmental area. Extent of activity shown at $P < 0.05$, uncorrected. (a): sagittal, (b,c): coronal, (d): transverse. Taken from reference [19].

to the between-group pool was seen as a measure of out-group hate. Oxytocin was then shown to increase in-group love, but not out-group hate. It also increased in-group trust (the expectancy that fellow group members would donate to the group) and not out-group distrust (the suspicion members of the other group would donate to the between-group pool).

Thus far, these findings are in line with the neuroeconomic findings from section 4.1 as they can be seen as reflecting generosity and trust, respectively. De Dreu, however, reasoned that out-group hate may be defensive in nature, much like the mate guarding of prairie voles discussed in section 3.2. A follow-up experiment therefore exposed the two groups to variations on the *prisoner's dilemma*[9], as depicted in figure 4.9. Mutual cooperation between groups would render the participants with more money than mutual non-cooperation, but not cooperating while the rival group does cooperate yields the highest reward. As non-cooperation can be due both to fear of non-cooperation of the rival group and to greed assuming cooperation of the other, De Dreu varied them independently by modifying payoffs.

Under influence of intranasally administered oxytocin, subjects were more prone to non-cooperation than placebo-treated subjects and this enhancement was strongest under conditions in which non-cooperation of the other group carried the biggest risk (the *high fear* conditions in figure 4.9). Oxytocin-treated non-cooperators also cited "protectionism" as an explanation for their behaviour more often than placebo-treated ones. Interestingly enough, out-group distrust was not affected by oxytocin, meaning the protectionist non-cooperation was more driven by concern for the own group than by an untrusting "pre-emptive strike" strategy.

These behavioural experiments argue in favour of a *group guarding* effect of oxytocin in human males. While by itself the prisoner's dilemma variant could be interpreted as maximizing personal gain, this does not fit with the non-competitive experiment. Also, this non-competitive experiment yielded results comparable with the work done by Michael Kosfeld and Paul Zak, discussed in section 4.1. Indeed, it seems oxytocin enhanced the group affiliation process, or at least the action in accordance of group bonding. Social psychological studies have illustrated the uncanny capacity of humans to feel part of a group and act in accordance with group interests: Muzafer Sherifs Robbers Cave experiments showed how bonded boys can engage in fierce, aggressive competition with other boys [237], while the infamous Stanford Prison Experiment of Philip Zambardo showed how quickly such group identification can escalate [107].

One of the views on group identity formation posits a phase in which the invidual becomes connected and a phase in which the individual acts according to the group's interests. The De Dreu studies do not show which process is affected by oxytocin, but it may be that both processes are, in neural terms, one. If group bonding in humans is anything like the pair-bonding seen in prairie voles, the group becomes a valued object much like food is. During the bonding process, the group interest may actually *become* the personal interest. Alternatively, the postulated separation in bonding and parochial altruism may hold. In this case, oxytocin may enhance the bonding process, the affiliative processes that act in accordance with group demands, or both. Interestingly enough, encouraging bonding processes (e.g. communication) before playing the modified prisoner's dilemma game has an oxytocin-like effect [105]. It will be interesting to explore the neural substrates of these effects, using for example neuroimaging, pharmacological manipulations or lesioned patients.

4.5 Summary

As in animals, oxytocin has been shown to induce a variety of prosocial behavioural effects in humans, in which the compound is usually applied through an intranasal spray. Using economic games, the neuropeptide was found to enhance trust and generosity in settings that required "mind-reading". Neuroimaging showed that oxytocinergic effects on the amygdala only occurred during the social con-

[9] The name of this dilemma stems from its original 1950 formulation in which two suspects are arrested for a crime and held completely separately. The police confront the suspects with the following terms: if one confesses while the other remains silent, the confessor goes free and the silent one faces a 10-year sentence. If both remain silent, they both get a six-month sentence. If both confess, each gets a five-year sentence. Their dilemma is clear: should they stay silent and hope for six months of jail time, or fess up and have the chance of getting away free?

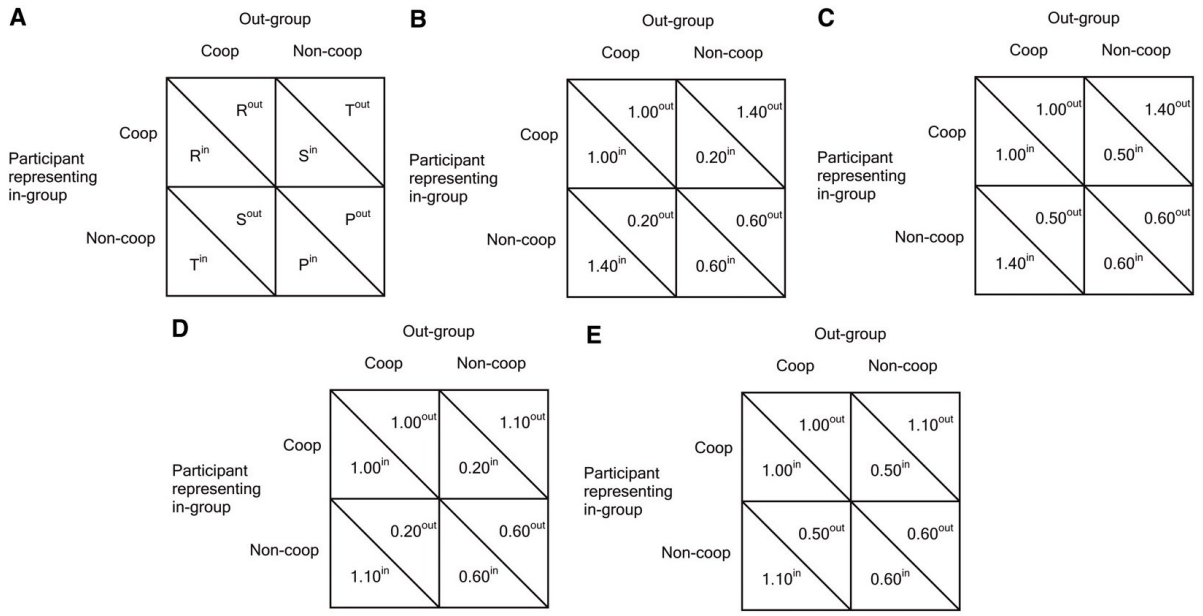

Figure 4.9: The parochial altruism test. Entries are payoffs in euros. Four general situations exist: temptation (T), reward (R), punishment (P) and sucker (S). **a** Payoff scheme. Payoffs to in-group (^{in}) are below the cell diagonal; payoffs to out-group (^{out}) are above it. **b** Situation of high greed ($T - R = 0.40$) and high fear ($P - S = 0.40$). **c** Situation of high greed (0.40) and low fear (0.10). **d** Situation of low greed (0.10) and high fear (0.40). **e** Situation of low greed (0.10) and low fear (0.10). Taken from reference [56].

tingency in a modified ultimatum game, suggesting a modality-specific action. Further studies showed the compound selectively improves memory for faces and stimulates social reinforcement learning, but not non-social reinforcement learning.

Many of these effects were argued to derive from a reduction of social anxiety, as seen through oxytocin-induced amygdala attenuation. Amygdala is not the only region in humans affected by oxytocin, however, and it may be the compound directly enhances sensory processing, as modulatory effects of oxytocin are seen throughout a tentative "face processing circuit".

As the prosocial, *cuddly* effect of ecstasy that is common in humans was shown to be mediated by oxytocin receptors in animals, oxytocin may also underlie romantic bonding or the neurobiologically closely related maternal bonding. Correlations of peripheral oxytocin levels with a variety of psychometric and relationship indexes supports this view, but paradoxical results have been obtained in the sphere of romantic bonding. One particular SNP of the *OXTR* gene, rs53576, has been associated with differences in attachment and social behaviour. In an interesting series of experiments, it was suggested oxytocin may even underlie group bonding and the exclusive, protectionist behaviour this entails. Since bonding - whether romantic, filial or paternal - generally involves increased perspective-taking, such a connecting role of oxytocin can be integrated with the "mind-reading" effects seen in the economic games.

Intravenous infusion of oxytocin was shown to improve the inference of emotions by people with an autism spectrum disorder. It also lowered the repetitive, compulsive aspects of the condition. As plasma oxytocin is lowered in autists, this has led to the idea that autism and other pervasive developmental disorders may be related to hypoactivity of the oxytocin system, a view that has not been convincingly corroborated by biomolecular studies yet. Using intranasal oxytocin administration, social engagement and emotion recognition could be enhanced in distinct subgroups of a population of

high-functioning autists and people with Asperger's syndrome.

Chapter 5

The meaning of it all

The previous chapters have examined the role of oxytocin in animal and human behaviour. Several mechanisms were suggested, such as enhancement of processing and storing of social cues, reduction of anxiety and activation of reward circuitry, contingent on social interaction. As oxytocin was shown to increase trust, generosity, emotion recognition, social reinforcement learning and group protectionism, it is tempting to speculate on how these functions evolved from the social behaviour mediated by oxytocin in more primitive species. At the same time, a molecular substrate for social cognition and behaviour opens the door to pharmacological intervention, potentially aiding psychological therapies aimed at improving social functioning.

5.1 Ecological validity

Before drawing such conclusions however, it is important to consider the relevancy of the aforementioned studies to real-life situations. They are good illustrations of what oxytocin can *potentially* do, but do they reflect the real endogenous function of oxytocin? To what extent do the experiments measure sociality as it occurs under natural circumstances? And can the effects from laboratory settings be applied in real life?

5.1.1 Concentration difficulties

In animals, application of oxytocin can be directed to distinct neural regions. Although not without difficulties, estimates of endogenous peptide concentrations can be made by measuring cerebrospinal fluid or extracellular concentrations using reverse dialysis. As a result, the difference between applied concentrations of oxytocin and the concentrations that occur naturally are known, within some margin of error. In humans, such experiments are often impossible to perform due to their invasive nature. As mentioned in the previous sections, oxytocin is applied either intranasally or through intravenous infusion. Cerebrospinal concentrations can under some circumstances be determined via lumbar puncture, but this procedure will generally not meet volunteer consent.

Estimations of oxytocin penetration into the central nervous system after intranasal application are based on an experiment in which vasopressin (among other peptides) was applied to human subjects in whom such access was possible [31]. Vasopressin levels in cerebrospinal fluid increased 10 minutes after application and continued to rise for some 80 minutes, while plasma levels hardly increased, suggesting the peptide followed a direct route to the central nervous system, probably by passing through intercellular clefts in the olfactory epithelium, followed by diffusion into the subarachnoid space. Assuming that the basic tail of vasopressin is not vital for this uptake process, this experiment can serve as a model for intranasal passage of oxytocin. Table 5.1 shows estimates of the cerebrospinal levels obtained in the aforementioned human studies, using this vasopressin model[1]. Note

[1]There are many criticisms to be pointed at using the vasopressin experiment in this way. First, entrance through the epithelium may depend strongly on the basicity of the peptide tail. Secondly, scaling uptake linearly with the applied amount of compound, as was done in the model, may not hold. Thirdly, basal vasopressin and oxytocin levels may not always be comparable. Fourthly, feedback effects on vasopressinergic and oxytocinergic neurons lining the arachnoid and ventricular spaces are not identical. The model used is therefore overly simplistic, but it is the best estimate

that these are estimates of ultimate levels - the vasopressin experiment showed a steady build-up to these levels over about 80 minutes. The oxytocin experiments discussed in the previous chapter were set up in such a way that plateau levels were reached during their performance (with the exception of the post-task oxytocin administration in reference [231]).

As noted in section 1.1.1, cerebrospinal fluid oxytocin levels at baseline are about 8 pM, meaning extrapolation from the vasopressin experiment yields double baseline concentrations. The significance of this depends on the endogenous role of cerebrospinal oxytocin. In the more traditional view, cerebrospinal fluid is the endpoint for neurochemicals from a stream that already acted on its neural targets. Taking this view would mean intranasally applied oxytocin elevates peptide levels in the brain's "waste bucket": volume effects from this space can occur, but only in periventricular regions, and in a manner that has nothing to do with the synaptic release that occurs endogenously.

There is another view, that emphasizes endogenous volume transmission over synaptic transmission and that considers the ventricular system and the arachnoid space to be a medium for hormonal messages [267]. In this view, intranasally applied oxytocin would add to an important oxytocin component and effectively doubling cerebrospinal concentrations could have great consequences. There are two main arguments to consider such a primary role for the ventricular system in oxytocin function. First, the main oxytocin synthesis sites are the paraventricular and supraoptic nuclei of the hypothalamus, which border the third ventricle and the arachnoid space, respectively. This makes them ideally positioned to release oxytocin into the cerebrospinal fluid directly[2]. Secondly, many oxytocin-receptive neurons in important recipient areas from the animals studies line the ventricular space. This does not include the amygdalar nuclei, but it does include the bed nucleus of the stria terminalis, which can be considered an important extension of the amygdala.

currently available.

[2]In fact, dendrites in both groups of nuclei are preferentially oriented towards the ventricular space. Also, dendritic release is accompanied by great positive feedback, thus ensuring mass release [187]: it has been argued 95% of oxytocin release occurs dendritically [223]

As the "waste bucket view" of cerebrospinal fluid does not seem to match the significant behavioural effects following intranasal spray use, the volume transmission role of ventricular oxytocin seems plausible. This then, would be the role that intranasally applied oxytocin mimicks, while ignoring the synaptic part of the oxytocin system. A comparison of the estimated levels shown in table 5.1 with known behaviour-related levels in animals lends credence to the idea that intranasal oxytocin can be compared to endogenous release: three- to five-fold increases in cerebrospinal fluid oxytocin levels have been found in maternally behaving sheep [141] and ejaculating rats [118], while two- to four-fold increases of baseline cerebrospinal oxytocin are estimated to be used in the behavioural studies.

