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Evolution of the Oxytocin and Vasopressin Systems in Humans and Great Apes

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Evolution of the Oxytocin and Vasopressin Systems in Humans and Great Apes

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M.A., Anthropology, Emory University, 2016
B.A., Anthropology and Psychology, University of Notre Dame, 2013

Advisor: James K. Rilling, Ph.D.

An abstract of
a dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Anthropology
2019

Abstract

Evolution of the Oxytocin and Vasopressin Systems in Humans and Great Apes

By Christina N. Rogers

Humans share a close phylogenetic relationship with chimpanzees and bonobos. We share approximately 98% of our genes with both species. Yet despite this similarity, there is a remarkable diversity of social behavior among the three species in mating systems, aggression, territoriality, and anxiety. A growing literature suggests a role for the neuropeptides oxytocin and vasopressin in the regulation of these behaviors. Most of this research has been done in rodents, and little is known about the basic neurobiology of the oxytocin and vasopressin systems in primates. This project explores variation in these systems in humans, chimpanzees, bonobos, and rhesus macaques, positing potential proximate mechanisms that may underlie variation in social behavior. First, this study examines the distribution of neurons producing oxytocin and vasopressin across the four species and the targets of their axonal fiber projections. We find a greater density of vasopressin fibers in chimpanzees than in bonobos or humans, which may be related to higher levels of aggression and territoriality in chimpanzees. We also find fiber projections into the cortex in great apes to a greater extent than rhesus macaques, suggesting a mechanism for spatially and temporally specific modulation by oxytocin and vasopressin of regions implicated in social cognitive processes such as empathy. Finally, we characterize the distribution of receptors in chimpanzees, comparing the results to published data from human studies. We find a lower density of oxytocin and vasopressin in reward areas as compared with humans, which may relate to differences in pair-bonding. We also find vasopressin receptors in the amygdala of chimpanzees, consistent with the presence of axonal fibers. These findings can add to anthropological theories of social behavior changes over the course of human evolution, such as the human self-domestication hypothesis and the cooperative breeding hypothesis.

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Acknowledgements

It is difficult to put into words the gratitude I feel for the incredible amount support I have been given over the last six years. First, I thank my advisor Dr. Jim Rilling for his endless encouragement and sometimes shockingly immediate feedback whenever I needed help. I have enjoyed many great conversations in his office, from formulating hypotheses early on to making sense of what my data meant years later. I had the special challenge of working at the boundary of multiple fields, and any time I struggled to navigate this, Jim was ready with advice or a connection.

My committee helped me grow as a scientist in their different ways. Dr. Todd Preuss has an unmatched intellectual curiosity and always made me feel like my ideas mattered and that I had something useful to contribute, even as a first year student struggling with my confidence. Dr. Larry Young continually inspires me with his ability to take thousands of studies with sometimes seemingly disparate findings and make them fit together in a cohesive theory, and helps me to understand the implications of my own findings in new and exciting ways. Dr. Dietrich Stout is one of the most broadly knowledgeable people I've ever met, and when I am mired in details of brain function on a molecular level, Dietz always keeps me grounded in anthropology and thinking more clearly about evolution. Dr. Adrian Jaeggi's enthusiasm and encouragement reminds me why I love this field, and his deep understanding of behavior was a much-needed complement to my focus on neurobiology.

I am indebted to Laney Graduate School as a whole, and particularly the anthropology department, for providing a fertile space for intellectual growth. I owe much to staff members, especially Lora McDonald, Jill Marshall, Kay Norgard, and Brian Banks, who helped me figure out requirements and funding. To the other anthropology graduate students, particularly my anthropology cohort - Sarah Whitaker, Ioulia Fenton, Shreyas Sreenath, Sara Kauko, and Tenzin Namdul - for making the highs higher and the lows bearable. Finally, to Dan Coppeto for being a great friend since the very beginning, a collaborator, and an endless source of knowledge. I will always remember shooting around crazy ideas

about oxytocin, vasopressin, and primate behavior at 3 A.M. while wrapping up an experiment or cutting a block of tissue.

The community at Yerkes National Primate Research Center has been a crucial part of my graduate training. I walked into Yerkes in 2014 with big ideas and zero wet lab experience. I am eternally grateful to Mary Ann Cree for making me feel welcome and giving me a home at Yerkes in the Preuss Lab, but also for endless logistical support, great friendship, and excellent pie recipes. To Jeromy Dooyema for teaching me pretty much everything I know about how to do science. To Ethan Siegel and Shweta Sahu, my undergraduate mentees with whom I shared the joys of discovery. To Olivia Zarella, who kept the fun alive even on the longest days of lab work. To the Young Lab, particularly Kiyoshi Inoue, Jamie LaPrairie, and Lorra Julian, for answering my millions of questions and teaching me how to do receptor autoradiography. To Ingrid Budreckas, Mark Hanfman, and the rest of the staff who helped me navigate the world of grants.

One of the most rewarding and engaging parts of my graduate education was my enrollment in the certificate program in the Center for Mind, Brain, and Culture. The faculty and staff of the CMBC significantly enriched my graduate experience, particularly Dr. Bob McCauley, Dr. Lynne Nygaard, and Tamara Beck. I thank my CMBC cohort - Eva Lewandowski, Marianne Florian, and Shensheng Wang - for many stimulating discussions and just as many laughs.

I owe a debt of gratitude to my funding sources for allowing me the freedom to explore the questions in my dissertation. First, to the Silvio O. Conte Center for Oxytocin and Social Cognition, for a pilot grant allowing me to try out receptor autoradiography in chimpanzees and get the variables right. Second, to the Leakey Foundation for believing in the importance of understanding our human past, and for providing me with a dissertation grant for living support and supplies.

Lastly, my greatest support of all came from my family and loved ones. To Mom, who is no longer here with us to see me finish my Ph.D., but always knew that I would. When I was going through my qualifying exam in 2016, she mailed me an entire box of frozen pizzas to help me survive it. That is one example of thousands of her incredible thoughtfulness, love, and support. I try every day to live her

example. To Dad, who would do anything for me and who always encouraged me to be a [rocket] scientist...brain science will have to do! To Paul and Daniela, the greatest and most supportive brother and sister-in-law imaginable, who are my partners in the tough times and the good times. Last to Michael, my best friend and my rock through the ups and downs and uncertainties of graduate school. Although Skyping from a lab at midnight might not be the ideal relationship quality time, it kept me sane in my busiest stretches. It is the great honor of my life to make you all proud.

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Introduction

"With those animals which were benefited by living in close association, the individuals which took the greatest pleasure in society would best escape various dangers, whilst those that cared least for their comrades, and lived solitary, would perish in greater numbers. With respect to the origin of the parental and filial affections, which apparently lie at the base of the social instincts...we may infer that it has been to a large extent through natural selection."

-Charles Darwin, The Descent of Man, 1871

Social Animals

Somewhere in east Africa, a naked mole-rat burrows underground, passing several other members of its colony of some eighty other individuals. This mole-rat is one of the very few breeding males that will mate with the queen, the only reproductively active female. All of the other mole-rats are sterile workers or reproductively suppressed females. They will all contribute to the care of the pups produced by the queen. Meanwhile, in southern Africa, a cape mole-rat - a relative of the naked mole-rat - crawls alone through a burrow system. The cape mole-rat has these tunnels all to itself, living a solitary life. By some unfortunate mistake, this mole-rat encounters an individual of its own species. It throws its head back and opens its jaws, chattering its teeth at the rival, finally scaring it away. It will react this way to conspecifics for its entire life, except for brief interludes during the breeding season.

The two species of mole-rat exhibit strikingly different social-behavioral strategies, yet both have survived for millions of years. The diversification and elaboration of animal species has produced remarkable variation in social behavior. This complicates and enriches the scientific endeavor to understand how behavior evolves. In social species, survival and reproduction depend not only on adaptation to the physical environment but adaptation to social surroundings. Humans live in large, complex social groups, as do many primates. The establishment and maintenance of social bonds are a crucial component of adaptive functioning within a social world.

Why do different species have different social behaviors? Such a question can be interpreted in various ways. In 1963, Niko Tinbergen formalized the field of ethology by clearly defining four questions necessary to paint a full picture of a behavior (Kapheim, 2018). These can be separated along an axis of causation (proximate versus ultimate) and object of study

(historical versus a contemporary snapshot of a behavior). Questions of *mechanism* ask what stimuli elicit a behavior, and how the behavior works on a molecular, physiological, and cognitive level. *Ontogeny* entails explaining the behavior in terms of its development, change with age, and the experiences necessary for the behavior to be expressed. *Adaptation* refers to how the behavior impacts an animal's chance of survival and reproduction, while *phylogeny* refers to how the behavior compares with that of related species, and how it might have developed over the course of evolution.

The bulk of this dissertation falls within the realm of *mechanism*. The neuroanatomy of the oxytocin and vasopressin systems will be explored in humans, chimpanzees, bonobos, and rhesus macaques. Thus, while the focus is on the neurobiological processes underlying the expression of social behavior, the comparative aspect of this research situates it within *phylogeny* as well. Importantly, the expression of these neuropeptides can change over the life course and in response to experience (*ontogeny*), and much concurrent research is outlining the adaptive value of the behavioral traits they promote (*adaptation*). The neuroanatomy of oxytocin and vasopressin will be placed in the context of this literature, painting a fuller picture of human and primate sociality.

Oxytocin and Vasopressin: Social Behavior across Taxa

Understanding the evolution and development of a particular social behavior is not simple. It involves close study of an animal's neurobiology, its genetics, its hormonal patterns, the details of its behavior and context of expression, the nature of the group structure in which it lives, and if possible, fossil evidence of morphological characteristics which indicate certain behaviors. Despite this complexity, some factors have emerged as influencing social behavior in

a wide range of animals. Perhaps the best known are two neuropeptides: oxytocin and vasopressin.

Two Neuropeptides

Two neuropeptides have been identified across diverse taxa to play a significant role in the regulation of social behavior. Oxytocin (OT) and arginine vasopressin (AVP) are known as nonapeptide hormones; they each have a nine amino acid sequence and differ at only the third and eighth positions (Albers, 2014). These neuropeptides, along with their homologs, are at least 700 million years old. OT and AVP are found in mammals (with lysipressin replacing AVP in pigs and some marsupials, and phenypressin in other marsupials), while in other vertebrates, the structurally similar vasotocin (AVP homolog), mesotocin, and isotocin (OT homolog) are found (Donaldson & Young, 2008). OT and AVP as two separate molecules are thought to have arisen from a gene duplication event occurring early in the vertebrate lineage ~600 million years ago (Acher & Chauvet, 1995). In mammals, OT and AVP genes are found on the same chromosome, where they are separated by a small region and transcribed in opposite directions (Burbach, Luckman, Murphy, & Gainer, 2001; Donaldson et al., 2008; Hara, Battey, & Gainer, 1990). Invertebrates have one peptide homologous to the OT/AVP of mammals - for example, annepressin is found in annelid worms, while connopressin is found in snails and leeches (Donaldson & Young, 2008). Remarkably, these singular OT/AVP-related peptides influence similar processes in invertebrates as in vertebrates and even mammals. For example, nematodes (*Caenorhabditis elegans*) produce nematocin, encoded by a similar gene (ntc-1) to those encoding the mammalian OT and AVP. Males lacking the peptide or its receptors show deficits in many reproductive behaviors such as mate search and mate recognition, while other motor behaviors remain intact (Garrison et al., 2012).

In basal vertebrates (anamnia), such as agnathans, fish, and amphibians, the homologs of OT and AVP are produced by magnocellular neurosecretory neurons in the wall of the third ventricle of the hypothalamus. In more advanced vertebrates - the amniotes, including reptiles, birds, and mammals - OT and AVP are produced in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus as well as the accessory nuclei in between them (Knobloch & Grinevich, 2014). Cells from these regions send axons to the posterior pituitary to be released into the bloodstream where they have physiological effects (Burbach, Young, & Russell, 2006). OT- and AVP- synthesizing neurons in the PVN and SON also release OT and AVP into the brain, where they can act on receptors to exert varied effects on social cognition and behavior. While the neuropeptide-producing cells and their projections are somewhat conserved across species, the distribution of receptors can vary drastically (Albers, 2014). Moreover, receptor localization correlates strongly with social cognition and behavior, such as sexual behavior, social attachment, aggression, and social recognition across multiple taxa (Albers, 2014; Anacker & Beery, 2013).

Before their influence on social behavior was understood, OT and AVP were identified for their physiological roles in the body. OT was initially labeled as a “maternal hormone” due to its facilitation of mammalian birth and milk ejection in lactation (Soloff, Alexandrova, & Fernstrom, 1979). OT receptors are found in the uterus, where the OT peptide binds to stimulate uterine contractions and facilitate labor. Receptors are also found in the mammary glands, where OT binds to stimulate milk ejection in response to suckling (Burbach et al., 2006). Broadly, OT has been found to promote maternal behavior and bonding in the brain, though it has effects in males as well as females. In contrast, AVP acts on blood vessels, which express the vasopressin v1a receptor (AVPR1a) to control vasoconstriction. It also acts on the v2 receptor (AVPR2) in

the kidneys to control water balance (Nielsen et al., 1995). In the brain, AVP is related to territoriality, aggression, but also social bonding in males (Johnson & Young, 2015). The functions of AVP in the brain and body do not seem to match up as readily as those of OT; however, some of the behaviors through which many animals communicate territoriality are scent-marking and urination. It may be that the evolutionarily ancient AVP system for bodily regulation was hijacked for social communication through the chemosenses (Freeman & Young, 2015).

Oxytocin, Vasopressin, and Social Behavior: Evidence from Rodent Studies

Pair-bonding

Monogamy is uncommon among mammals, occurring in about 9% of mammalian species, compared to 90% of bird species (Walum & Young, 2018). Much of our knowledge about the neurobiology of pair-bonding comes from the monogamous prairie vole (*Microtus ochrogaster*). Prairie voles form long-lasting pair bonds in which both sexes provide care to offspring. Perhaps the most well-studied roles of OT and AVP are their facilitation of the pair bond in monogamous versus non-monogamous voles.

These studies indicate that species-specific receptor distributions for OT and AVP can underlie species-typical bonding behaviors. In the monogamous prairie vole, OT receptor (OXTR) density is highest in the prelimbic cortex, bed nucleus of the stria terminalis (BNST), nucleus accumbens, midline nuclei of the thalamus, and lateral amygdala, all of which show sparse or absent binding for OXTR in the polygynous montane vole (*Microtus montanus*). In the latter species, OXTR were localized to the lateral septum, ventromedial hypothalamus, and

cortical nucleus of the amygdala (Insel & Shapiro, 1992). Moreover, the distributions of these receptors in two more species, the monogamous pine vole (*Microtus pinetorum*), and the polygynous meadow vole (*Microtus pennsylvanicus*), were found to largely reflect the same patterns (Insel, Wang, & Ferris, 1994).

Further investigation revealed species-specific distributions of AVP receptors (AVPR1a) in these same species (Insel et al., 1994). Subsequently, it was determined that AVPR1a in the ventral pallidum and lateral septum of the male prairie vole, and OXTR in the nucleus accumbens and prefrontal cortex of the female prairie vole, specifically facilitate pair-bonding (Johnson & Young, 2015; Lim, Hammock, & Young, 2004; Young & Wang, 2004; Young, Lim, Gingrich, & Insel, 2001). Moreover, receptor antagonists in these areas block partner-preference formation (Lim et al., 2004; Liu, Curtis, & Wang, 2001; Young et al., 2001). Importantly, individual variation in the density of OXTR in the nucleus accumbens in prairie voles is associated with variation in alloparental (Olazábal & Young, 2006) and monogamy-related behavior (Ophir, Gessel, Zheng, & Phelps, 2012).