Intravenous infusion is an entirely different matter. As mentioned in section 1.1.1, oxytocin does not readily cross the blood-brain barrier. However, the fact that the behavioural effects mentioned in section 4.3 occur means that the compound does pass into the central nervous system, possibly through active transport. As of now, no estimates of central uptake via infusion are available.

5.1.2 The sociality of faces

The facial memory and social reinforcement experiments show dependencies on oxytocin that are tempting to interpret in the framework of the peptide being a "sociality hormone". However, operationalizing social stimuli as pictures of faces while assuming other photographs or coloured dots are non-social may be premature. There is more difference between a face and a coloured dot than sociality alone. Some of these differences may derive from faces being social, such as their relative salience, while others may relate to picture symmetry or stimulus feature detail.

However, if oxytocin were to act on highly salient cues selectively, this would not argue in favour of it being sociality-specific. Similarly, if the peptide acts on a brain region that processes stimuli with reflection symmetry, sociality-specificity would not follow. While the social reinforcement learning experiment is consistent with an exclusively social view of oxytocin, it cannot be considered as evidence.

Table 5.1: Estimated oxytocin uptake in the intranasal oxytocin spray studies discussed in this thesis. Modeled on the vasopressin uptake data from reference [31], the expected concentration of oxytocin in cerebrospinal fluid (CSF) after 80 minutes is shown. Loading time signifies the amount of time between intranasal administration and initiation of the oxytocin-dependent experimental part. Calculations were done assuming 600 International Units (IU) per mg of vasopressin and 500 IU per mg oxytocin.

Study	Reference	Application	Peptide (μmol)	CSF (pM)	Loading time (min)
Transnasal vasopressin	[31]	40 IU (0.067 mg)	0.062	18	n/a
		80 IU (0.13 mg)	0.120	37	n/a
Trust game	[149]	24 IU (0.048 mg)	0.048	14	50
fMRI trust Game	[20]	24 IU (0.048 mg)	0.048	14	50
Ultimatum game	[282]	40 IU (0.080 mg)	0.079	24	60
Face recognition	[103]	24 IU (0.048 mg)	0.048	14	45
Face recognition	[217]	24 IU (0.048 mg)	0.048	14	40
Face recognition	[231]	20 IU (0.040 mg)	0.040	12	30, 1440
Eye gaze	[102]	24 IU (0.048 mg)	0.048	14	45
Fear processing	[146]	27 IU (0.054 mg)	0.054	16	30
fMRI face processing	[65], [67]	24 IU (0.048 mg)	0.048	14	45
Reinforcement learning	[120]	24 IU (0.048 mg)	0.048	14	45
Pain empathy	[239]	32 IU (0.064 mg)	0.064	19	45
Reading mind in the eyes	[66]	24 IU (0.048 mg)	0.048	14	45
Parochial altruism	[56]	24 IU (0.048 mg)	0.048	14	30
Ball toss game	[4]	24 IU (0.048 mg)	0.048	14	50
Face perception	[4]	24 IU (0.048 mg)	0.048	14	50

Much the same reasoning goes for the use of non-facial stimuli - a typical image database contains a random collection of objects and places, but these are rarely matched with faces for symmetry, luminance, detail and the plethora of other image properties that may be relevant for visual processing.

5.1.3 Taking it to the streets

Regardless of how well it mimicks endogenous action, the behavioural effects of intranasal oxytocin are undeniable. Yet, the question remains if experimental setups can be translated to the real world. Oxytocin may increase generosity in an ultimatum game, but will it do so during a real economic transaction, where the stakes may be higher, past experience may be more salient and negotiations take place through all sorts of posturing and promising? The simplest view would be that the role of oxytocin is additive: it decreases any distrust and increases any trust. True cognitive dynamics may be of a wholly different kind, however, as interaction effects between oxytocin and other trust signals could occur. For example, it may be that if an initial oxytocin-induced feeling of trust is violated by an untrustworthy move, trust decreases more rapidly than it would by the untrustworthy move alone. Using a subtle variation on the trust game paradigm, recent work by Moïra Mikolajczak indeed suggests oxytocin's trust-inducing effects are not additive - no measureable increase in transfer was found toward trustees which were previously described as untrustworthy [183].

An even clearer example is the study on parochial altruism. This study served as a reminder of what Garett Hardin dubbed *modern tribalism* [108]: the tendency of groups to both include and exclude. It is not clear, however, to what extent performance in a prisoner's dilemma game can be extended to workfloor behaviour, or the motivations of terrorist cells. Both can be real world examples of modern tribalism, but they do not necessarily involve the same psychological or neural mechanisms as are at play during the prisoner's dilemma game[3].

[3]This is a critique that applies not only to the prisoner's dilemma game, or just the game theoretical paradigms of

If the experimental findings are extrapolated to claims about *human nature*, not just behaviour in western societies, an additional complexity arises. From visual processing to performance in economic games, cross-cultural differences have been found that implicate a rather malleable human cognition [112]. Once academic behavioural findings - mostly performed on undergraduate students of the behavioural sciences - are used to make inferences on the evolution of cognition, it should be realised the pool of sampled data is biased indeed.

While the controlled laboratory settings single out a potential contribution of oxytocin to a wide range of functions, this should not be immediately taken to mean that oxytocin is important, or even involved in these functions if they occur in daily life. That being said, research on oxytocin in both animals and humans has the potential to clarify the picture, not only of neuropeptide function, but of sociality itself.

5.2 Implications

5.2.1 The social signalling hypothesis revisited

In the Interlude, a proposal was put forward that considered oxytocin to be a signal, informing brain regions that the current environmental context is social in nature. This social signalling hypothesis fits with the finding from animal studies that the neuropeptide acts on general-purpose circuitry, activating or inhibiting them as required by ecology and organism state. The notion that the effects of intranasally applied oxytocin mimick functions normally mediated by ventricular transport of the neuropeptide seems to be in line with this idea: volume transmission is by definition not targeted and its temporal or even molar structure is unlikely to contain any more information than a binary cue that context is social, or pleasurably social.

Such volume transmission of a sociality signal to general-purpose regions cannot account for the finding that social reinforcement was enhanced selectively, though (see section 4.2). If intranasally applied oxytocin would enhance general memory by falsely giving the impression of a good or important social context, it should have done equally for faces and non-faces. Similarly, the experiments on trust and generosity discussed in section 4.1 contained a non-interpersonal control condition. If oxytocin acted on a general-purpose substrate, performance in this non-social condition should be affected as much as that in the social condition.

These results indicate oxytocin acts on a neural substrate that specifically processes social information - at least under the operationalizations of sociality as used in the cited studies. Sociality may be determined in the region(s) on which oxytocin acts directly, or in upstream circuits. Whichever is the case, the dissociation found for oxytocin effects on social and non-social processing suggests a *social module* in the mind[4]. This idea is not new: social modules have been proposed by evolutionary psychologists John Tooby and Lea Cosmides to explain reasoning skills [44] and more recently cognitive neuroscientist Ralph Adolphs has put himself to the task of mapping the "social brain" [2], a proposed network of brain regions that are devoted to sociocognitive processing. Both lines are inspired by what is known as the *social brain hypothesis*, the idea that the evolutionary growth spurt of brain size and cognitive capacity that led to the rise of *Homo sapiens* occurred under selective pressure of an increasingly complex social environment [274][5].

Proposed modularity is most often based on a number of key sociocognitive skills in which *Homo sapiens* is especially proficient among the known living primates. These skills are often grouped under the blanket term *theory of mind*. As mentioned in section 4.3, autism and related conditions have been conceptualized as a deficit in the neural apparatus underlying formation of such theory of mind and oxytocin attenuates some of their symptoms.

experimental economics. Behavioural sciences often employ game-like tasks. Insofar as these are not skill tests, but probes into the motivations and strategies of people, they assume what could be called the *Grand Theft Auto fallacy*: that people behave in daily life as they do in games.

[4]Introduced into 1980s cognitive science by Jerry Fodor, the core idea of a *modular mind* holds that functionally specialized - i.e. domain-specific - units underlie cognitive functioning. For example, if a language module would exist, a part of the mind would exclusively be dedicated to processing language. A consequence of this would be that language could be lost without affecting other aspects of cognition. For this reason, theorists often cite lesion studies if debating a proposed modularity.

[5]The social brain hypothesis does not immediately imply modularity. Increased social complexity could also lead to selection of improved general cognition.

If the idea that oxytocin enhances sociality-specific cognition holds, it is tempting to extend the social signalling hypothesis to include action on social modules. Oxytocin transmission would then not only put general-purpose circuits in "social mode", but also regulate sociality-specific brain regions.

5.2.2 The social brain

The question then, is which regions *are* sociality-specific? Ralph Adolphs has suggested a number of regions to be involved in the social cognition found in humans (see table 5.2), but they have been shown to be involved in non-social processes as well. This might mean that they are not social modules, but it could also be that current techniques lack the spatial resolution or specificity that is necessary to disentangle modules. In fact, although rarely considered by modularists, it may be that modules are topographically dispersed and seemingly non-contiguous. As in language, music and other domains of cognition, the modularity of sociality is subject of an ongoing debate among social cognition researchers.

Oxytocin may prove to be an excellent probe for social modularity. If the neuropeptide is "modular" itself, its binding sites may help elucidate the constituent regions of the social brain. If it turns out not to be modular, it is likely still revealing, as social processing can then (partly) be interpreted in light of the general-purpose regions on which it acts.

5.2.3 Sympathy and the origin of morality

If future research strengthens the idea that human bonding - whether romantic or maternal - is mediated by oxytocin and if the interpretation of oxytocin as a modulator of *theory of mind*-like capacities holds, an interesting view on humanity comes to light. It has long been claimed that the mind-reading and empathizing that humans display is beyond anything found in other denizens of the animal kingdom. The human capacity for sympathy may be unrivalled and, building on the experiments and speculation discussed above, may have developed in accordance with the oxytocin system.

In the view of Scottish Enlightenment philosopher David Hume, such sympathy is a proper basis of ethics [119] - our ideas of right and wrong can often, if not always, be understood as part of a sentimental effort to take the suffering of others into account[6]. The idea sits well with the social brain hypothesis and the evolutionary development of theory of mind - the evolution of morality could go hand in hand with increasing social complexity. Could oxytocin then, as a modulator of trust and possibly mind-reading, play a role in this evolution? Could the development of a system that enhanced earthworm egg-laying provide a naturalistic account of the evolution of ethical systems?

It is an admittedly speculative view, but it is one espoused by Canadian-American philosopher Patricia Churchland [42]. While leaving space for cultural, ecological and historical contingencies to shape moral evolution, she follows the Humean argument and considers the oxytocin system to lie at the heart of it. Without disregarding the caveats of ecological validity from section 5.1, there is indeed reason to do so. The oxytocin view of morality would consider sympathy to be an extension of romantic and parent-offspring bonding, integrating the family or the clan into "loved ones". As the definition of the group abstracts - from clan to village, from village to city-state, from city-state to nation, and from nation to global village - so does the applicability of *in-group love*. This historical process did not go smoothly, as innumerable accounts of warfare and group violence show. Where the prairie vole complements its pair-bonding with *mate guarding*, humans may complement their sympathy and communal bonding with *out-group hate*.

The way in which *in-group love* increased its scope is a subject of considerable debate. One might claim, like Adam Smith did, that humans have an innate propensity to sympathize with those like them and that only ecological or cultural demands can put them to consider others as "out-group". It could also be, and this is more in line with the philosophy of David Hume, that the development of institutions which provide security (e.g., the rule of law or the state protectorate) stimulate

[6]This idea cannot be attributed exclusively to David Hume. His ideas were a refinement of the earlier work of Anthony Cooper, the Third Earl of Shaftesbury and of Francis Hutcheson, a Scottish utilitarian philosopher not unlike Hume. Adam Smith, a good friend of Hume, developed the idea further in *A Theory of Moral Sentiments* [243].

Table 5.2: .

Brain region	Sociality-specific association [2]	Non-social association
Fusiform Gyrus	Specialized Face processing	Expertise feature discrimination [89]
Superior Temporal Gyrus	Voice/face integration	Cross-modal integration [23]
Amygdala	Facial affect processing	Emotional/salient processing [283]
Orbitofrontal Cortex	Social norms/regret	Reward expectation [233]
Ventral striatum	Facial attractiveness	Subjective aspects of reward [136]
Anterior Cingulate Cortex	Regulating social emotions	Aspects of cognitive control [196]
Insula	Empathy, moral sentiment	Pain, disgust, feeling [238]
Temporoparietal Junction	Theory of mind abilities, moral reasoning	Temporal order judgment [54]

trust of strangers[7]. A thorough discussion of these and other ideas on the historical evolution of sympathy and morality is beyond the scope of this text, but it is interesting to consider them in relation to the biological bonding mechanisms elaborated in the previous chapters.

5.2.4 *Homo addictus*

The idea that human sympathy follows extension of the bonding system puts its roots in the mesolimbic dopamine pathway and the associated pair-bonding circuit, which can be summarized as an oxytocin-boosted reward system. The emergence of such a system can be understood as an economical convergence between reproduction-related neural circuits and the reinforcement system, which makes organisms seek food and other rewarding stimuli, while avoiding harmful ones. This convergence carries a risk, however. Much like pair-bonding may have co-opted mother-offspring bonding during evolutionary history - and much like human group behaviour may have co-opted the pair-bonding system - the existence of agonistic oxytocin-dopamine interactions leaves the reward system more vulnerable to chemical hijacking[8]. Indeed, some drugs of abuse have been associated with oxytocinergic action. MDMA was mentioned before, but γ-hydroxybutyrate[9] (GHB) administration also leads to oxytocin receptor activation as witnessed by increased levels of receptor mRNA [266].