Parental behavior

The mother-infant bond is a crucial component of mammalian sociality, and it may provide the neurobiological scaffold from which other forms of social bonds have been built (Carter, 1998). As mentioned above, OT plays a role in (live) birth and lactation - both of which are unique to mammals. In many mammals, the event of giving birth causes previously aversive infant stimuli to become attractive (Rilling & Young, 2014). OT is necessary for the expression of maternal behaviors; intracerebroventricular administration of OT in virgin rats can induce maternal behavior (Pedersen & Prange, 1979), while the introduction of an OT antagonist in

post-partum rats inhibits maternal behavior (Van Leengoed, Kerker, & Swanson, 1987). Both OT and AVP are also implicated in maternal aggression, which refers to a mother defending her offspring against a potential intruder. This drive is unique in lactating females; virgin female rats almost never attack an intruder (Bosch, 2013). Finally, AVP is involved in paternal defense of the young in prairie voles (Kenkel et al., 2012), and an AVP antagonist injected into the lateral septum has been shown to reduce paternal care in this species (Numan & Insel, 2003).

Social Recognition

There is considerable evidence for the involvement of OT and AVP on social recognition in a variety of rodent species. Mice lacking the OT gene cannot develop social memory, but other forms of memory remain intact (Ferguson et al., 2000). Moreover, OT acting in the medial amygdala is necessary for social recognition in mice (Ferguson, Aldag, Insel, & Young, 2001). In rats, OT receptor deletion in the anterior olfactory cortex results in impairment of social recognition (Oettl et al., 2016). AVP has also been shown to facilitate social memory in rats, specifically in the septum (Dantzer, Koob, Bluthé, & Le Moal, 1988; Le Moal, Dantzer, Michaud, & Koob, 1987). In mice, knockout of the AVP v1a receptor leads to profound impairment of social recognition as well as a reduction of anxiety-like behaviors (Bielsky, Hu, Szegda, Westphal, & Young, 2004).

Aggression

AVP has been associated across rodent species with territorial and aggressive behavior, particularly in males (Donaldson & Young, 2008; Ferris et al., 1997). Indeed, selective aggression is part of the typical pair-bonding behaviors in male monogamous prairie voles

(Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). In particular, AVP in the anterior hypothalamus has been linked to aggression in pair-bonded prairie voles (Gobrogge, Liu, Jia, & Wang, 2007) as well as hamsters (Ferris & Potegal, 1988), and local AVP injected into the amygdala has been associated with intermale aggression in rats (Koolhaas, Van Den Brink, Roozendaal, & Boorsma, 1990).

Reducing anxiety and fear

As mentioned above, AVP v1a receptor knockout mice show a reduction of anxiety-like behaviors (Bielsky et al., 2004). However, the relationship between AVP and anxiety may be more complicated and context-specific. In California mice, inhibition of v1a receptors in the BNST of males and nucleus accumbens of females was anxiogenic in a social defeat paradigm (Duque-Wilckens et al., 2016). OT is generally anxiolytic and is released during sexual intercourse (Carmichael et al., 1987). One study found that in rats, centrally released OT mediates mating-induced sedation and calmness in males (Waldherr & Neumann, 2007). Moreover, OT has been found to attenuate amygdala reactivity to fear (Labuschagne et al., 2010). Consolation behavior in prairie voles towards a stressed partner is dependent on OXTR in the cingulate cortex (Burkett et al., 2016).

Mechanisms of Action

In mammals, OT and AVP are primarily produced in the PVN and SON of the hypothalamus. The peptides must reach receptors in the body and brain to exert their effects. One OT and three AVP (V1a, V1b, V2) receptors have been identified, all of which are part of the G-protein coupled receptor superfamily (Barberis & Tribollet, 1996; Caldwell & Young, 2006; Hasunuma et al., 2013). OT and V1a receptors are expressed throughout the mammalian brain,

while V1b is restricted to areas of the hippocampus, hypothalamus, and amygdala (Stevenson & Caldwell, 2012; Young, Li, Wersinger, & Palkovits, 2006), and evidence for V2 in the brain is unclear (Foletta, Brown, & Young, 2002; Hirasawa, Hashimoto, & Tsujimoto, 1994; Hirasawa, Nakayama, et al., 1994; Kato, Igarashi, Hirasawa, Tsujimoto, & Kobayashi, 1995; Vargas et al., 2009). It is likely that some of the effects of OT and AVP on behavior might arise through cross-talk among these receptors (Albers, 2014); both OT acting on AVP v1a receptors (Schorscher-Petcu et al., 2010; Song et al., 2014) and vice versa (Song, Larkin, Malley, & Albers, 2016) have been observed in rodents.

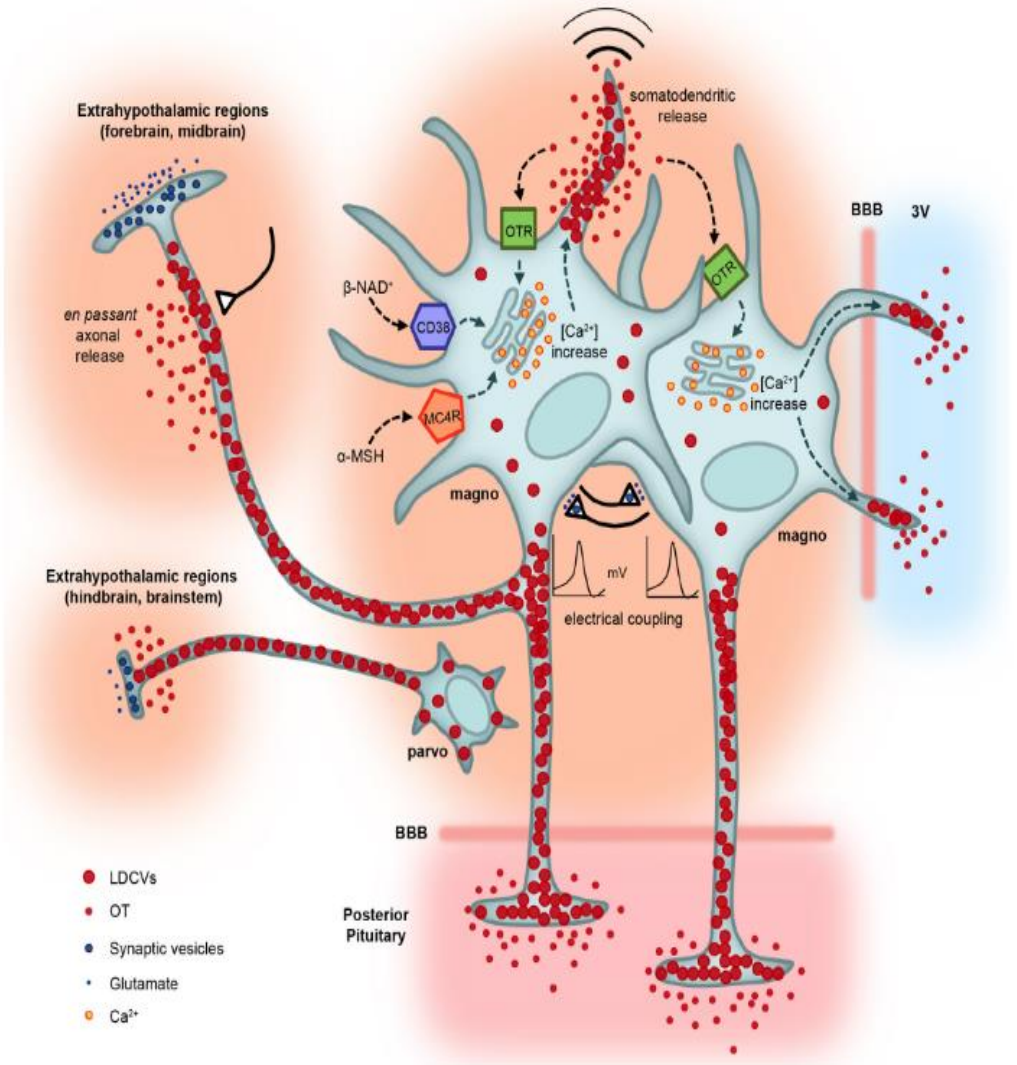
How do OT and AVP reach these receptors, often far from their site of production in the hypothalamus (Figure 1.1)? Magnocellular OT and AVP neurons send axons into the posterior pituitary to release these peptides into the blood circulation. OT and AVP can also be released from the dendrites of these neurons, which can contact the third ventricle. Upon their somatodendritic release, OT and AVP can diffuse through the brain in a global rather than targeted fashion. OT has a half-life of about 20 minutes (Mens, Laczi, Tonnaer, de Kloet, & van Wimersma Greidanus, 1983); it is unlikely that OT and AVP released from dendrites of hypothalamic cells reaches distant extrahypothalamic regions to achieve temporally specific effects (Knobloch & Grinevich, 2014).

OT- and AVP-producing cells also send axons into the brain. Parvocellular OT and AVP neurons send projections toward the brainstem and spinal cord, forming synaptic contacts with local neurons (Swanson & Sawchenko, 1983). The parvocellular cells do not release OT and AVP into the bloodstream like the magnocellular neurons do. Release of OT and AVP from magnocellular hypothalamic neurons into the forebrain is unlikely to occur at synapses of axonal terminals (Knobloch & Grinevich, 2014). First, the onset of electrophysiological and behavioral

responses to release is delayed, suggesting a process on a longer temporal scale than synaptic release. Also, in the few OT neuron synapses found in the SON and ventromedial hypothalamus, large dense-core vesicles containing OT or AVP are not found in the active zones of pre-synapses and OT receptors are not found in the postsynaptic membrane.

It is more likely that OT and AVP are released from axons *en passant* (from the axon stem rather than at axon terminals). Early studies have overlooked the presence of OT and AVP axons in forebrain regions, which is unsurprising given their sparsity (Knobloch et al., 2012). However, each OT or AVP large dense core vesicle contains an enormous number of molecules: about ~85,000 (Leng & Ludwig, 2008). Both OT and AVP have high affinities for their receptors, in the nanomolar range (Akerlund et al., 1999). Altogether, this suggests that even with a few fibers, OT and AVP release can exert significant effects within a brain area.

*Figure 1.1***.** Mechanisms of signaling. Figure reproduced from (Johnson & Young, 2017). Oxytocin neuron function and mechanisms of release are displayed. Magnocellular OT axons project to the posterior pituitary, but also innervate extrahypothalamic forebrain and midbrain regions. OT can be released from dendrites as well, which can also contact the third ventricle. Parvocellular OT regions project mainly to hindbrain and brainstem regions, and axon collaterals from parvocellular OT neurons can excite and trigger release from magnocellular OT neurons in the SON. OT is stored in large dense core vesicles found in soma, dendrites, and axons (but not axon terminals), thus it is thought to be released from axons *en passant*.



Oxytocin and Vasopressin: Systems within Systems

Interactions with Other Neuromodulators

There are other neurotransmitters, hormones, etc., involved in social behavior, and several brain regions besides the hypothalamus where OT/AVP is produced. Where does OT/AVP fit in to the broader attempt to understand social behavior?

OT and AVP interact with a number of other brain systems. Interplay between OT/AVP and the dopamine reward system is necessary in many species to facilitate pair-bonding. The presence of OT or AVP v1a receptors in reward pathways is a common characteristic across several monogamous mammal species (Walum & Young, 2018), and OT pathway genes in humans are highly coexpressed with those for dopamine as well as acetylcholine (Quintana et al., 2019). In a variety of species, OT receptors are found in regions high in acetylcholine, and OT may interact with acetylcholine to orient attention to social stimuli (Demeter & Sarter, 2013). OT also interacts with serotonin to promote social reward: in mice, OT receptor- containing cells in the dorsal raphe nucleus send serotonin projections to the nucleus accumbens to promote social reward (Dölen, Darvishzadeh, Huang, & Malenka, 2013). OT and AVP are both regulated by gonadal steroid hormones in early development as well as in adulthood. Gonadectomy reduces AVP cell number and fibers in the medial amygdala and BNST of adult rats, both in neonatal and adult individuals (Bingham & Viau, 2008; De Vries, Buijs, & Van Leeuwen, 1984; Rood et al., 2013; Wang & De Vries, 1995).

Interactions within Brain Networks

Research concerning the effects of OT and AVP on social behavior often proceeds in the tradition of connecting a single region to a single behavior. However, in the last couple of

decades, there have been great advances in our understanding of brain networks and how social behaviors might be emergent from these networks. The parts of the brain that have been associated with OT and AVP belong to established networks that operate in a complex manner. Thus the roles of OT and AVP in social behavior are best considered in the context of neural circuits.

As first proposed by Newman (1999), the Social Behavior Network (SBN) is composed of the extended amygdala, the lateral septum, the periaqueductal gray, and three hypothalamic regions: the medial pre-optic area, the ventromedial hypothalamus, and the anterior hypothalamus. The Social Behavior Network is more tightly defined than many previous concepts such as the "limbic system", the makeup of which has no generally accepted definition (Heimer & Van Hoesen, 2006). Newman's criteria for inclusion in the Social Behavior Network are as follows: all regions are reciprocally connected, all regions contain neurons with gonadal hormone receptors, and each region has been identified as a site of regulation or activation of more than one social behavior. Importantly, OT and/or AVP has been found to exert effects in each of these regions in rodent species (Albers & Cooper, 1995; Albers, & Ferris, 1985; Ferris, Albers, Wesolowski, Goldman, & Luman, 1984; Hennessey & Alberts, 1992; Irvin, Szot, Dorsa, Potegal, & Ferris, 1990).

The Social Behavioral Network interacts with the mesolimbic dopamine system (O'Connell & Hofmann, 2011). Specifically, this refers to the dopaminergic pathways leading from the ventral tegmental area (VTA) to the nucleus accumbens. Moreover, the pathway leads back to the VTA, both directly and through the ventral pallidum (VP). When adding in the connections from the VTA to the medial prefrontal cortex, as well as from the medial prefrontal cortex to the nucleus accumbens and VTA, it is referred to as the mesocorticolimbic pathway

(Shizgal & Hyman, 2013). These regions and connections are considered to be part of the brain's "reward system", an evolutionarily conserved neural circuit where the salience of an external stimulus is evaluated and is incorporated into motivation to seek reward and avoid aversion (Deco & Rolls, 2005; Wickens, Budd, Hyland, & Arbuthnott, 2007). The reward pathway has been identified as the neural substrate of drug addiction (Everitt & Robbins, 2005), and thus a substantial portion of research on the nucleus accumbens investigates reward or addiction.

O'Connell and Hofmann have theorized that the Social Behavior Network and the mesolimbic dopamine system should be considered together as a Social Decision-Making Network. Because social decision-making requires an evaluation of the salience of external stimuli in order to select an adaptive behavioral response, the mesolimbic reward system and SBN are best understood as "an integrated and evolutionarily ancient social decision-making (SDM) network that regulates and implements responses to salient stimuli (both social and nonsocial)" (O'Connell & Hofmann, 2011).

Social attachment and pair-bonding are behaviors which involve brain regions in both the mesolimbic reward pathway and the Social Behavior Network. The aforementioned OT and AVP V1a receptor distribution in monogamous prairie voles and promiscuous montane voles differs in mesolimbic reward areas (Burkett & Young, 2012). Evolution may accomplish pair-bonding in different species by introducing neuropeptides into regions of the reward system. A recent model proposed by Johnson and Young includes dopamine release to modulate reward learning and salience of social cues, and neuropeptide release to facilitate and encode the sensory signature of a social partner into the amygdala and ultimately the ventrostriatopallidal system where it is integrated with the reward of mating (Johnson & Young, 2015). Pair-bonding may essentially be a linkage of the neural encoding of partner cues via OT and AVP with the brain's

reward system (Walum & Young, 2018). The OT and AVP in pair-bonding provides an example of how the Social Behavior Network and mesolimbic reward system interact to form a Social Decision-Making network.

By Newman's as well as O'Connell and Hofmann's definitions, the SBN and mesolimbic reward system share two nodes: the lateral septum and BNST (Newman, 1999; O'Connell & Hofmann, 2011). O'Connell and Hofmann propose that these regions are well positioned to serve as relay stations mediating information about stimulus salience into adaptive behavioral output. As mentioned above, AVP in the lateral septum plays an important role in pair-bonding. Infusions of an AVP antagonist to the AVP V1a receptor also impair social recognition (Landgraf et al., 1995; Liebsch, Wotjak, Landgraf, & Engelmann, 1996), while infusions of AVP into the lateral septum facilitates social memory (Dantzer et al., 1988), and expression of the V1a receptor in the lateral septum rescues social recognition in V1a knockout mice (Bielsky, Hu, Ren, Terwilliger, & Young, 2005). The role of neuropeptides in the BNST has been mentioned above with regard to the BNST as a component of the extended amygdala. The BNST is also a region with consistent sex differences in rats; AVP innervation of the BNST and medial amygdala is higher in males, influenced by gonadal hormones during the prenatal period of development (Caffe, Van Leeuwen, & Luiten, 1987; van Leeuwen, Caffe, & De Vries, 1985). Thus the study of neuropeptides within the Social Decision-Making network can shed light on how this network can produce diverging behaviors in males and females.