Moreover, it is not implausible that the development of ever more complex forms of bonding, which all need distinct processing, has led to an expansion of the reward circuitry and its downstream effects. The subjective experience of love - be it romantic, parental or group-based - may be a relatively new adaptation to further reinforce bonding-related behaviour. This would not only lead to the possibility of large-scale economies and moral systems, but also to an increasing amount of sites at which drugs of abuse can act. It is known virtually all such drugs act primarily on mesolimbic structures[10] and, as noted in section 4.4.2, the neural activation pattern associated with cocaine euphoria is comparable to that of a mother seeing her child. Drug addiction can be seen as a particularly strong form of bonding - there's even a self-sacrificial element in the junkie foregoing his physical constitution for access to his drug of choice.

Perhaps human civilization - its economy, its morality - derives from a hyperdeveloped system of compassion, such as David Hume and Patricia Churchland hint at. This neural capacity for love, however, may have come at the price of an enslaving sensitivity to all compounds that exogenously drive the system. Hopeless romantics are heard to proclaim that love is an addiction, but it may be a neurobiologically more reasonable stance that addiction is a love.

It should be stressed that this reasoning applies at the species level. At the individual level, variation in the "bonding system" does not necessar-

[7] Cognitive anthropologist Joseph Henrich recently proposed markets may serve a similar role, institutionalizing trust behaviour as measured by economic games [111].

[8] A comparison of the efficacy with which amphetamine increases nucleus accumbens dopamine in the prairie and meadow voles of chapter 3 yielded that monogamous voles are more sensitive to the drug [49].

[9] GHB was once used to promote child delivery, just like oxytocin, as it acts as an oxytocin receptor agonist both peripherally and centrally.

[10] Drug action at the mesolimbic level has been associated with the *wanting* found in addicts, that may be disassociable from subjective *liking* [26].

ily equate with variation in drug sensitivity. Indeed, one may speculate that humans who are brought up in environments severely lacking secure attachment, such as the orphans mentioned in section 4.4.2, can have a poorly developed neural system of bonding but are at increased risk of drugs abuse, perhaps precisely because oxytocin-induced dopamine release is impaired and because a compensatory mesolimbic activator is sought out.

5.3 Applications

From the scientist's perspective, research on the behavioural effects of oxytocin is only just beginning and the speculation of the peptide as an explanation for several social cognitive questions is a mere motivation. The commercial sector, however, has already jumped at the opportunity to market an "all-natural" compound that increases trust and prosocial behaviour. Carrying such enticing names as *Liquid Trust*, oxytocin sprays are sold as perfumes that are claimed to boost success at job interviews, business meetings and in relationships (see figure 5.1).

Such sprays are very unlikely to be effective. At a molecular weight of more than 1017 g mol^{-1}, the volatility of oxytocin has not even been determined. It is implausible any oxytocin worn on the body ever reaches the nasal cavities of others, let alone their central nervous system. Even if someone were to inhale oxytocin off the skin at contact, this will in no way equate to the 20-40 pharmacological units applied in the studies shown in table 5.1.

That is not to say there is no application at all for oxytocin sprays, or other forms of peptide intake. Clinical, therapeutical and consumer use of oxytocin for its central effects await field testing.

5.3.1 Clinical applications

The most obvious clinical application of oxytocin has been discussed in section 4.3. Autists or people with Asperger's syndrome may benefit from oxytocin's effect of reducing repetitive behaviour, improving performance on facial processing tasks and promoting prosocial behaviour, as measured in the virtual ball tossing game. It will be interesting to see whether emotional recognition in a laboratory setting can be translated to daily life. One of

Figure 5.1: Oxytocin is marketed as a perfume that will increase sex appeal and trustworthiness. It is highly unlikely the spray will have any such effect.

the first challenges, however, will be to move away from infusion and towards intranasal administration. Reproducing the infusion-based findings using an oxytocin spray could pave the way to "field studies" with higher ecological validity. This may be easier said than done: the distribution of oxytocin across the central nervous may be entirely different from that attained by intravenous infusion. Intranasal doses that mimick the infusion effects may introduce side-effects that were not visible in the pilots using intravenous administration. However, the results on face processing and social engagement found in high-functioning autists and people with Asperger's syndrome after intranasal oxytocin administration are promising.

Another clinical case that may benefit from oxytocin effects are people with social phobia. If the interpretation of social anxiety reduction is correct, such patients could benefit of oxytocin. Indeed, a recent trial combining intranasal oxytocin and exposure therapy found short-term improvements over placebo [101]. A related role for oxytocin

would be in the treatment of depression, which is associated with negatively biased facial affect processing and hyperactivity of the amygdala [87]. Although the anxiolytic effects of oxytocin in animals have long been known and can be extended to typical animal models of anti-depressant function (for a recent study, see [39]), the usefulness of oxytocin in depression treatment has not been investigated in humans yet.

Given the proposed interaction of the oxytocin-dopamine system with drugs of abuse, oxytocin may prove to be relevant for battling substance addiction, as well. In rats, oxytocin treatment has been argued to decrease methamphetamine addiction-related behaviour [36]. Whether this happens through occlusion of drug-induced monoamine transmission or whether oxytocin blocks drug action can not be ascertained from the current literature, but it will prove interesting to extend these findings to human trials.

5.3.2 Therapeutical applications

As mentioned in section 4.3, people with (high-functioning) autism or Asperger Syndrome may benefit from an adjunct use of oxytocin in social skills training. The enhancement of social reinforcement learning may aid them in the acquisition of social norms. This application is not limited to people with autism spectrum disorder, however - therapies aimed at alleviating social norm violations in children with conduct disorder could also profit off oxytocin administration.

The trust induction of oxytocin may be rendered useless by signals that lower trust (see section 5.1.3) but situations exist in which the will to trust exists, but realization of that trust is poor. Relationship therapy may find use for a compound that has been shown to increase trusting behaviour and may also promote interpersonal bonding. The fact that marital discussion of conflict became more positive under oxytocin administration, as mentioned in section 4.4.1, suggests that such applications are realistic. If the latter can be shown to hold true in future studies, oxytocin may also be applicable in other settings where bonding is to be promoted, such as in adoption processes or other challenges for parent-child bonding.

5.3.3 Consumer applications

The consumer buying *Liquid Trust* to become more succesful in love and business may be wrong to use it as an odourant, but intranasal administration could help him overcome moderate forms of social anxiety. This, in turn, could increase performance during public speaking and other social stressful circumstances.

Given the role of oxytocin in "in-group love" (see section 4.4.3), its application may also serve organizations that seek to promote cooperation among its members. Although it remains to be seen whether oxytocin is a sensible replacement for more traditional forms of group bonding, future developments may prove it to be a viable tool to boost intergroup trust and productivity.

5.4 Summary

Research on oxytocin in humans has only just started in earnest and challenges remain to translate research findings into applications and theoretical frameworks. The fact that intranasal oxytocin administration affects behaviour suggests a functional role for volume transmission of the compound through the ventricular system.

The seemingly sociality-specific role of oxytocin in humans suggests such volume transmission does not only act on general-purpose circuits, but also on social modules, in line with arguments from evolutionary psychology that cognitive complexity increased as an adaptation to social challenges, yielding sociality-specific brain circuits. Oxytocin may prove to be an excellent probe for such social modules.

The notion that many forms of bonding and even mind-reading interact with the oxytocin system has led to the suggestion that the mesolimbic circuit plays a central role in the evolution of not only maternal and romantic love, but also sympathy and the emergence of more complex facets of human sociality, such as the cooperation, coordination and division of labour that underlies human economies. Sympathy may even, in line with Scottish Enlightenment philosophers like David Hume and Adam Smith, provide the foundation of morality. Thus, ever-increasing functionality of the mesolimbic system may provide a naturalistic account of the emer-

gence of ethics. The dark side of this love system may be increased sensitivity to its activation by drugs of abuse.

Many of the promising applications of oxytocin still need to be translated to clinical and societal settings, but they may range from treatment of social phobia, depression and addiction to attenuation of symptoms of autism. Therapies aimed at improving relationships or social norm functioning may also benefit of oxytocin.

Chapter 6

Conclusion

Oxytocin's role in bonding processes has not been completely translated to humans, but it is plausible that the effects oxytocin does have in humans are related to bonding. The enhancement of trust, generosity, mind-reading, facial affect processing and in-group love can all be interpreted in light of a evolutionarily extended *love circuit*. That being said, a number of questions remain open for future animal and human research.

6.1 Future research

6.1.1 Animal research

Many of the biochemical details on oxytocin action are lacking. How does oxytocin add to dopaminergic transmission in prairie voles? What does the oxytocin-mediated regulation of nucleus accumbens by medial prefrontal cortex entail? Many of the complex interactions of oxytocin with other chemicals have been left out of this thesis, but their elucidation will prove vital to proper use of the compound in humans. For example, the anxiolytic role of oxytocin is likely to occur not only through direct inhibition of amygdalar nuclei, but also through interactions with the hypothalamic–pituitary–adrenal axis. If oxytocin is to be used in treatment of depression, addiction and anxiety, detailed animal models of its neural effects are necessary.

Some regions have been convincingly argued to be vital to oxytocinergic effects on cognition, but it is not always clear how. Why do mice rely so strongly on oxytocin levels in the medial nucleus of the amygdala to succesfully recognize conspecifics? Does the amygdala serve as a substrate of memory, or is recognition-induced behaviour specifically impaired?

The regulation of oxytocin release takes place at many levels, from DNA methylation and (hormonal) regulation through neurotransmitter feedback effects to behavioural induction. Mapping all these effects will prove a major, but important challenge. An especially interesting hypothesis to explore is if an established social memory in rodents can induce oxytocin release upon activation by itself. Despite the large body of knowledge on effects of oxytocin release, knowledge on its causes are limited to positive autofeedback, tactile input and the effects of exogenous compounds.

This latter question is especially important in species that rely more on distal investigation than do rodents. Non-human primates may be excellent model organisms to explore visual and cognition-driven stimulation of oxytocinergic cells. Using plasma levels as a proxy for central oxytocin, it has been found vocalizations can drive oxytocin release in humans [234], but such research lacks neural details. Non-human primates may also provide an interesting link in relation to the proposed social evolution. Given the rich social structures found in a number of primate species, the development of in-group love may be observed by researching them through the lens of the oxytocin system. It will also prove interesting to repeat the neuroimaging work on romantic love in pair-bonded coppery titi monkeys. The PET results mentioned in section 3.4 showed little overlap with the human data, but this may be because baseline glucose uptake was measured, instead of task-related activation.

A comparative approach can also explore the thesis that animals more capable of bonding are also more sensitive to drugs acting on the mesolimbic

circuitry. While amphetamine reinforcement in different vole species supports the view [49], it will be interesting to see if the finding can be extended across more wide-ranging species (and drugs). As ethological data on sociality already exists and drug sensitivity has been explored across species, many of the necessary data is likely to be available and awaiting integration.

A final exciting thing to investigate is whether the apparent social modularity of oxytocin that is found in humans can be extended to animals. In the current explanation of oxytocin-enhanced encoding, all incoming odours are treated equally. It would be interesting to see if stimuli that are demonstrably "social", such as vocalizations or maternal odours, are preferentially consolidated under oxytocin influence, as compared to non-social odours or sounds.

6.1.2 Human research

Besides the trials needed to apply current knowledge to clinical settings, as discussed in section 5.3.1, more active research on the effect of oxytocin in humans is needed in order to test the ideas put forward in the previous chapters. To properly interpret the behavioural and neuroimaging results obtained in human research, knowledge of oxytocin binding sites is vital. As of now, a discrepancy exists between these findings and autoradiographical work. In the early 1990s, Fabienne Loup and colleagues mapped oxytocin binding sites in the brains of middle-aged to elderly subjects, but the lack of binding found in limbic and neocortical areas makes the neuroimaging results difficult to explain. Use of a younger subject pool and more recent autoradiographical technology may shed light on the current paradox.

Binding sites are not the whole story - only those regions effected by intranasal administration of oxytocin are relevant to understand the data. A better view of these regions may also add or subtract plausibility from the ventricular version of the social signalling hypothesis. To get such a map, radionuclide labelled oxytocin could be applied, with subsequent PET measurement. If tests indicate receptor binding and transport is not affected, PET should provide a neural map with which to interpret the behavioural and neuroimaging results from the previous chapters.

Given the role of oxytocin in animal pair-bonding and maternal care, it is not outrageous to claim oxytocin is involved in romantic and maternal love in humans. However, current imaging results only show an overlap with the mesolimbic system, which by itself does not mean oxytocin is involved. Manipulations showing a role of the peptide in other forms of bonding than those involved in parochial altruism is important to ground the idea of continuous development of the oxytocin system in more firm, empirical footing.

As mentioned in section 5.1.2, the operationalization of sociality is less than perfect. It is difficult to think of a paradigm that bypasses all criticism, but using a number of different operationalizations can lead to convincing, convergent evidence. For example, reproducing the findings on facial stimuli within the auditory modality would go a long way in ascertaining the effects of oxytocin are not due to trivial stimulus features. Alternatively, proper control experiments may seek to find non-social enhancement by oxytocin. For example, the option of symmetrical features processing given in 5.1.2 could be investigated by offering such stimuli and testing the effect of oxytocin.

As in animals, the stimuli that lead to oxytocin release need to be elucidated. Especially in humans, feelings of affection and prosocial behaviour can be elicited by auditory and visual cues. As mentioned, recent research has suggested vocalization to upregulate oxytocin in humans. Understanding what processes underlie such release will likely involve both animal and human studies. One particularly exciting idea is to follow oxytocin release from the hypothalamus using magnetic resonance spectroscopy. Feasibility studies will need to be done to investigate how viable this option is.

Given the dependence of oxytocin effects on social status in squirrel monkeys (see section 3.4), it will also be interesting to investigate the effect of status in humans. Current experiments put subjects in novel (social) environments, which may preclude dominant behaviour. Repeating the economic games using established social hierarchies may yield wholly different effects of oxytocin on behaviour.

A final point that needs further exploration is the biochemical significance of the rs53576 polymorphism. Given its relation to behavioural indices and brain structure, it is worth investigating how

the SNP affects the transcription and translation of the *OXTR* gene.