OT and AVP exert complex and varied effects on social behavior, the diversity of which can only be understood with an appreciation of the wider networks in which they operate. A network-level perspective on OT and AVP is therefore invaluable in the effort to demystify their role in the evolution of social behavior across species, from rodents to primates. Evidence for the

effects of OT and AVP on social behavior have come mainly from rodent studies. How might these neuropeptides affect human behavior, as well as the behavior of our closest ape relatives and primates in general? What is known about behavioral variation among primates, and how might we expect OT and AVP systems to contribute to this variation?

Socio-behavioral and Neurobiological Differences among Humans, Chimpanzees, and Bonobos

A high degree of sociality is common to most primate species, and is seen among humans and our closest relatives, the chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*). The last common ancestor of humans and the genus *Pan* lived approximately 7-8 million years ago (Langergraber et al., 2012), while the chimpanzee and bonobo lines split from each other approximately 1.5–2.6 million years ago, with possible admixture between 200,000-550,000 years ago (de Manuel et al., 2016; Langergraber et al., 2012). 1.7% of the human genome is more similar to chimpanzees than to bonobos, while 1.6% is more similar to bonobos than to chimpanzees (Prüfer et al., 2012). Both chimpanzees and bonobos are found in sub-Saharan Africa, though their ranges do not overlap. Bonobos are confined to a heavily forested region south of the Congo River, while chimpanzees occupy many diverse habitats north of the river, where most groups experience competition with sympatric gorillas (Malenky & Wrangham, 1994; Wrangham & Peterson, 1996; Wrangham, 1993). Ecological pressures are thought to have led to divergence in chimpanzees and bonobos in socio-behavioral traits such as mating patterns, aggression, and anxiety. What exactly are the differences, and how do they compare to human behavior? What neurobiological and hormonal differences might support the divergence in behavior among the three species?

Behavioral Differences: Aggression

It is generally accepted that chimpanzees display more intense within-group aggression than bonobos. Male chimpanzees aggressively compete for dominance rank, which allows them access to resources and mating (Goodall, 1986; Muller, Kahlenberg, Emery Thompson, & Wrangham, 2007). Often, male chimpanzees engage in elaborate displays which can build up to physical aggression inflicted upon conspecifics (Watts, 1998). All chimpanzee males, even low-ranking males, are dominant to all females, and adolescent males have been observed to target adult females (Goodall, 1986). Chimpanzees routinely engage in coalitionary aggression, when "at least two individuals jointly direct aggression at one or more conspecific targets" (Gilby et al., 2013). Aggression among females can also be severe, though it is less frequent than male aggression (Pusey et al., 2008). Both male and female chimpanzees have been observed to commit within-group infanticide (Townsend, Slocombe, Emery Thompson, & Zuberbühler, 2007).

In contrast, bonobos display much lower within-group aggression. Bonobos have less overt behavioral signs to express dominance. Bonobo displays are much less intense than those of chimpanzees, and they rarely end in aggressive contact (Fruth & Hohmann, 2003; Kano, 1992). Male bonobos do not form alliances with each other to monopolize matings (Fruth & Hohmann, 2003; Furuichi, 1997, 2011). In fact, the strongest bonds male bonobos form are with their mothers (Kano, 1992; Surbeck, Mundry, & Hohmann, 2011). Male-female alliances are relatively frequent in bonobos, and male aggression towards females is low and often countered by a coalition of females (Hohmann & Fruth, 2003; Kano, 1992; Parish, 1996). Female bonobos are dominant to males as measured by feeding priority in all observed bonobo groups (White & Wood, 2007). Reconciliation following aggression is more common and more often initiated by

the aggressor in bonobos than chimpanzees (Goodall, 1986; Parish, de Waal, & Haig, 2000; de Waal, 1987).

It is difficult to posit a species-typical level of aggression for humans, given the extent to which human behavior is modified by social learning and cultural input. Moreover, the issue is complicated by socially learned regulations of behavior – norms, rules, laws – achieved through third-party rewards and punishments (Hill, Barton, & Hurtado, 2009). Nevertheless, anthropologists often look to modern hunter-gatherer societies, which certainly have complex norms and rules, but for whom aspects of life, such as environment and group size, are more similar to the way of life for most of human evolution than those of agricultural and post-agricultural societies. One study attempted to classify aggressive acts among humans in a subset of twenty-one independent mobile forager band societies (MFBS) taken from the Standard Cross-Cultural Sample (Fry & Soderberg, 2013). More than half of events resulting in lethal aggression were perpetrated by lone individuals, while 36% percent of lethal events took place within the local band and 85% percent took place within the same larger societal group. Importantly, this study only recorded lethal aggression; however, many aggressive encounters do not result in fatality. Nevertheless, the fact that lethal aggression does occur is important and stands in contrast to bonobos, which have never been recorded to kill (Wilson et al., 2014).

Between-group aggression is also more severe among chimpanzees than among bonobos. Chimpanzees are highly territorial. Male chimpanzees form groups to patrol the boundaries of their territories, both defending their home range from intruders and attacking vulnerable neighbors (Goodall, 1986; Mitani, Watts, & Amsler, 2010; Wrangham, 1999). Chimpanzees are very xenophobic; inter-group encounters are hostile without exception and can be lethal (Sobolewski, Brown, & Mitani, 2012). Occasionally chimpanzee males will kill the infants of

neighboring mothers or solitary males (Wrangham, 1999). In captive chimpanzees, the introduction of new members to a social group must be managed very closely and carefully, and fatalities can occur because of conspecific aggression (de Waal, 1986).

Bonobos also have hostile intergroup encounters in which both males and females display at outsiders (Kano, 1992); however, intergroup encounters have never been observed to be lethal (Wilson et al., 2014). Moreover, these interactions usually do not always result in aggressive contact. The highest recorded rate of intergroup bonobo encounters resulting in physical aggression is 8 of 23 encounters at the Lomako field site in 1993-1998 (Hohmann & Fruth, 2002). In stark contrast to chimpanzees, bonobos do not conduct border patrols and do not commit infanticide within or between groups (Furuichi, 2011). The most common result of bonobo intergroup encounters is one group leaving the contested area without incident (Hohmann & Fruth, 2002). Intergroup encounters in bonobos have even been observed to be prosocial, with stranger bonobos sitting in close proximity, playing, sharing food, or even engaging in sexual activity (Fruth & Hohmann, 2018; Furuichi, 2011; Idani, 1991). In captivity, new bonobos can be introduced to a social group without issue, even vulnerable individuals such as infants (Hare, Wobber, & Wrangham, 2012).

Human societies, on the whole, tend to show a range of variation in intergroup encounters. Fry and Soderberg's survey revealed that 15% of lethally aggressive events involve persons from outside the larger society, such as colonists, neighboring indigenous cultures, or missionaries. About a third of killings among human foragers involved members outside the immediate band. Meanwhile, in chimpanzees, 44% of killings involve neighboring communities (Wilson et al., 2014). Half of all societies surveyed had no lethal events involving more than one perpetrator, standing in contrast to Pinker's (2011) assertion that warfare is prevalent among

hunter-gatherers and Wrangham and Glowacki's (2012) claim that humans have an evolved tendency to form coalitions and kill members of neighboring groups. Fry and Soderberg (2013) insist that hunter-gatherers aren't particularly warlike; it appears from their data that hunter-gatherer coalitionary killing may range from being virtually absent to approximating the rates of chimpanzees. Ethnographic data from the Yanomami suggest between-group human aggression can be extraordinarily high; moreover, the Yanomami defy notions of xenophobia, as the vast majority of fighting occurs between individuals who know each other well (Ferguson, 2001; Thorpe, 2003). Among the Yanomami, those killed are often relatives in closely related villages with whom good relations were previously shared. Importantly, an increase in the intensity of warfare may be related to periods of Western presence (Ferguson, 2001).

Behavioral Differences: Social Tolerance

Social tolerance is an aspect of primate relationships defined as "the propensity to be in proximity to conspecifics around valuable resources with little or no aggression" (Cronin, De Groot, & Stevens, 2015; Cronin, van Leeuwen, Vreeman, & Haun, 2014). Social tolerance contrasts with conflict avoidance and conflict escalation through aggression, other possible outcomes of coexistence near valuable resources (de Waal, 1996). Hare et al. highlighted social tolerance as another characteristic on which chimpanzees and bonobos differ (Hare, Melis, Woods, Hastings, & Wrangham, 2007). In one study, these researchers tested the extent to which bonobos and chimpanzees would come into close proximity to group mates and co-feed when presented with a food source that could be monopolized. Hare et al. found that bonobos co-fed significantly more often than chimps. Furthermore, in another study, Hare and Kwetuenda (2010) found that bonobos, alone in a room with a food source, would open a door to allow another bonobo in more often than they would open a door to an empty room. This and other

reports have led some researchers to conclude that bonobos are particularly socially tolerant and that this may lend them a higher ability for cooperation (Hare et al., 2007, 2012).

However, there is a significant deal of conflicting evidence about a species-typical level of social tolerance in bonobos and chimpanzees. While studies by Hare and colleagues provide unequivocal support for high levels in bonobos, several other studies fail to find a difference (Bullinger, Burkart, Melis, & Tomasello, 2013) and some even find that bonobos are less socially tolerant in a feeding context (Jaeggi, Stevens, & van Schaik, 2010; de Waal, 1992). In a recent set of studies, Cronin et al. tested chimpanzee and bonobo behavior in a group-level rather than dyadic paradigm (Cronin et al., 2015, 2014). Peanuts, a highly preferred food, were scattered among the chimpanzee and later the bonobo enclosure in two conditions: one in which the peanuts could be easily removed from the ground and monopolized, and one in which the peanuts were mixed in with leaf litter and could not be removed. The researchers found that bonobos were not more socially tolerant than chimpanzees, and, when possible, preferred to quickly collect nuts and then disperse from conspecifics to consume them in a conflict avoidant strategy.

Social tolerance as measured by co-feeding in bonobos and chimpanzees can perhaps be compared to food sharing in humans, though it is important to note that human sharing behavior is facilitated by culturally constructed relationships and extended kinship (Bogin, Bragg, & Kuzawa, 2014; Kaplan, Hooper, & Gurven, 2009). When sharing occurs in most primate species, including great apes, it is mostly done passively through tolerated theft. In contrast, human hunter-gatherer societies are marked by a high degree of food sharing than can occur through all possible patterns, including proactive provisioning (Jaeggi & Gurven, 2013). Human hunter-gatherers commonly rely on food sharing to buffer risk and engage in divisions of labor to

increase production efficiency. The use of food sharing to solve adaptive problems indicates a high degree of social tolerance and may be related to unique aspects of human cooperative psychology (Jaeggi & Gurven, 2013).

Behavioral Differences: Mating and Socio-sexual Behavior

Chimpanzees and bonobos both live in multi-male, multi-female societies in which individuals mate with multiple members of the opposite sex. While there may be some relatively stable male-female associations (Langergraber, Mitani, Watts, & Vigilant, 2013), neither species forms exclusive pair bonds or shows significant paternal care. While in chimpanzees, males rank higher than all females, bonobo females typically rank highest in the group (Surbeck & Hohmann, 2013), and females have been described as dominant or co-dominant. Bonobos also have higher degrees of female-female bonding than chimpanzees (Sommer, Bauer, Fowler, & Ortmann, 2011; White, 1996). Overall, bonobos have more relaxed dominance hierarchies than chimpanzees, likely due to lower feeding competition among females and lower mating competition among males (Jaeggi, Boose, White, & Gurven, 2016).

The promiscuity of bonobos is one of their most well-known characteristics. Bonobos have also been described as "more nervous" than chimpanzees (de Waal, 1996), and they show greater stress hormone response to feeding competition (Wobber et al., 2010). It has been proposed that bonobos engage in sexual activity in all age and sex combinations to relieve tension (de Waal, 1990). After conflict, bonobos who receive sexual consolatory behavior exhibit lower rates of self-scratching, an indicator of stress (Clay & de Waal, 2015). This connection between sexual activity and stress alleviation has not been described in chimpanzees, though they may use other forms of affiliative contact for a similar purpose (de Waal, 1989).

The human mating system is characterized by flexibility and varies across cultures. Despite this variation, stable mating relationships are widespread (Quinlan, 2008). It is notable that humans can engage in pair-bonding behavior, and all societies have the institution of marriage (Chapais, 2013; Walker, Hill, Flinn, & Ellsworth, 2011). In the Standard Cross-Cultural Sample (a representative sample of 186 pre-industrial societies), polyandry is rare (1%) and polygyny is common (82%), but the majority of marriages, even in polygynous societies, are monogamous (Murdock & White, 1969). Indeed, monogamy is often conceived as the minimal attainable form of polygyny (Chapais, 2013; Gurven & Hill, 2009; Hooper, Ross, Gavrilets, & Mulder, 2016). Across cultures, the stability of marriage is greater when both sexes contribute equally to subsistence (Quinlan & Quinlan, 2007). While there is a sexual division of labor in hunter-gatherer societies, humans are marked by a considerable degree of male parental care, lacking in other great ape species (Storey & Ziegler, 2016).

It should be emphasized that as an economic and legal institution, marriage can only be indirect evidence for actual human sexual behavior. Extra-pair copulations certainly occur; attempts to estimate their frequency are difficult and have provided different conclusions (Anderson, 2006; Scelza, 2011). Nevertheless, aspects of human physiology suggest humans may have a long evolutionary history of pair-bonding behavior: we are less sexually dimorphic than species with high male-male competition for and monopolization of females such as gorillas (Mitani, Gros-Louis, & Manson, 1996), and human testes size is smaller than that of highly promiscuous species such as chimpanzees and bonobos (Preston, Stevenson, Pemberton, Coltman, & Wilson, 2003). These observations suggest that even though not all humans engage in pair-bonding, humans possess the biological capacity to facilitate pair-bonding to a greater degree than chimpanzees or bonobos.

Neurobiological, psychological and hormonal differences

What biological characteristics might contribute to the social behavioral variation among hominoids outlined above? A few studies have attempted to explore neuroanatomical differences between chimpanzees and bonobos. A comparative neuroimaging study revealed that, compared to chimpanzees, bonobos have more gray matter in the part of the amygdala that includes the central nucleus, a region implicated in fear and anxiety which activates the hypothalamic-pituitary-adrenal axis and autonomic nervous system (Davis, 1998; Rilling et al., 2012). Bonobos also have more neuropil in the central and accessory basal nuclei of the amygdala than chimpanzees (Issa et al., 2018). Neuropil refers to the space between neurons and glial cell bodies, containing axons, dendrites, and the processes of glial cells; a higher proportion of neuropil suggests a greater interconnectedness among neurons. Notably, in this same study, bonobos and chimpanzees did not differ in the amount of Von Economo neurons, which are found in frontoinsular and cingulate cortex, and are hypothesized to play a role in social cognition (Allman, Tetreault, Hakeem, & Park, 2011). However, granular insular cortex is enlarged in chimpanzees compared with bonobos, while dysgranular and agranular insular cortex are larger in bonobos (Bauernfeind et al., 2013; Staes et al., 2018). This is intriguing, given that fMRI studies in humans associate the more posterior granular insula with somatosensory visceral processing and the anterior agranular insula with emotional and social awareness (Flynn, 1999; Gu, Hof, Friston, & Fan, 2013). In both chimpanzees and bonobos, the basal nucleus of the amygdala contains the highest amount of neurons, while in humans the lateral nucleus, which receives input from the cortex, is the largest (Barger et al., 2012).