6.2 Theses

6.2.1 A core oxytocin system, associated with social behaviour, is conserved across species

This claim is a general one and is hard to prove definitely. The fact that oxytocin is a mammalian neuropeptide speaks against it in a trivial sense. More importantly however, oxytocin has indeed been associated with social (i.e., interpersonal) behaviour in all mammals in which it has been investigated. Homologs of the compound, which were not subject of this thesis, serve similar functions in non-mammals.

To say a *core* oxytocin system is preserved fits with the fact that the supraoptic and paraventricular nuclei of the hypothalamus are consistently shown to be the source of endogenous oxytocin, with oxytocin receptor distribution varying across species to fit ecological demands. Indeed, the ventricular release and the interaction with a number of key regions, including the amygdala, point to an evolutionarily ancient system that is common to all mammals.

However, it is important to add that the innervation of this core system and its synaptic targets may not have been conserved across evolution. Indeed, while somatosensory input underlies rodent oxytocin release, other forms of input are likely to be important to primates. Current studies suggest a preserved system of synthesis and release, complemented by a flexible expression of oxytocin receptors that explains behavioural variety. Connectivity data is sparse, however and the exact wiring of the core system may prove to vary greatly across species.

6.2.2 Species-specific sociality can be understood through oxytocin action

This thesis has focused on the social role of oxytocin, but as previously mentioned it is not the only molecule that plays this part. Mate guarding is a social behaviour that is related to vasopressin. Oxytocin knock-out mice show some maternal behaviour and interactions with other mice. Even the clear species-specific behaviour of male prairie vole pair-bonding can be manipulated by alteration of vasopressin levels - a finding most dramatically illustrated by the viral insertion of *Avpr1a* in the promiscuous male meadow vole ventral pallidum, rendering it monogamous. The role of oxytocin is a social one, but it is certainly not the only molecule regulating such behaviour across species. Therefore, not all (species-specific) sociality can be understood by focusing on oxytocin.

6.2.3 Oxytocin signals sociality and acts only on general-purpose circuits

The notion of oxytocin as a molecule signalling social context fits with current empirical findings. In animals, results can be explained as oxytocin affecting general-purpose circuits. However, this may be because the thesis of social modularity of oxytocin in animals has not been explicitly investigated.

Whether social modularity exists in humans is the subject of a fierce debate. The studies on oxytocin in humans suggest it to be the case, but this could be an artifact of the paradigms that were used to operationalize "sociality".

6.2.4 Autism, social phobia and attachment disorders can be conceptualized as oxytocin system disorders

Some evidence connects autism with polymorphisms of the *OXTR* gene and with its regulation, which may explain why oxytocin administration alleviates a number of the symptoms encountered in autists. Low oxytocin plasma levels also support the thesis. Still, insights connecting these polymorphisms to the condition are lacking and are awaiting further neurodevelopmental work.

In a way, conceptualizing autism as the consequence of a poorly developed oxytocin system seems to be a return to the days when attachment theorists, inspired by Freudian thought, explained the condition by blaming "refrigerator moms". The fact that the social deficits of Harry Harlow's rhesus monkeys, which were cited as biological proof of

attachment theory back then, are now interpreted in a similar oxytocin-centered way as autism (see section 3.4), only adds to this suspicion. It should be emphasized, however, that deficits of the oxytocin system can be caused in several ways. Apparently, very poor early-life experiences *can* be a cause, as the orphan studies show. However, genetic and neurodevelopmental processes outside the reach of upbringing are just as likely to affect the oxytocin system and, if the two are indeed strongly related, the development of autism.

A similar reasoning goes for attachment disorders. Some dimensions of attachment have been associated with the oxytocin and the *OXT* gene. Social phobia, while possibly treatable with oxytocin, has not been shown to relate to deviant oxytocin levels, or related genes.

6.2.5 Addiction is a love

Given the relevance of the mesolimbic system for different forms of love, the thesis that drugs of abuse hijack systems that evolved for bonding is tempting. The only study to date that looked into such an effect compared methamphetamine reinforcement in monogamous and promiscuous voles and indeed found that the monogamous species was more "at risk" for addiction.

6.2.6 Man is a soft machine

In William S. Burroughs 1961 beat novel *The Soft Machine*, man is portrayed as an organism seeking to abuse his own metabolism for pleasure through chemical means. Such drug abuse seems to stand far from the poem of Leo Vroman with which this thesis opened, but given the interactions between oxytocin, dopamine and addictive substances, the love-seeking human from Vroman does not drift far from the darker vision Burroughs espoused.

To say that man is a soft machine is to emphasize a specialized neural machinery, dedicated to maternal, romantic and group love. This view, while still in need of firmer empirical grounding, is plausible in light of the possibility that oxytocin does not only interact strongly with the mesolimbic dopamine system in humans, but also affects brain regions in a seemingly sociality-specific way, thus connecting natural reinforcement with a wide array of affiliative processes. This may not be different than in other social mammals, but the findings that oxytocin also affects communal bonding and high-level social cognition suggests that the oxytocin system may be especially well-developed in humans.

Indeed, if it is true that increasing interactions between the oxytocinergic and mesolimbic dopamine system underlie the evolution of empathy, morality and that complex systems of economy and government follow from them, the most striking aspects of humanity can be understood in terms of a hyperdeveloped love system. In that case, man is indeed a soft machine.

Acknowledgments

The writing of this literature thesis, part of the MSc programme *Brain and Cognitive Sciences* at the University of Amsterdam, greatly benefited from the advice and encouragement given by Wim Ghijsen and the aid of Peter Burbach, to whom I would like to extend my gratitude. I would also like to thank Kendall Rattner, who proofread the manuscript and pointed out a number of Dutchisms in my English, and Diana Deca, who patiently listened to my rantings on oxytocin, Scottish Enlightenment and the evolution of morality, before directing me to the work of Patricia Churchland. Yordy van der Werff, who gave me his time and effort by designing the cover art and Paul Foch-Gatrell, who made some of his excellent wildlife photography available to me, also deserve my gratitude.

Apart from those helping me write *The Soft Machine*, I would also like to thank the people that allowed me to start it in the first place. Hans Matthijs and Iris Hettelingh deserve special mention, as my academic Odyssey would have ended prematurely without their help. I am also indebted to Cyriel Pennartz and Marijn van Wingerden, under whose mentorship I could transform myself from a biochemist to a student of the brain. The unconditional support from my friends and family has been invaluable throughout all these years, too, and will be forever appreciated. I thank you all, for your well-developed oxytocin systems.

Vincent Tijms, August 2010

Bibliography

[1] R. A. Adan, J. J. Cox, J. P. van Kats, and J. P. Burbach. Thyroid hormone regulates the oxytocin gene. *The Journal of Biological Chemistry*, 267(6):3771–7, Feb. 1992.

[2] R. Adolphs. The social brain: neural basis of social knowledge. *Annual Review of Psychology*, 60:693–716, Jan. 2009.

[3] R. Adolphs, D. Tranel, and A. R. Damasio. The human amygdala in social judgment. *Nature*, 393(6684):470–4, June 1998.

[4] E. Andari, J.-R. Duhamel, T. Zalla, E. Herbrecht, M. Leboyer, and A. Sirigu. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America*, 107(9):4389–94, Mar. 2010.

[5] B. J. Aragona, Y. Liu, J. T. Curtis, F. K. Stephan, and Z. Wang. A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. *The Journal of Neuroscience*, 23(8):3483–90, Apr. 2003.

[6] B. J. Aragona, Y. Liu, Y. J. Yu, J. T. Curtis, J. M. Detwiler, T. R. Insel, and Z. Wang. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nature Neuroscience*, 9(1):133–9, Jan. 2006.

[7] B. J. Aragona and Z. Wang. Opposing regulation of pair bond formation by cAMP signaling within the nucleus accumbens shell. *The Journal of Neuroscience*, 27(48):13352–6, Nov. 2007.

[8] M. A. Arbib. From monkey-like action recognition to human language: An evolutionary framework for neurolinguistics. *Behavioral and Brain Sciences*, 28(02):105–124, Apr. 2005.

[9] A. Aron, H. Fisher, D. J. Mashek, G. Strong, H. Li, and L. L. Brown. Reward, motivation, and emotion systems associated with early-stage intense romantic love. *Journal of Neurophysiology*, 94(1):327–37, July 2005.

[10] M. J. Bakermans-Kranenburg and M. H. van IJzendoorn. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Social Cognitive and Affective Neuroscience*, 3(2):128–34, June 2008.

[11] K. L. Bales, W. A. Mason, C. Catana, S. R. Cherry, and S. P. Mendoza. Neural correlates of pair-bonding in a monogamous primate. *Brain Research*, 1184:245–53, Dec. 2007.

[12] D. Bannerman, M. Lemaire, S. Beggs, J. Rawlins, and S. Iversen. Cytotoxic lesions of the hippocampus increase social investigation but do not impair social-recognition memory. *Experimental Brain Research*, 138(1):100–109, Apr. 2001.

[13] D. M. Bannerman, M. Lemaire, B. K. Yee, S. D. Iversen, C. J. P. Oswald, M. A. Good, and J. N. P. Rawlins. Selective cytotoxic lesions of the retrohippocampal region produce a mild deficit in social recognition memory. *Experimental Brain Research*, 142(3):395–401, Feb. 2002.

[14] S. Baron-Cohen. Does the autistic child have a "theory of mind" ? *Cognition*, 21(1):37–46, Oct. 1985.

[15] S. Baron-Cohen. The amygdala theory of autism. *Neuroscience & Biobehavioral Reviews*, 24(3):355–364, May 2000.

[16] S. Baron-Cohen. The extreme male brain theory of autism. *Trends in Cognitive Sciences*, 6(6):248–254, June 2002.

[17] S. Baron-Cohen. Autism: the empathizing-systemizing (E-S) theory. *Annals of the New York Academy of Sciences*, 1156:68–80, Mar. 2009.

[18] A. Bartels and S. Zeki. The neural basis of romantic love. *Neuroreport*, 11(17):3829–34, Nov. 2000.

[19] A. Bartels and S. Zeki. The neural correlates of maternal and romantic love. *NeuroImage*, 21(3):1155–66, Mar. 2004.

[20] T. Baumgartner, M. Heinrichs, A. Vonlanthen, U. Fischbacher, and E. Fehr. Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron*, 58(4):639–50, May 2008.

[21] M. G. Baxter and E. A. Murray. The amygdala and reward. *Nature Reviews Neuroscience*, 3(7):563–73, July 2002.

[22] F. A. Beach and J. Jaynes. Studies of Maternal Retrieving in Rats I: Recognition of Young. *Journal of Mammalogy*, 37(2):177 – 180, 1956.

[23] M. S. Beauchamp, K. E. Lee, B. D. Argall, and A. Martin. Integration of Auditory and Visual Information about Objects in Superior Temporal Sulcus. *Neuron*, 41(5):809–823, Mar. 2004.

[24] A. Bechara and A. Damasio. The somatic marker hypothesis: A neural theory of economic decision. *Games and Economic Behavior*, 52(2):336–372, Aug. 2005.

[25] L. Bellusio. A Map of Pheromone Receptor Activation in the Mammalian Brain. *Cell*, 97(2):209–220, Apr. 1999.

[26] K. Berridge. Food reward: Brain substrates of wanting and liking. *Neuroscience & Biobehavioral Reviews*, 20(1):1–25, 1996.

[27] D. Blum. *Love at Goon Park: Harry Harlow and the Science of Affection*. Berkley Books, 2004.

[28] R. Bluthe and R. Dantzer. Role of the vomeronasal system in vasopressinergic modulation of social recognition in rats. *Brain Research*, 604(1-2):205–210, Feb. 1993.

[29] M. L. Boccia, A.-P. S. Goursaud, J. Bachevalier, K. D. Anderson, and C. A. Pedersen. Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. *Hormones and Behavior*, 52(3):344–51, Sept. 2007.

[30] I. Bohnet and R. Zeckhauser. Trust, risk and betrayal. *Journal of Economic Behavior & Organization*, 55(4):467–484, Dec. 2004.

[31] J. Born, T. Lange, W. Kern, G. P. McGregor, U. Bickel, and H. L. Fehm. Sniffing neuropeptides: a transnasal approach to the human brain. *Nature Neuroscience*, 5(6):514–6, June 2002.

[32] H. C. Breiter, R. L. Gollub, R. M. Weisskoff, D. N. Kennedy, N. Makris, J. D. Berke, J. M. Goodman, H. L. Kantor, D. R. Gastfriend, J. P. Riorden, R. T. Mathew, B. R. Rosen, and S. E. Hyman. Acute effects of cocaine on human brain activity and emotion. *Neuron*, 19(3):591–611, Sept. 1997.

[33] H. M. Bruce. An Exteroceptive Block to Pregnancy in the Mouse. *Nature*, 184(4680):105–105, July 1959.

[34] R. Buijs, G. de Vries, and F. van Leeuwen. Distribution and synaptic release of oxytocin in the central nervous system. *Oxytocin: Clinical and Laboratory Aspects*, pages 77–86, 1985.

[35] M. S. Carmichael, R. Humbert, J. Dixen, G. Palmisano, W. Greenleaf, and J. M. Davidson. Plasma oxytocin increases in the human sexual response. *The Journal of Clinical Endocrinology and Metabolism*, 64(1):27–31, Jan. 1987.

[36] D. S. Carson, J. L. Cornish, A. J. Guastella, G. E. Hunt, and I. S. McGregor.

Oxytocin decreases methamphetamine self-administration, methamphetamine hyperactivity, and relapse to methamphetamine-seeking behaviour in rats. *Neuropharmacology*, 58(1):38–43, Jan. 2010.

[37] F. Champagne, J. Diorio, S. Sharma, and M. J. Meaney. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 98(22):12736–41, Oct. 2001.