Also relevant are reports of how bonobos and chimpanzees compare on basic psychological skills that contribute to social behavior. Bonobos perform better than chimpanzees

on tests of social cognition, such as comprehension of communicative signaling and gaze following (Herrmann, Hare, Call, & Tomasello, 2010; Kano & Call, 2014). Chimpanzees outperform bonobos on tasks involving physical cognition, such as spatial memory (Rosati & Hare, 2012) and tool use (Herrmann et al., 2010), and wild chimpanzees employ diverse tool use behavior in the wild, while bonobos do not (Gruber, Clay, & Zuberbühler, 2010; Koops, Furuichi, & Hashimoto, 2015). Interestingly, bonobos follow human gaze better than chimpanzees (Kano & Call, 2014), but bonobos do not engage in "gaze alternation" - shifting gaze between an object of interest and a communicative partner, in this case a human experimenter - nearly as much as chimpanzees. However, chimpanzees do not frequently gaze alternate until adulthood, while humans will do it early in development (Lucca, MacLean, & Hare, 2018).

Finally, differences have been reported among chimpanzees and bonobos in their hormonal profiles, and the way hormones relate to behavior. In one study, bonobos showed an increase in cortisol in response to feeding competition, while chimpanzees showed an increase in testosterone (Wobber et al., 2010). A few other studies have explored potential differences in sex hormones. The ratio of the second to fourth digits of the hand has been put forth as an indicator of prenatal androgen exposure. Bonobos have a higher, and more human-like, ratio than chimpanzees, suggesting they experience less prenatal androgens than chimpanzees (McIntyre et al., 2009). Bonobo and chimpanzee males experience a spike in urinary testosterone levels around 8 years of age, while bonobo females experience an increase in urinary testosterone three years before chimpanzee females (Behringer, Deschner, Deimel, Stevens, & Hohmann, 2014), suggesting they may undergo puberty earlier, perhaps related to their younger age of dispersal. At the same time, both male and female bonobos have prolonged heightened levels of urinary

thyroid hormone compared with chimpanzees, indicating developmental delay in bonobos (Behringer, Hohmann, Stevens, Weltring, & Deschner, 2012). On a battery of 16 cognitive tasks, higher levels of testosterone were related to higher performance in several tasks among chimpanzees, including tasks assessing physical and spatial cognition, while no correlation was found between testosterone and cognitive performance in bonobos (Wobber & Herrmann, 2015).

The Oxytocin and Vasopressin Systems in Non-human Primate Brains

Neuroanatomy

Immunoreactive Cell Bodies and Fibers

Most research concerning OT and AVP has been performed in rodents. However, there is a small body of literature investigating the OT and AVP system in primates. While the neuropeptide-producing magnocellular neurons of the hypothalamus have been well-studied, there is a surprising paucity of studies on the projections of OT and AVP neurons to different brain areas (Knobloch & Grinevich, 2014).

Immunoreactive AVP cells have been found in the common marmosets (*Callithrix jacchus*) in the bed nucleus of the stria terminalis (BNST), as well as the lateral hypothalamus and suprachiasmatic nucleus, while OT cells have been found in the BNST and medial amygdala. Immunoreactive OT fibers have been found the nucleus accumbens of the basal ganglia in common marmosets (Wang, Moody, Newman, & Insel, 1997), an area implicated in pair-bonding in rodent as well as primate neurobiological studies (Freeman et al., 2014). In Japanese macaques (*Macaca fuscata*), AVP- and OT-containing fibers are found in several nuclei of the hypothalamus as well as the BNST (Kawata & Sano, 1982). The long-tailed macaque (*Macaca fascicularis*) exhibits extra-hypothalamic AVP fibers in forebrain regions

including the amygdala, hippocampus, and BNST, but reportedly has no such forebrain OT fibers (Caffé, Van Ryen, Van der Woude, & Van Leeuwen, 1989).

Receptors

As described earlier, receptor distribution is species-specific and often mediates the relationship between neuropeptides and social behavior. The localization of OXTR and AVPR1a has been examined in a few select primate species using receptor autoradiography for ligand binding. All primates appear to have OXTR or AVPR1a in areas important for visual orienting to stimuli, such as the superior colliculus and nucleus basalis of Meynert (Freeman & Young, 2016). Outside these areas, there is significant variation.

Rhesus macaques, a polyandrous and rigidly hierarchical species of catarrhine monkeys, showed no binding for either OXTR or AVPR1a in the basal ganglia. Interestingly, this brain region encompasses the reward system pathways implicated in prairie vole pair-bonding. Macaques show dense binding for both OXTR and AVPR1a in the BNST, along with AVPR1a binding for the medial and central amygdala. Macaques show significant binding for both OXTR and AVPR1a in the cortex (Freeman, Inoue, Smith, Goodman, & Young, 2014; Young, Toloczko, & Insel, 1999).

Marmosets, a cooperative breeding species of platyrrhine monkey, have a significant presence of OXTR and AVPR1a in the basal ganglia, dense AVPR1a in the medial amygdala and bed nucleus of the stria terminalis (BNST), and moderate OXTR in layer 6 of the cerebral cortex (Schorscher-Petcu, Dupré, & Tribollet, 2009; Wang et al., 1997). Finally, the coppery titi monkey, a monogamous platyrrhine, shows widespread AVPR1a receptor binding within the basal ganglia. The titi monkey also has AVPR1a in the central amygdala, and OXTR in the

hippocampus. Notably, many species of platyrrhines have a derived form of the OT peptide, with a substitution of a leucine to a proline in amino acid position 8 (Lee et al., 2011).

Oxytocin and Non-human Primate Behavior

In non-human primates, OT has been connected to social affiliation, pair-bonding and parental behavior, and reduced anxiety. In rhesus macaques, which do not form pair bonds, intranasal OT increases infants' affiliative communicative gestures (Simpson et al., 2014). In chimpanzees, which are also non-monogamous, urinary OT is higher after a grooming session, particularly with a strongly socially bonded partner (Crockford et al., 2013). Elevated levels of urinary OT has also been connected to meat sharing and greater coordination of border patrolling behavior in chimpanzees (Samuni et al., 2016; Wittig et al., 2014). Interestingly, OT reduces prosocial behavior towards strangers in marmosets (Mustoe, Cavanaugh, Harnisch, Thompson, & French, 2015), suggesting OT may not universally increase prosociality, but rather it may modulate behavior differently depending on context.

In marmosets, intranasal OT increases the rate of huddling with a monogamous partner and increases partner-seeking behavior over contact with a stranger. An OT receptor antagonist administered orally decreases food-sharing behavior between partners (Smith, Agmo, Birnie, & French, 2010). OT also increases tolerance of an adult male marmoset toward its offspring as measured by transfer of food (Saito & Nakamura, 2011). OT also enhances responsiveness to infant stimuli in adult marmosets (Taylor & French, 2015) and reduces sociosexual behavior toward opposite-sex strangers (Cavanaugh, Mustoe, Taylor, & French, 2014). In another monogamous species, tamarins, urinary OT levels are related to variance in grooming behaviors in females and sexual behavior in males (Snowdon et al., 2010).

Finally, multiple lines of evidence suggest that OT is anxiolytic in primates. In squirrel monkeys, intranasal OT leads to lower concentrations of adrenocorticotropic hormone (ACTH), a component of the stress response, after 90 minutes of social isolation (Parker, Buckmaster, Schatzberg, & Lyons, 2005). In rhesus macaques, OT alters fMRI responses to emotional expressions, selectively reducing reactivity to fearful and aggressive faces in face-responsive regions. While viewing these faces, OT reduces coupling between the amygdala and the occipital and inferior temporal cortex (Liu et al., 2015). OT also has been shown to blunt social vigilance in rhesus macaques (Ebitz & Platt, 2013).

Vasopressin and Non-human Primate Behavior

Like OT, AVP has been connected to pair-bonding and parental behavior in primates. In the coppery titi monkey, males who have received a high dosage of intranasal AVP contact their partners more frequently than a stranger (Jarcho, Mendoza, Mason, Yang, & Bales, 2011). Also like OT, AVP enhances responsiveness to infant stimuli in adult marmosets (Taylor & French, 2015). Marmoset fathers experience a higher expression of AVP v1a receptors in the frontal cortex after fatherhood (Kozorovitskiy, Hughes, Lee, & Gould, 2006).