[38] F. A. Champagne, P. Chretien, C. W. Stevenson, T. Y. Zhang, A. Gratton, and M. J. Meaney. Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *The Journal of Neuroscience*, 24(17):4113–23, Apr. 2004.

[39] S. Chaviaras, P. Mak, D. Ralph, L. Krishnan, and J. H. Broadbear. Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test. *Psychopharmacology*, 210(1):35–43, May 2010.

[40] S. A. Cheetham, M. D. Thom, F. Jury, W. E. R. Ollier, R. J. Beynon, and J. L. Hurst. The genetic basis of individual-recognition signals in the mouse. *Current Biology*, 17(20):1771–1777, 2007.

[41] E. Choleris, S. R. Little, J. A. Mong, S. V. Puram, R. Langer, and D. W. Pfaff. Microparticle-based delivery of oxytocin receptor antisense DNA in the medial amygdala blocks social recognition in female mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104(11):4670–5, Mar. 2007.

[42] P. S. Churchland. The impact of neuroscience on philosophy. *Neuron*, 60(3):409–11, Nov. 2008.

[43] H. Cohen, Z. Kaplan, N. Kozlovsky, Y. Gidron, M. A. Matar, and J. Zohar. Hippocampal microinfusion of oxytocin attenuates the behavioural response to stress by means of dynamic interplay with the glucocorticoid-catecholamine responses. *Journal of Neuroendocrinology*, 22(8):889–904, Aug. 2010.

[44] L. Cosmides. The logic of social exchange: has natural selection shaped how humans reason? Studies with the Wason selection task. *Cognition*, 31(3):187–276, Apr. 1989.

[45] B. Costa, S. Pini, P. Gabelloni, M. Abelli, L. Lari, A. Cardini, M. Muti, C. Gesi, S. Landi, S. Galderisi, A. Mucci, A. Lucacchini, G. B. Cassano, and C. Martini. Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology*, 34(10):1506–14, Nov. 2009.

[46] B. Costa, S. Pini, C. Martini, M. Abelli, P. Gabelloni, O. Ciampi, M. Muti, C. Gesi, L. Lari, A. Cardini, A. Mucci, P. Bucci, A. Lucacchini, and G. B. Cassano. Mutation analysis of oxytocin gene in individuals with adult separation anxiety. *Psychiatry Research*, 168(2):87–93, July 2009.

[47] J. P. Curley, E. R. Jordan, W. T. Swaney, A. Izraelit, S. Kammel, and F. A. Champagne. The meaning of weaning: influence of the weaning period on behavioral development in mice. *Developmental Neuroscience*, 31(4):318–31, Jan. 2009.

[48] J. T. Curtis and Z. Wang. Ventral tegmental area involvement in pair bonding in male prairie voles. *Physiology & Behavior*, 86(3):338–46, Oct. 2005.

[49] J. T. Curtis and Z. Wang. Amphetamine effects in microtine rodents: a comparative study using monogamous and promiscuous vole species. *Neuroscience*, 148(4):857–66, Oct. 2007.

[50] B. S. Cushing, J. O. Martin, L. J. Young, and C. S. Carter. The effects of peptides on partner preference formation are predicted by habitat in prairie voles. *Hormones and Behavior*, 39(1):48–58, Feb. 2001.

[51] A. P. Da Costa, C. De La Riva, R. Guevara-Guzman, and K. M. Kendrick. C-fos and c-jun in the paraventricular nucleus play a role in regulating peptide gene expression, oxytocin and glutamate release, and maternal behaviour. *The European Journal of Neuroscience*, 11(7):2199–210, July 1999.

[52] S. Z. Z. Da Yu Lin, E. Block, and L. C. Katz. Encoding social signals in the mouse main olfactory bulb. *Nature*, 434(7032):470–477, 2005.

[53] H. H. Dale. The Action of Extracts of the Pituitary Body. *The Biochemical Journal*, 4(9):427–47, Jan. 1909.

[54] B. Davis, J. Christie, and C. Rorden. Temporal order judgments activate temporal parietal junction. *The Journal of Neuroscience*, 29(10):3182–8, Mar. 2009.

[55] M. Davis, D. L. Walker, L. Miles, and C. Grillon. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*, 35(1):105–35, Jan. 2010.

[56] C. K. W. De Dreu, L. L. Greer, M. J. J. Handgraaf, S. Shalvi, G. A. Van Kleef, M. Baas, F. S. Ten Velden, E. Van Dijk, and S. W. W. Feith. The Neuropeptide Oxytocin Regulates Parochial Altruism in Intergroup Conflict Among Humans. *Science*, 328(5984):1408–1411, June 2010.

[57] D. De Wied. The influence of the posterior and intermediate lobe of the pituitary peptides on the maintenance of a conditioned avoidance response in rats. *International journal of neuropharmacology*, 4:157–67, May 1965.

[58] D. de Wied, M. Diamant, and M. Fodor. Central nervous system effects of the neurohypophyseal hormones and related peptides. *Frontiers in Neuroendocrinology*, 14(4):251–302, Oct. 1993.

[59] M. R. Delgado, R. H. Frank, and E. A. Phelps. Perceptions of moral character modulate the neural systems of reward during the trust game. *Nature Neuroscience*, 8(11):1611–8, Nov. 2005.

[60] T. L. Dellovade, Y.-S. Zhu, and D. W. Pfaff. Thyroid Hormones and Estrogen Affect Oxytocin Gene Expression in Hypothalamic Neurons. *Journal of Neuroendocrinology*, 11(1):1–10, Jan. 1999.

[61] M. Demitrack, M. Lesem, S. Listwak, H. Brandt, D. Jimerson, and P. Gold. CSF oxytocin in anorexia nervosa and bulimia nervosa: clinical and pathophysiologic considerations. *American Journal of Psychiatry*, 147(7):882–886, 1990.

[62] I. Dinstein, J. L. Gardner, M. Jazayeri, and D. J. Heeger. Executed and observed movements have different distributed representations in human aIPS. *The Journal of Neuroscience*, 28(44):11231–9, Oct. 2008.

[63] B. Ditzen, M. Schaer, B. Gabriel, G. Bodenmann, U. Ehlert, and M. Heinrichs. Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biological Psychiatry*, 65(9):728–31, May 2009.

[64] D. E. Dluzen, S. Muraoka, M. Engelmann, K. Ebner, and R. Landgraf. Oxytocin induces preservation of social recognition in male rats by activating alpha-adrenoceptors of the olfactory bulb. *European Journal of Neuroscience*, 12(2):760–766, Feb. 2000.

[65] G. Domes, M. Heinrichs, J. Gläscher, C. Büchel, D. F. Braus, and S. C. Herpertz. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biological Psychiatry*, 62(10):1187–90, 2007.

[66] G. Domes, M. Heinrichs, A. Michel, C. Berger, and S. C. Herpertz. Oxytocin improves "mind-reading" in humans. *Biological Psychiatry*, 61(6):731–3, Mar. 2007.

[67] G. Domes, A. Lischke, C. Berger, A. Grossmann, K. Hauenstein, M. Heinrichs, and S. C. Herpertz. Effects of intranasal oxytocin on emotional face processing in women. *Psychoneuroendocrinology*, 35(1):83–93, Jan. 2010.

[68] V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis.

The Synthesis of Oxytocin1. *Journal of the American Chemical Society*, 76(12):3115–3121, 1954.

[69] V. du Vigneaud, C. Ressler, and S. Trippett. The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. *Journal of Biological Chemistry*, 205(2):949–957, 1953.

[70] P. Duchamp-Viret. Odor Response Properties of Rat Olfactory Receptor Neurons. *Science*, 284(5423):2171–2174, June 1999.

[71] C. Dulac and A. T. Torello. Molecular detection of pheromone signals in mammals: from genes to behaviour. *Nature Reviews Neuroscience*, 4(7):551–62, July 2003.

[72] C. Dulac and S. Wagner. Genetic analysis of brain circuits underlying pheromone signaling. *Annual Review of Genetics*, 40:449–67, Jan. 2006.

[73] G. J. H. Dumont, F. C. G. J. Sweep, R. van Der Steen, R. Hermsen, A. R. T. Donders, D. J. Touw, J. M. A. van Gerven, J. K. Buitelaar, and R. J. Verkes. Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxymethamphetamine) administration. *Social Neuroscience*, 4(4):359–66, Jan. 2009.

[74] I. Dziobek, K. Rogers, S. Fleck, M. Bahnemann, H. R. Heekeren, O. T. Wolf, and A. Convit. Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). *Journal of Autism and Developmental Disorders*, 38(3):464–73, Mar. 2008.

[75] M. Engelmann, K. Ebner, C. T. Wotjak, and R. Landgraf. Endogenous oxytocin is involved in short-term olfactory memory in female rats. *Behavioural Brain Research*, 90(1):89–94, Jan. 1998.

[76] L.-Y. Fang, R.-D. Quan, and H. Kaba. Oxytocin facilitates the induction of long-term potentiation in the accessory olfactory bulb. *Neuroscience Letters*, 438(2):133–7, June 2008.

[77] R. Feldman, A. Weller, O. Zagoory-Sharon, and A. Levine. Evidence for a neuroendocrinological foundation of human affiliation: plasma oxytocin levels across pregnancy and the postpartum period predict mother-infant bonding. *Psychological Science*, 18(11):965–70, Nov. 2007.

[78] J. N. Ferguson, J. M. Aldag, T. R. Insel, and L. J. Young. Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience*, 21(20):8278–85, Oct. 2001.

[79] J. N. Ferguson, L. J. Young, E. Hearn, M. M. Matzuk, T. R. Insel, and J. T. Winslow. Social amnesia in mice lacking the oxytocin gene. *Nature Genetics*, 25:284–288, 2000.

[80] B. Ferrier, D. Kennet, and M. Devlin. Influence of oxytocin on human memory processes. *Life Sciences*, 27(24):2311–2317, Dec. 1980.

[81] H. E. Fisher. Evolution of human serial pairbonding. *American Journal of Physical Anthropology*, 78(3):331–54, Mar. 1989.

[82] D. D. Francis. Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat. *Science*, 286(5442):1155–1158, Nov. 1999.

[83] D. D. Francis, F. C. Champagne, and M. J. Meaney. Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Journal of Neuroendocrinology*, 12(12):1145–8, Dec. 2000.

[84] D. D. Francis, L. J. Young, M. J. Meaney, and T. R. Insel. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. *Journal of Neuroendocrinology*, 14:349–353, 2002.

[85] D. Freedberg and V. Gallese. Motion, emotion and empathy in esthetic experience. *Trends in Cognitive Sciences*, 11(5):197–203, May 2007.

[86] A. B. W. Fries, T. E. Ziegler, J. R. Kurian, S. Jacoris, and S. D. Pollak. Early experience

in humans is associated with changes in neuropeptides critical for regulating social behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 102(47):17237–40, Nov. 2005.

[87] C. H. Y. Fu, S. C. R. Williams, A. J. Cleare, M. J. Brammer, N. D. Walsh, J. Kim, C. M. Andrew, E. M. Pich, P. M. Williams, L. J. Reed, M. T. Mitterschiffthaler, J. Suckling, and E. T. Bullmore. Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Archives of general psychiatry*, 61(9):877–89, Sept. 2004.

[88] M. Gamer, B. Zurowski, and C. Büchel. Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 107(20):9400–5, Apr. 2010.

[89] I. Gauthier, P. Skudlarski, J. C. Gore, and A. W. Anderson. Expertise for cars and birds recruits brain areas involved in face recognition. *Nature Neuroscience*, 3(2):191–7, Feb. 2000.

[90] M. S. George, T. A. Ketter, P. I. Parekh, B. Horwitz, P. Herscovitch, and R. M. Post. Brain activity during transient sadness and happiness in healthy women. *The American Journal of Psychiatry*, 152(3):341–51, Mar. 1995.

[91] O. Gillath, P. R. Shaver, J.-M. Baek, and D. S. Chun. Genetic correlates of adult attachment style. *Personality and Social Psychology Bulletin*, 34(10):1396–405, Oct. 2008.

[92] G. Gimpl and F. Fahrenholz. Human oxytocin receptors in cholesterol-rich vs. cholesterol-poor microdomains of the plasma membrane. *European Journal of Biochemistry*, 267(9):2483–97, May 2000.

[93] G. Gimpl and F. Fahrenholz. The oxytocin receptor system: structure, function, and regulation. *Physiological Reviews*, 81(2):629–83, Apr. 2001.

[94] K. L. Gobrogge, Y. Liu, X. Jia, and Z. Wang. Anterior hypothalamic neural activation and neurochemical associations with aggression in pair-bonded male prairie voles. *The Journal of Comparative Neurology*, 502(6):1109–22, June 2007.

[95] K. L. Gobrogge, Y. Liu, L. J. Young, and Z. Wang. Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *Proceedings of the National Academy of Sciences of the United States of America*, 106(45):19144–9, Nov. 2009.

[96] H. Gray. *Anatomy of the human body*. Lea & Febiger, 1918.

[97] L. Green, D. Fein, C. Modahl, C. Feinstein, L. Waterhouse, and M. Morris. Oxytocin and autistic disorder: alterations in peptide forms. *Biological Psychiatry*, 50(8):609–13, Oct. 2001.

[98] S. G. Gregory, J. J. Connelly, A. J. Towers, J. Johnson, D. Biscocho, C. A. Markunas, C. Lintas, R. K. Abramson, H. H. Wright, P. Ellis, C. F. Langford, G. Worley, G. R. Delong, S. K. Murphy, M. L. Cuccaro, A. Persico, and M. A. Pericak-Vance. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC medicine*, 7:62, Jan. 2009.

[99] K. M. Grewen, S. S. Girdler, J. Amico, and K. C. Light. Effects of partner support on resting oxytocin, cortisol, norepinephrine, and blood pressure before and after warm partner contact. *Psychosomatic Medicine*, 67(4):531–8, Jan. 2005.

[100] C. G. Gross. Single neuron studies of inferior temporal cortex. *Neuropsychologia*, 46(3):841–52, Feb. 2008.

[101] A. J. Guastella, A. L. Howard, M. R. Dadds, P. Mitchell, and D. S. Carson. A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. *Psychoneuroendocrinology*, 34(6):917–23, July 2009.

[102] A. J. Guastella, P. B. Mitchell, and M. R. Dadds. Oxytocin increases gaze to the eye region of human faces. *Biological Psychiatry*, 63(1):3–5, Jan. 2008.

[103] A. J. Guastella, P. B. Mitchell, and F. Mathews. Oxytocin enhances the encoding of positive social memories in humans. *Biological Psychiatry*, 64(3):256–8, Aug. 2008.

[104] B. Güroglu, W. van Den Bos, S. A. R. B. Rombouts, and E. A. Crone. Unfair? It depends: Neural correlates of fairness in social context. *Social Cognitive and Affective Neuroscience*, Mar. 2010.

[105] N. Halevy, G. Bornstein, and L. Sagiv. "In-group love" and "out-group hate" as motives for individual participation in intergroup conflict: a new game paradigm. *Psychological Science*, 19(4):405–11, Apr. 2008.

[106] M. Halpern. Structure and function of the vomeronasal system: an update. *Progress in Neurobiology*, 70(3):245–318, June 2003.

[107] C. Haney, C. Banks, and P. Zimbardo. Interpersonal dynamics in a simulated prison. *International Journal of Criminology and Penology*, 1:29, 1973.

[108] G. Hardin. Population, Biology, and the Law. *Journal of Urban Law*, 48:563, 1970.

[109] H. F. Harlow, R. O. Dodsworth, and M. K. Harlow. Total social isolation in monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, 54(1):90–7, July 1965.

[110] C. Heim, L. J. Young, D. J. Newport, T. Mletzko, A. H. Miller, and C. B. Nemeroff. Lower CSF oxytocin concentrations in women with a history of childhood abuse. *Molecular Psychiatry*, 14(10):954–8, Oct. 2009.

[111] J. Henrich, J. Ensminger, R. McElreath, A. Barr, C. Barrett, A. Bolyanatz, J. C. Cardenas, M. Gurven, E. Gwako, N. Henrich, C. Lesorogol, F. Marlowe, D. Tracer, and J. Ziker. Markets, religion, community size, and the evolution of fairness and punishment. *Science*, 327(5972):1480–4, Mar. 2010.

[112] J. Henrich, S. J. Heine, and A. Norenzayan. The weirdest people in the world? *The Behavioral and Brain Sciences*, 33(2-3):1–23, June 2010.

[113] E. L. Hill. Executive dysfunction in autism. *Trends in Cognitive Sciences*, 8(1):26–32, Jan. 2004.

[114] E. Hollander, J. Bartz, W. Chaplin, A. Phillips, J. Sumner, L. Soorya, E. Anagnostou, and S. Wasserman. Oxytocin increases retention of social cognition in autism. *Biological Psychiatry*, 61(4):498–503, 2007.

[115] E. Hollander, S. Novotny, M. Hanratty, R. Yaffe, C. M. DeCaria, B. R. Aronowitz, and S. Mosovich. Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology*, 28(1):193–8, Jan. 2003.

[116] J. House, K. Landis, and D. Umberson. Social relationships and health. *Science*, 241(4865):540–545, July 1988.

[117] D. Huber, P. Veinante, and R. Stoop. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science*, 308(5719):245–8, May 2005.

[118] A. Hughes, B. Everitt, S. Lightman, and K. Todd. Oxytocin in the central nervous system and sexual behaviour in male rats. *Brain Research*, 414(1):133–137, June 1987.

[119] D. Hume. *An enquiry concerning the principles of morals*. Printed for A. Millar, 1751.

[120] R. Hurlemann, A. Patin, O. A. Onur, M. X. Cohen, T. Baumgartner, S. Metzler, I. Dziobek, J. Gallinat, M. Wagner, W. Maier, and K. M. Kendrick. Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *The Journal of Neuroscience*, 30(14):4999–5007, Apr. 2010.

[121] R. Hurlemann, H. Walter, A. K. Rehme, J. Kukolja, S. C. Santoro, C. Schmidt, K. Schnell, F. Musshoff, C. Keysers, W. Maier, K. M. Kendrick, and O. A. Onur.

Human amygdala reactivity is diminished by the beta-noradrenergic antagonist propranolol. *Psychological Medicine*, pages 1–10, Jan. 2010.

[122] S. Ikemoto. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Research Reviews*, 56(1):27–78, Nov. 2007.

[123] T. Insel, Z. Wang, and C. Ferris. Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *Journal of Neuroscience*, 14(9):5381–5392, 1994.

[124] T. R. Insel. Oxytocin Receptor Distribution Reflects Social Organization in Monogamous and Polygamous Voles. *Proceedings of the National Academy of Sciences*, 89(13):5981–5985, July 1992.

[125] T. R. Insel, L. Young, D. M. Witt, and D. Crews. Gonadal Steroids have Paradoxical Effects on Brain Oxytocin Receptors. *Journal of Neuroendocrinology*, 5(6):619–628, Dec. 1993.

[126] S. Jacob, C. W. Brune, C. S. Carter, B. L. Leventhal, C. Lord, and E. H. Cook. Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. *Neuroscience Letters*, 417(1):6–9, Apr. 2007.

[127] E. Jolkkonen and A. Pitknen. Intrinsic connections of the rat amygdaloid complex: Projections originating in the central nucleus. *The Journal of Comparative Neurology*, 395(1):53–72, May 1998.

[128] H. Jorgensen, A. Kjaer, U. Knigge, M. Moller, and J. Warberg. Serotonin stimulates hypothalamic mRNA expression and local release of neurohypophysial peptides. *Journal of Neuroendocrinology*, 15(6):564–71, June 2003.

[129] H. Kaba, A. Rosser, and B. Keverne. Neural basis of olfactory memory in the context of pregnancy block. *Neuroscience*, 32(3):657–62, Jan. 1989.

[130] N. Kanwisher, J. McDermott, and M. M. Chun. The Fusiform Face Area: A Module in Human Extrastriate Cortex Specialized for Face Perception. *Journal of Neuroscience*, 17(11):4302–4311, 1997.

[131] K. P. Kaut and M. D. Bunsey. The effects of lesions to the rat hippocampus or rhinal cortex on olfactory and spatial memory: Retrograde and anterograde findings. *Cognitive, Affective, & Behavioral Neuroscience*, 1(3):270–286, Sept. 2001.

[132] S. Kelemenova, E. Schmidtova, A. Ficek, P. Celec, A. Kubranska, and D. Ostatnikova. Polymorphisms of candidate genes in Slovak autistic patients. *Psychiatric Genetics*, 20(4):137–9, Aug. 2010.

[133] M. Keller, M. Meurisse, and F. Lévy. Mapping the neural substrates involved in maternal responsiveness and lamb olfactory memory in parturient ewes using Fos imaging. *Behavioral Neuroscience*, 118(6):1274–84, Dec. 2004.

[134] M. Keller, M. Meurisse, P. Poindron, R. Nowak, G. Ferreira, M. Shayit, and F. Lévy. Maternal experience influences the establishment of visual/auditory, but not olfactory recognition of the newborn lamb by ewes at parturition. *Developmental Psychobiology*, 43(3):167–76, Nov. 2003.

[135] M. Keller, G. Perrin, M. Meurisse, G. Ferreira, and F. Lévy. Cortical and medial amygdala are both involved in the formation of olfactory offspring memory in sheep. *The European Journal of Neuroscience*, 20(12):3433–41, Dec. 2004.

[136] A. Kelley, V. Bakshi, S. Haber, T. Steininger, M. Will, and M. Zhang. Opioid modulation of taste hedonics within the ventral striatum. *Physiology & Behavior*, 76(3):365–377, July 2002.

[137] K. M. Kendrick. Neurobiological correlates of visual and olfactory recognition in sheep. *Behavioural Processes*, 33(1-2):89–111, Dec. 1994.

[138] K. M. Kendrick. Facial and vocal discrimination in sheep. *Animal Behaviour*, 49(6):1665–1676, June 1995.

[139] K. M. Kendrick. Oxytocin, motherhood and bonding. *Experimental Physiology*, 85 Spec No:111S–124S, Mar. 2000.

[140] K. M. Kendrick, R. Guevara-Guzman, J. Zorrilla, M. R. Hinton, K. D. Broad, M. Mimmack, and S. Ohkura. Formation of olfactory memories mediated by nitric oxide. *Nature*, 388(6643):670–674, Aug. 1997.

[141] K. M. Kendrick, E. B. Keverne, and B. A. Baldwin. Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. *Neuroendocrinology*, 46(1):56–61, 1987.

[142] K. M. Kendrick, F. Lévy, and E. B. Keverne. Changes in the sensory processing of olfactory signals induced by birth in sleep. *Science*, 256(5058):833–6, May 1992.

[143] T. Kimura, Y. Makino, F. Saji, M. Takemura, T. Inoue, T. Kikuchi, Y. Kubota, C. Azuma, T. Nobunaga, and Y. Tokugawa. Molecular characterization of a cloned human oxytocin receptor. *European Journal of Endocrinology*, 131(4):385–90, Oct. 1994.

[144] T. Kimura, O. Tanizawa, K. Mori, M. J. Brownstein, and H. Okayama. Structure and expression of a human oxytocin receptor. *Nature*, 356(6369):526–9, Apr. 1992.

[145] B. King-Casas, D. Tomlin, C. Anen, C. F. Camerer, S. R. Quartz, and P. R. Montague. Getting to know you: reputation and trust in a two-person economic exchange. *Science*, 308(5718):78–83, Apr. 2005.

[146] P. Kirsch, C. Esslinger, Q. Chen, D. Mier, S. Lis, S. Siddhanti, H. Gruppe, V. S. Mattay, B. Gallhofer, and A. Meyer-Lindenberg. Oxytocin modulates neural circuitry for social cognition and fear in humans. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(49):11489–11493, Dec. 2005.

[147] N. M. Kleinhans, T. Richards, L. Sterling, K. C. Stegbauer, R. Mahurin, L. C. Johnson, J. Greenson, G. Dawson, and E. Aylward. Abnormal functional connectivity in autism spectrum disorders during face processing. *Brain*, 131(Pt 4):1000–12, Apr. 2008.

[148] J. H. Kogan, P. W. Franklandand, and A. J. Silva. Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus*, 10(1):47–56, 2000.

[149] M. Kosfeld, M. Heinrichs, P. J. Zak, U. Fischbacher, and E. Fehr. Oxytocin increases trust in humans. *Nature*, 435(7042):673–6, June 2005.

[150] K. Kramer, B. Cushing, C. Carter, J. Wu, and M. Ottinger. Sex and species differences in plasma oxytocin using an enzyme immunoassay. *Canadian Journal of Zoology*, 82(8):7.

[151] Y. Kubota, T. Kimura, K. Hashimoto, Y. Tokugawa, K. Nobunaga, C. Azuma, F. Saji, and Y. Murata. Structure and expression of the mouse oxytocin receptor gene. *Molecular and Cellular Endocrinology*, 124(1-2):25–32, Nov. 1996.

[152] R. Landgraf, I. Neumann, J. A. Russell, and Q. J. Pittman. Push-pull perfusion and microdialysis studies of central oxytocin and vasopressin release in freely moving rats during pregnancy, parturition, and lactation. *Annals of the New York Academy of Sciences*, 652:326–39, June 1992.

[153] R. Landgraf and I. D. Neumann. Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. *Frontiers in Neuroendocrinology*, 25(3-4):150–76, 2004.

[154] A. Larrazolo-López, K. M. Kendrick, M. Aburto-Arciniega, V. Arriaga-Avila, S. Morimoto, M. Frias, and R. Guevara-Guzmán. Vaginocervical stimulation enhances social recognition memory in rats via oxytocin release in the olfactory bulb. *Neuroscience*, 152(3):585–93, Mar. 2008.

[155] J. LeDoux. The amygdala. *Current biology*, 17(20):868–74, Oct. 2007.

[156] A. Lee. Mother rats bar-press for pups: effects of lesions of the mpoa and limbic sites on maternal behavior and operant responding for pup-reinforcement. *Behavioural Brain Research*, 100(1-2):15–31, Apr. 1999.

[157] T. Leinders-Zufall, P. Brennan, P. Widmayer, P. C. S, A. Maul-Pavicic, M. Jäger, X.-H. Li, H. Breer, F. Zufall, and T. Boehm. MHC class I peptides as chemosensory signals in the vomeronasal organ. *Science*, 306(5698):1033–7, Nov. 2004.

[158] T. Leinders-Zufall, A. P. Lane, A. C. Puche, W. Ma, M. V. Novotny, M. T. Shipley, and F. Zufall. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature*, 405(6788):792–6, June 2000.

[159] B. E. Leonard and M. Tuite. Anatomical, physiological, and behavioral aspects of olfactory bulbectomy in the rat. *International Review of Neurobiology*, 22:251–86, Jan. 1981.

[160] F. Lévy, R. Gervais, U. Kindermann, P. Orgeur, and V. Piketty. Importance of beta-noradrenergic receptors in the olfactory bulb of sheep for recognition of lambs. *Behavioral Neuroscience*, 104(3):464–9, June 1990.

[161] F. Lévy, A. Locatelli, V. Piketty, Y. Tillet, and P. Poindron. Involvement of the main but not the accessory olfactory system in maternal behavior of primiparous and multiparous ewes. *Physiology & Behavior*, 57(1):97–104, Jan. 1995.

[162] B. G. Leypold, C. R. Yu, T. Leinders-Zufall, M. M. Kim, F. Zufall, and R. Axel. Altered sexual and social behaviors in trp2 mutant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 99(9):6376–81, Apr. 2002.

[163] K. C. Light, K. M. Grewen, and J. A. Amico. More frequent partner hugs and higher oxytocin levels are linked to lower blood pressure and heart rate in premenopausal women. *Biological Psychology*, 69(1):5–21, Apr. 2005.

[164] M. Lim, Z. Wang, D. Olazábal, X. Ren, E. Terwilliger, and L. Young. Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene., 2004.

[165] M. M. Lim and L. J. Young. Vasopressin-dependent neural circuits underlying pair bond formation in the monogamous prairie vole. *Neuroscience*, 125(1):35–45, Jan. 2004.

[166] Y. Liu. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience*, 121(3):537–544, Oct. 2003.

[167] Y. Liu, B. J. Aragona, K. A. Young, D. M. Dietz, M. Kabbaj, M. Mazei-Robison, E. J. Nestler, and Z. Wang. Nucleus accumbens dopamine mediates amphetamine-induced impairment of social bonding in a monogamous rodent species. *Proceedings of the National Academy of Sciences of the United States of America*, 107(3):1217–22, Jan. 2010.

[168] F. Loup, E. Tribollet, M. Dubois-Dauphin, and J. J. Dreifuss. Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. *Brain Research*, 555(2):220–32, Aug. 1991.

[169] M. J. Lucht, S. Barnow, C. Sonnenfeld, A. Rosenberger, H. J. Grabe, W. Schroeder, H. Völzke, H. J. Freyberger, F. H. Herrmann, H. Kroemer, and D. Rosskopf. Associations between the oxytocin receptor gene (OXTR) and affect, loneliness and intelligence in normal subjects. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 33(5):860–6, Aug. 2009.

[170] M. Luo, M. S. Fee, and L. C. Katz. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. *Science*, 299(5610):1196–201, Feb. 2003.

[171] J. E. Magnusson and A. S. Fleming. Rat pups are reinforcing to the maternal rat: role of sensory cues. *Psychobiology*, 23(1):69–75, 1995.

[172] F. Managò, C. Castellano, A. Oliverio, A. Mele, and E. De Leonibus. Role of dopamine receptors subtypes, D1-like and D2-like, within the nucleus accumbens subregions, core and shell, on memory consolidation in the one-trial inhibitory avoidance task. *Learning & Memory*, 16(1):46–52, Jan. 2009.

[173] J. Matochik. Role of the main olfactory system in recognition between individual spiny mice. *Physiology & Behavior*, 42(3):217–222, 1988.

[174] B. J. Mattson, S. Williams, J. S. Rosenblatt, and J. I. Morrell. Comparison of two positive reinforcing stimuli: pups and cocaine throughout the postpartum period. *Behavioral Neuroscience*, 115(3):683–94, June 2001.

[175] K. McBride. Does Intranasal Application of Zinc Sulfate Produce Anosmia in the Mouse? An Olfactometric and Anatomical Study. *Chemical Senses*, 28(8):659–670, Oct. 2003.

[176] A. McDonald. Cortical pathways to the mammalian amygdala. *Progress in Neurobiology*, 55(3):257–332, June 1998.

[177] B. B. McEwen. Brain–Fluid Barriers: Relevance for Theoretical Controversies Regarding Vasopressin and Oxytocin Memory Research. *Advances in Pharmacology*, 50:531–592, 2004.

[178] M. R. Melis, A. Argiolas, R. Stancampiano, and G. L. Gessa. Effect of apomorphine on oxytocin concentrations in different brain areas and plasma of male rats. *European Journal of Pharmacology*, 182(1):101–7, June 1990.

[179] M. Meredith. Vomeronasal, Olfactory, Hormonal Convergence in the Brain: Cooperation or Coincidence? *Annals of the New York Academy of Sciences*, 855(1):349–361, Nov. 1998.

[180] A. Meyer-Lindenberg. Impact of prosocial neuropeptides on human brain function. In I. D. Neumann and R. Landgraf, editors, *Advances in Vasopressin and Oxytocin* {–} *From Genes to Behaviour to Disease*, volume 170, chapter Progress i, pages 463–470. Elsevier, 2008.

[181] A. Meyer-Lindenberg, B. Kolachana, B. Gold, A. Olsh, K. K. Nicodemus, V. Mattay, M. Dean, and D. R. Weinberger. Genetic variants in AVPR1A linked to autism predict amygdala activation and personality traits in healthy humans. *Molecular Psychiatry*, 14(10):968–75, Oct. 2009.

[182] Y. Mihov, S. Mayer, F. Musshoff, W. Maier, K. M. Kendrick, and R. Hurlemann. Facilitation of learning by social-emotional feedback in humans is beta-noradrenergic-dependent. *Neuropsychologia*, May 2010.

[183] M. Mikolajczak, J. J. Gross, A. Lane, O. Corneille, P. de Timary, and O. Luminet. Oxytocin Makes People Trusting, Not Gullible. *Psychological Science*, 21(8), July 2010.

[184] C. Modahl, L. Green, D. Fein, M. Morris, L. Waterhouse, C. Feinstein, and H. Levin. Plasma oxytocin levels in autistic children. *Biological Psychiatry*, 43(4):270–277, Feb. 1998.

[185] P. Mombaerts, F. Wang, C. Dulac, S. K. Chao, A. Nemes, M. Mendelsohn, J. Edmondson, and R. Axel. Visualizing an olfactory sensory map. *Cell*, 87(4):675–86, Nov. 1996.

[186] S. Moriceau and R. M. Sullivan. Unique neural circuitry for neonatal olfactory learning. *The Journal of Neuroscience*, 24(5):1182–9, Mar. 2004.

[187] J. F. Morris and M. Ludwig. Magnocellular dendrites: prototypic receiver/transmitters. *Journal of Neuroendocrinology*, 16(4):403–8, Apr. 2004.

[188] S. D. Munger, T. Leinders-Zufall, and F. Zufall. Subsystem organization of the mammalian sense of smell. *Annual Review of Physiology*, 71:115–140, 2009.

[189] Y. Muroi, T. Ishii, S. Komori, and M. Nishimura. A competitive effect of androgen signaling on male mouse attraction to

volatile female mouse odors. *Physiology & Behavior*, 87(1):199–205, 2006.

[190] R. Nowak, T. M. Murphy, D. R. Lindsay, P. Alster, R. Andersson, and K. Uvnäs-Moberg. Development of a preferential relationship with the mother by the newborn lamb: importance of the sucking activity. *Physiology & Behavior*, 62(4):681–8, Oct. 1997.

[191] M. Numan, M. J. Numan, N. Pliakou, D. S. Stolzenberg, O. J. Mullins, J. M. Murphy, and C. D. Smith. The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal behavior in rats. *Behavioral Neuroscience*, 119(6):1588–604, Dec. 2005.

[192] L. M. Oberman, J. A. Pineda, and V. S. Ramachandran. The human mirror neuron system: a link between action observation and social skills. *Social Cognitive and Affective Neuroscience*, 2(1):62–6, Mar. 2007.

[193] A. Ohman, K. Carlsson, D. Lundqvist, and M. Ingvar. On the unconscious subcortical origin of human fear. *Physiology & Behavior*, 92(1-2):180–5, Sept. 2007.

[194] T. Otto and H. Eichenbaum. Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behavioral Neuroscience*, 106(5):762–75, Oct. 1992.

[195] T. Oumi, K. Ukena, O. Matsushima, T. Ikeda, T. Fujita, H. Minakata, and K. Nomoto. Annetocin, an annelid oxytocin-related peptide, induces egg-laying behavior in the earthworm Eisenia foetida. *The Journal of Experimental Zoology*, 276(2):151–156, Oct. 1996.

[196] T. Paus. Primate anterior cingulate cortex: where motor control, drive and cognition interface. *Nature Reviews Neuroscience*, 2(6):417–24, June 2001.

[197] C. Pedersen and M. Boccia. Oxytocin antagonism alters rat dams' oral grooming and upright posturing over pups. *Physiology & Behavior*, 80(2-3):233–241, Nov. 2003.

[198] C. A. Pedersen. Induction of Maternal Behavior in Virgin Rats after Intracerebroventricular Administration of Oxytocin. *Proceedings of the National Academy of Sciences*, 76(12):6661–6665, Dec. 1979.

[199] C. A. Pedersen, S. V. Vadlamudi, M. L. Boccia, and J. A. Amico. Maternal behavior deficits in nulliparous oxytocin knockout mice. *Genes, Brain, and Behavior*, 5(3):274–81, Apr. 2006.

[200] A. Petrulis, P. Alvarez, and H. Eichenbaum. Neural correlates of social odor recognition and the representation of individual distinctive social odors within entorhinal cortex and ventral subiculum. *Neuroscience*, 130(1):259–74, Jan. 2005.

[201] A. Petrulis and H. Eichenbaum. The perirhinal–entorhinal cortex, but not the hippocampus, is critical for expression of individual recognition in the context of the Coolidge effect. *Neuroscience*, 122(3):599–607, 2003.

[202] H. Pfister and J. Muir. Influence of Exogeneously Administered Oxytocin on Central Noradrenaline, Dopamine and Serotonin Levels Following Psychological Stress in Nulliparous Female Rats (Rattus Norvegicus); International Journal of Neuroscience - 45(3-4):Pages 221-229 - Informa. *International Journal of Neuroscience*, pages 221–229, 1989.

[203] R. C. Pierce and V. Kumaresan. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Biobehavioral Reviews*, 30(2):215–38, Jan. 2006.

[204] L. Pitkow, C. Sharer, X. Ren, T. Insel, E. Terwilliger, and L. Young. Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole. *Journal of Neuroscience*, pages 7392–6, 2001.

[205] P. Poindron, F. Lévy, and D. Krehbiel. Genital, olfactory, and endocrine interactions in the development of maternal behaviour in the parturient ewe. *Psychoneuroendocrinology*, 13(1-2):99–125, 1988.

[206] P. Popik and J. M. van Ree. Oxytocin but not vasopressin facilitates social recognition following injection into the medial preoptic area of the rat brain. *European Neuropsychopharmacology*, 1(4):555–60, Dec. 1991.

[207] P. Popik, J. Vetulani, A. Bisaga, and J. M. van Ree. Recognition cue in the rat's social memory paradigm. *Journal of Basic and Clinical Physiology and Pharmacology*, 2(4):315–27.

[208] Z. M. Prichard, A. J. Mackinnon, A. F. Jorm, and S. Easteal. AVPR1A and OXTR polymorphisms are associated with sexual and reproductive behavioral phenotypes in humans. *Human Mutation*, 28(11):1150, Nov. 2007.

[209] P. Pro-Sistiaga, A. Mohedano-Moriano, I. Ubeda-Bañon, M. Del Mar Arroyo-Jimenez, P. Marcos, E. Artacho-Pérula, C. Crespo, R. Insausti, and A. Martinez-Marcos. Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. *The Journal of Comparative Neurology*, 504(4):346–62, Oct. 2007.

[210] M. Raggenbass. Vasopressin- and oxytocin-induced activity in the central nervous system: electrophysiological studies using in-vitro systems. *Progress in Neurobiology*, 64(3):307–326, June 2001.

[211] S. Rangel and M. Leon. Early odor preference training increases olfactory bulb norepinephrine. *Brain Research Developmental Brain Research*, 85(2):187–91, Apr. 1995.

[212] P. A. Rao, D. C. Beidel, and M. J. Murray. Social skills interventions for children with Asperger's syndrome or high-functioning autism: a review and recommendations. *Journal of Autism and Developmental Disorders*, 38(2):353–61, Feb. 2008.

[213] L. P. Renaud and C. W. Bourque. Neurophysiology and neuropharmacology of hypothalamic magnocellular neurons secreting vasopressin and oxytocin. *Progress in Neurobiology*, 36(2):131–69, Jan. 1991.

[214] S. M. Reynolds and K. C. Berridge. Positive and Negative Motivation in Nucleus Accumbens Shell: Bivalent Rostrocaudal Gradients for GABA-Elicited Eating, Taste "Liking"/"Disliking" Reactions, Place Preference/Avoidance, and Fear. *Journal of Neuroscience*, 22(16):7308–7320, 2002.

[215] P. Richard, F. Moos, and M. J. Freund-Mercier. Central effects of oxytocin. *Physiological Reviews*, 71(2):331–370, 1991.

[216] P. R. Riley, A. P. Flint, D. R. Abayasekara, and H. J. Stewart. Structure and expression of an ovine endometrial oxytocin receptor cDNA. *Journal of Molecular Endocrinology*, 15(2):195–202, Oct. 1995.

[217] U. Rimmele, K. Hediger, M. Heinrichs, and P. Klaver. Oxytocin makes a face in memory familiar. *The Journal of Neuroscience*, 29(1):38–42, Jan. 2009.

[218] S. M. Rodrigues, L. R. Saslow, N. Garcia, O. P. John, and D. Keltner. Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 106(50):21437–41, Dec. 2009.

[219] B. Roozendaal, G. Schoorlemmer, J. Koolhaas, and B. Bohus. Cardiac, neuroendocrine, and behavioral effects of central amygdaloid vasopressinergic and oxytocinergic mechanisms under stress-free conditions in rats. *Brain Research Bulletin*, 32(6):573–579, 1993.

[220] L. Rosenblum. Differing concentrations of corticotropin-releasing factor and oxytocin in the cerebrospinal fluid of bonnet and pigtail macaques. *Psychoneuroendocrinology*, 27(6):651–660, Aug. 2002.

[221] H. E. Ross, C. D. Cole, Y. Smith, I. D. Neumann, R. Landgraf, A. Z. Murphy, and L. J.

Young. Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. *Neuroscience*, 162(4):892–903, Sept. 2009.

[222] H. E. Ross, S. M. Freeman, L. L. Spiegel, X. Ren, E. F. Terwilliger, and L. J. Young. Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(5):1312–8, Feb. 2009.

[223] E. Rossoni, J. Feng, B. Tirozzi, D. Brown, G. Leng, and F. Moos. Emergent synchronous bursting of oxytocin neuronal network. *Public Library of Science Computational Biology*, 4(7):e1000123, Jan. 2008.

[224] F. Rozen, C. Russo, D. Banville, and H. H. Zingg. Structure, characterization, and expression of the rat oxytocin receptor gene. *Proceedings of the National Academy of Sciences of the United States of America*, 92(1):200–4, Jan. 1995.

[225] A. H. Rutgers, M. J. Bakermans-Kranenburg, M. H. van Ijzendoorn, and I. A. van Berckelaer-Onnes. Autism and attachment: a meta-analytic review. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 45(6):1123–34, Sept. 2004.

[226] C. A. Salvatore, C. J. Woyden, M. T. Guidotti, D. J. Pettibone, and M. A. Jacobson. Cloning and expression of the rhesus monkey oxytocin receptor. *Journal of Receptor and Signal Transduction Research*, 18(1):15–24, Jan. 1998.

[227] G. Sánchez-Andrade, B. M. James, and K. M. Kendrick. Neural encoding of olfactory recognition memory. *The Journal of reproduction and development*, 51(5):547–58, Oct. 2005.

[228] A. G. Sanfey, J. K. Rilling, J. A. Aronson, L. E. Nystrom, and J. D. Cohen. The neural basis of economic decision-making in the Ultimatum Game. *Science*, 300(5626):1755–8, June 2003.

[229] Z. Sarnyai. Oxytocin and neuroadaptation to cocaine. *Progress in Brain Research*, 119:449–66, Jan. 1998.

[230] E. Sausville, D. Carney, and J. Battey. The human vasopressin gene is linked to the oxytocin gene and is selectively expressed in a cultured lung cancer cell line. *The Journal of Biological Chemistry*, 260(18):10236–41, Aug. 1985.

[231] E. Savaskan, R. Ehrhardt, A. Schulz, M. Walter, and H. Schächinger. Post-learning intranasal oxytocin modulates human memory for facial identity. *Psychoneuroendocrinology*, 33(3):368–74, Apr. 2008.

[232] F. Scalia and S. S. Winans. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *The Journal of Comparative Neurology*, 161(1):31–55, May 1975.

[233] G. Schoenbaum and G. R. Esber. How do you (estimate you will) like them apples? Integration as a defining trait of orbitofrontal function. *Current Opinion in Neurobiology*, 20(2):205–211, 2010.

[234] L. J. Seltzer, T. E. Ziegler, and S. D. Pollak. Social vocalizations can release oxytocin in humans. *Proceedings of the Royal Society B: Biological Sciences*, May 2010.

[235] A. Senju, V. Southgate, S. White, and U. Frith. Mindblind eyes: an absence of spontaneous theory of mind in Asperger syndrome. *Science*, 325(5942):883–5, Aug. 2009.

[236] D. K. Shahrokh, T.-Y. Zhang, J. Diorio, A. Gratton, and M. J. Meaney. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. *Endocrinology*, 151(5):2276–86, May 2010.

[237] M. Sherif, O. Harvey, B. White, W. Hood, and C. Sherif. *Intergroup Conflict and cooperation: The Robbers Cave experiments*. University Book Exchange, Norman, Oklahoma, 1961.

[238] T. Singer, H. D. Critchley, and K. Preuschoff. A common role of insula in feelings, empathy and uncertainty. *Trends in Cognitive Sciences*, 13(8):334–40, Aug. 2009.

[239] T. Singer, R. Snozzi, G. Bird, P. Petrovic, G. Silani, M. Heinrichs, and R. J. Dolan. Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. *Emotion*, 8(6):781–91, Dec. 2008.

[240] D. H. Skuse and L. Gallagher. Dopaminergic-neuropeptide interactions in the social brain. *Trends in Cognitive Sciences*, 13(1):27–35, 2009.

[241] B. Slotnick. Initiation of maternal behavior in pregnant nulliparous rats. *Hormones and Behavior*, 4(1-2):53–59, June 1973.

[242] M. D. Smeltzer, J. T. Curtis, B. J. Aragona, and Z. Wang. Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. *Neuroscience Letters*, 394(2):146–51, Mar. 2006.

[243] A. Smith. *The theory of moral sentiments*. 1759.

[244] A. S. Smith, A. Agmo, A. K. Birnie, and J. A. French. Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, Callithrix penicillata. *Hormones and Behavior*, 57(2):255–62, Feb. 2010.

[245] K. S. Smith and K. C. Berridge. The ventral pallidum and hedonic reward: neurochemical maps of sucrose "liking" and food intake. *The Journal of Neuroscience*, 25(38):8637–49, Sept. 2005.

[246] M. Spehr, K. R. Kelliher, X.-H. Li, T. Boehm, T. Leinders-Zufall, and F. Zufall. Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. *The Journal of Neuroscience*, 26(7):1961–70, Feb. 2006.

[247] M. L. Spezio, P.-Y. S. Huang, F. Castelli, and R. Adolphs. Amygdala damage impairs eye contact during conversations with real people. *The Journal of Neuroscience*, 27(15):3994–7, Apr. 2007.

[248] A. S. Squires, R. Peddle, S. J. Milway, and C. W. Harley. Cytotoxic lesions of the hippocampus do not impair social recognition memory in socially housed rats. *Neurobiology of Learning and Memory*, 85(1):95–101, Jan. 2006.

[249] P. Straub. An experimental investigation of ultimatum games: information, fairness, expectations, and lowest acceptable offers. *Journal of Economic Behavior & Organization*, 27(3):345–364, Aug. 1995.

[250] D. F. Swaab. Sexual orientation and its basis in brain structure and function. *Proceedings of the National Academy of Sciences of the United States of America*, 105(30):10273–4, July 2008.

[251] S. Takami and P. P. Graziadei. Light microscopic Golgi study of mitral/tufted cells in the accessory olfactory bulb of the adult rat. *The Journal of Comparative Neurology*, 311(1):65–83, Sept. 1991.

[252] K. E. Tansey, K. J. Brookes, M. J. Hill, L. E. Cochrane, M. Gill, D. Skuse, C. Correia, A. Vicente, L. Kent, L. Gallagher, and R. J. L. Anney. Oxytocin receptor (OXTR) does not play a major role in the aetiology of autism: genetic and molecular studies. *Neuroscience Letters*, 474(3):163–7, May 2010.

[253] S. E. Taylor, G. C. Gonzaga, L. C. Klein, P. Hu, G. A. Greendale, and T. E. Seeman. Relation of oxytocin to psychological stress responses and hypothalamic-pituitary-adrenocortical axis activity in older women. *Psychosomatic Medicine*, 68(2):238–45, Jan. 2006.

[254] M. G. Terenzi and C. D. Ingram. Oxytocin-induced excitation of neurones in the rat central and medial amygdaloid nuclei. *Neuroscience*, 134(1):345–54, Jan. 2005.

[255] M. R. Thompson, P. D. Callaghan, G. E. Hunt, J. L. Cornish, and I. S. McGregor.

A role for oxytocin and 5-HT(1A) receptors in the prosocial effects of 3,4 methylenedioxymethamphetamine ("ecstasy"). *Neuroscience*, 146(2):509–14, May 2007.

[256] R. N. Thompson, R. McMillon, A. Napier, and K. S. Wekesa. Pregnancy block by MHC class I peptides is mediated via the production of inositol 1,4,5-trisphosphate in the mouse vomeronasal organ. *The Journal of Experimental Biology*, 210(Pt 8):1406–12, Apr. 2007.

[257] D. H. Thor and W. R. Holloway. Social memory of the male laboratory rat. *Journal of Comparative and Physiological Psychology*, 96(6):1000–1006, 1982.

[258] K. Tomizawa, N. Iga, Y.-F. Lu, A. Moriwaki, M. Matsushita, S.-T. Li, O. Miyamoto, T. Itano, and H. Matsui. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nature Neuroscience*, 6(4):384–90, Apr. 2003.

[259] M. Tops, J. M. van Peer, and J. Korf. Individual differences in emotional expressivity predict oxytocin responses to cortisol administration: relevance to breast cancer? *Biological Psychology*, 75(2):119–23, May 2007.

[260] H. Tost, B. Kolachana, S. Hakimi, H. Lemaitre, B. A. Verchinski, V. S. Mattay, D. R. Weinberger, and A. Meyer-Lindenberg. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. *Proceedings of the National Academy of Sciences of the United States of America*, 107(31):13936–13941, July 2010.

[261] E. Tribollet, M. Dubois-Dauphin, J. J. Dreifuss, C. Barberis, and S. Jard. Oxytocin receptors in the central nervous system. Distribution, development, and species differences. *Annals of the New York Academy of Sciences*, 652:29–38, June 1992.

[262] D. Y. Tsao, W. A. Freiwald, R. B. H. Tootell, and M. S. Livingstone. A cortical region consisting entirely of face-selective cells. *Science*, 311(5761):670–4, Feb. 2006.

[263] C. Unkelbach, A. J. Guastella, and J. P. Forgas. Oxytocin selectively facilitates recognition of positive sex and relationship words. *Psychological Science*, 19(11):1092–4, Nov. 2008.

[264] L. D. Van de Kar, A. Javed, Y. Zhang, F. Serres, D. K. Raap, and T. S. Gray. 5-HT2A Receptors Stimulate ACTH, Corticosterone, Oxytocin, Renin, and Prolactin Release and Activate Hypothalamic CRF and Oxytocin-Expressing Cells. *Journal of Neuroscience*, 21(10):3572–3579, 2001.

[265] E. van Leengoed, E. Kerker, and H. H. Swanson. Inhibition of post-partum maternal behaviour in the rat by injecting an oxytocin antagonist into the cerebral ventricles. *Journal of Endocrinology*, 112(2):275–282, Feb. 1987.

[266] P. S. van Nieuwenhuijzen, L. E. Long, G. E. Hunt, J. C. Arnold, and I. S. McGregor. Residual social, memory and oxytocin-related changes in rats following repeated exposure to gamma-hydroxybutyrate (GHB), 3,4-methylenedioxymethamphetamine (MDMA) or their combination. *Psychopharmacology*, pages 1–12, Aug. 2010.

[267] J. G. Veening, T. de Jong, and H. P. Barendregt. Oxytocin-messages via the cerebrospinal fluid: Behavioral effects; a review. *Physiology & Behavior*, May 2010.

[268] P. Veinante and M.-J. Freund-Mercier. Distribution of oxytocin- and vasopressin-binding sites in the rat extended amygdala: a histoautoradiographic study. *The Journal of Comparative Neurology*, 383(3):305–325, July 1997.

[269] E. Vul, C. Harris, P. Winkielman, and H. Pashler. Puzzlingly High Correlations in fMRI Studies of Emotion, Personality, and Social Cognition. *Perspectives on Psychological Science*, 4(3):274–290, May 2009.

[270] S. Wagner, A. L. Gresser, A. T. Torello, and C. Dulac. A multireceptor genetic approach uncovers an ordered integration of VNO sensory inputs in the accessory olfactory bulb. *Neuron*, 50(5):697–709, June 2006.

[271] H. Walum, L. Westberg, S. Henningsson, J. M. Neiderhiser, D. Reiss, W. Igl, J. M. Ganiban, E. L. Spotts, N. L. Pedersen, E. Eriksson, and P. Lichtenstein. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 105(37):14153–6, Sept. 2008.

[272] Z. Wang, G. Yu, C. Cascio, Y. Liu, B. Gingrich, and T. R. Insel. Dopamine D2 Receptor-Mediated Regulation of Partner Preferences in Female Prairie Voles (Microtus ochrogaster): A Mechanism for Pair Bonding?*1. *Behavioral Neuroscience*, 113(3):9, 1999.

[273] S. R. Wersinger, J. L. Temple, H. K. Caldwell, and W. S. Young. Inactivation of the oxytocin and the vasopressin (Avp) 1b receptor genes, but not the Avp 1a receptor gene, differentially impairs the Bruce effect in laboratory mice (Mus musculus). *Endocrinology*, 149(1):116–21, Jan. 2008.

[274] A. Whiten and R. W. Byrne. *Machiavellian intelligence II: extensions and evaluations, Volume 2*. Cambridge University Press, 1997.

[275] J. Winking, H. Kaplan, M. Gurven, and S. Rucas. Why do men marry and why do they stray? *Proceedings of the Royal Society B*, 274(1618):1643–9, July 2007.

[276] J. Winslow and T. Insel. Social status in pairs of male squirrel monkeys determines the behavioral response to central oxytocin administration. *Journal of Neuroscience*, 11(7):2032–2038, 1991.

[277] J. T. Winslow. Neuropeptides and nonhuman primate social deficits associated with pathogenic rearing experience. *International Journal of Developmental Neuroscience*, 23(2-3):245–51.

[278] J. T. Winslow, N. Hastings, C. S. Carter, C. R. Harbaugh, and T. R. Insel. A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, 365(6446):545–8, Oct. 1993.

[279] S. Wu, M. Jia, Y. Ruan, J. Liu, Y. Guo, M. Shuang, X. Gong, Y. Zhang, X. Yang, and D. Zhang. Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biological Psychiatry*, 58(1):74–7, July 2005.

[280] L. J. Young and Z. Wang. The neurobiology of pair bonding. *Nature Neuroscience*, 7(10):1048–54, 2004.

[281] P. J. Zak, R. Kurzban, and W. T. Matzner. Oxytocin is associated with human trustworthiness. *Hormones and Behavior*, 48(5):522–7, Dec. 2005.

[282] P. J. Zak, A. A. Stanton, and S. Ahmadi. Oxytocin increases generosity in humans. *Public Library of Science One*, 2(11):e1128, Jan. 2007.

[283] D. Zald. The human amygdala and the emotional evaluation of sensory stimuli. *Brain Research Reviews*, 41(1):88–123, Jan. 2003.

[284] H. H. Zingg and D. L. Lefebvre. Oxytocin mRNA: increase of polyadenylate tail size during pregnancy and lactation. *Molecular and Cellular Endocrinology*, 65(1-2):59–62, Aug. 1989.