While the nucleotide sequence within the coding region of AVPR1a is relatively conserved across mammalian taxa, there is variability in many species in the 5 prime microsatellite within the regulatory region of the AVPR1a gene (Hammock & Young, 2005). Variation in the length of these microsatellites have been shown to affect monogamy, paternal care, and social interest, likely by affecting receptor expression in the brain (Hammock, Lim, Nair, & Young, 2005).

In chimpanzees, there is a polymorphic deletion of ~360 base pairs in the 5 prime regulatory region of the vasopressin 1a receptor gene (Donaldson et al., 2008). This is referred to

as the DupB microsatellite, and it contains the RS3 allele, which has been linked with variation in social bonding (Donaldson et al., 2008). Chimpanzees are the only great apes polymorphic for a complete deletion of this microsatellite (Staes et al., 2014). The deletion is most common among chimpanzees of West African origin, with an estimated 70% having a complete deletion, while 62% of east African chimps in the wild retain the DupB microsatellite (Anestis et al., 2014).

A handful of studies have investigated social behavioral associations of the DupB polymorphism of AVPR1a in chimpanzees. Chimpanzees with the presence of DupB (DupB+ genotype) may have "smart" personality types, successfully using coalitions in aggressive encounters, receiving grooming, and initiating play (Anestis et al., 2014). There is evidence that males with DubB+ genotypes are more dominant and less conscientious (Hopkins, Donaldson, & Young, 2012; Latzman, Hopkins, Keebaugh, & Young, 2014), or more sociable (Staes et al., 2015). Importantly, bonobos, while being closely related to chimpanzees, do not have the deletion of DupB (Donaldson et al., 2008; Staes et al., 2014).

The Oxytocin and Vasopressin Systems in Human Brains

Though the effects of OT and AVP on human behavior are varied and complex, several themes emerge. OT appears to facilitate social reward, social cognition and social approach and may play a role in social attachment. OT also modulates amygdala reactivity to social stimuli, with potential for anxiety reduction. The prosocial effects of OT may be context dependent, depending on individual characteristics as well as in-group status of an interaction partner. AVP also shows some association with pair-bonding behavior as well as increased cooperation but appears to increase reactivity to threat and enhance anxiety. Importantly, there are significant sex

differences in the behavioral effects of these neuropeptides, and this may be especially true of AVP.

Neuroanatomy

A 1980 study claimed that rats and humans had identical OT and AVP extra-hypothalamic projections; however, this study only investigated the brainstem and upper spinal cord in each species (Sofroniew, 1980). Fliers found few VP fibers in the lateral septum and BNST, as well as few AVP and OT fibers in the amygdala and hippocampus (Fliers, Guldenaar, Wal, & Swaab, 1986). Dense networks of AVP and OT fibers were found in the locus coeruleus, and AVP neurons, but not OT neurons, in the BNST. The presence of fibers in the human locus coeruleus differs from that of rats, demonstrating that identical neuroanatomy of the OT/AVP system could not be assumed.

A few studies have attempted to establish the distribution of OXTR and AVPR1a in human brains. Loup et al. (1991) used receptor autoradiography for both OXTR and AVPR1a, but this study was confounded by the fact that these peptides, due to their high structural similarity, can bind to each other's receptors in primates. For the aforementioned rhesus macaque and titi monkey studies, Freeman et al. developed a competitive binding protocol to solve this issue (Freeman et al., 2014). However, this protocol has not yet been used on human brains outside of a limited set of regions (Freeman et al., 2018). Nevertheless, Loup et al. reported light binding for OXTR in the basal ganglia (globus pallidus, ventral pallidum, islands of Calleja), nucleus basalis of Meynert, diagonal band, ventral lateral septum, as well as AVPR1a binding in the BNST, basal amygdala, dorsal lateral septum, midline thalamic nuclei, and dentate gyrus.

By contrast, Boccia (2013) used immunohistochemistry to attempt to localize OXTR (AVPR1a was not included). Immunohistochemistry for these receptors is of dubious reliability, however; one study examined six different antibodies for OXTR and found that all produced identical, positive staining in wild-type and OXTR knockout mice (Yoshida et al., 2009). Boccia found no OXTR in the basal ganglia but did find a significant presence of OXTR in the amygdala. Additionally, Boccia found no OXTR in the superior colliculus. All three of these findings are opposed to that of Loup (1991).

Freeman used receptor autoradiography with a competitive binding protocol to examine OXTR and AVPR1a in the human brainstem, finding OXTR binding in the spinal trigeminal nucleus, a conserved pattern across primates, and AVPR1a in the nucleus prepositus, important for eye gaze stabilization (Freeman, Smith, Goodman, & Bales, 2016). Freeman also compared a large sample of forebrain sections from post-mortem brains from neurotypical controls with those of people with autism spectrum disorder (Freeman et al., 2018). Controls showed dense OXTR binding in the nucleus basalis of Meynert as well as the ventral pallidum, with low binding in the superior colliculus, globus pallidus, and periaqueductal gray. AVPR1a was not detected in any of these regions. People with autism had lower OXTR in ventral pallidum and higher OXTR in the nucleus basalis of Meynert than controls.

Oxytocin and Human Behavior

OT plays a role in several aspects of human social cognition and social approach. The ability to infer the mental state of others is a key characteristic of adaptive social interaction. Several studies have investigated the role of OT in this process. One study examined the effects of a single dose of 24 IU of intranasally administered OT on the ability to infer the mental state of others from facial cues, specifically the eye region of facial expressions, finding that OT

improved accuracy on this measure (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007). There is evidence that OT also increases the number and duration of gazes toward the eye region of human faces showing neutral affect (Guastella, Mitchell, & Dadds, 2008). However, in other studies, OT did not enhance attention to angry faces (Guastella, Carson, Dadds, Mitchell, & Cox, 2009), and induced positive bias in emotion identification (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009). OT also may slow reaction times for facial fear recognition and reduce misclassifications of positive emotions as negative ones (Di Simplicio et al., 2009). Thus there is support for the hypothesis that OT is involved in facial processing in humans, though it may depend on other factors, such as the valence of the stimulus.

OT also affects social memory and recognition. Intranasal OT selectively modulates implicit memory for socially relevant versus neutral words (Heinrichs, Meinlschmidt, Wippich, Ehlert, & Hellhammer, 2004), and has been shown to enhance immediate and delayed recognition for face identity even when administered post-learning (Savaskan, Ehrhardt, Schulz, Walter, & Schächinger, 2008). There is mixed evidence regarding whether OT enhances memory selectively for particular emotions. In the previous study, face identity memory was only affected for angry or neutral expressions. However, other studies show that intranasal OT specifically enhances recognition for happy faces (Guastella et al., 2009), or enhances memory for all faces regardless of affect, but not non-social stimuli (Rimmele, Hediger, Heinrichs, & Klaver, 2009). OT reportedly increases the false alarm rate for identifying faces as familiar (Rimmele et al., 2009). Clearly, further research is needed to determine the characteristics of these experiments or the participants which may have resulted in these discrepancies.

The effects of OT on the accurate identification of facial expressions as well as the enhancement of social memory may be related to the direction of attention to social stimuli,

increase of the salience of social stimuli, or some combination of these. OT promotes gaze to the eye region of faces (Gamer, Zurowski, & Büchel, 2010; Guastella et al., 2008). This may underlie increased salience of and attention to social stimuli. An opposing view argues that OT actually reduces the salience of social stimuli (Ebitz & Platt, 2013). Indeed there is some evidence that OT blunts gaze to emotional faces in some circumstances (Domes et al., 2013). Ebitz and Platt draw a distinction between directing attention and sustaining attention, claiming that OT may interact with these processes differently.

A significant body of literature implicates OT in the promotion of prosocial interactions. OT acts in the mesolimbic dopamine system to mediate rewarding properties of social interactions, and may do so in a sex dependent manner (Borland, Rilling, Frantz, & Albers, 2019). This has been observed in the context of same-sex social interaction and cooperation (Feng et al., 2015; Groppe et al., 2013) as well as in pair-bonding behavior and parenting. The involvement of OT in pair-bonding is well-established in animal studies (Walum & Young, 2018). There is some evidence that OT is involved in romantic attachment in humans as well. In one study, experimenters administered OT and measure the distance male subjects kept from an attractive female. OT increased the preferred distance for males in monogamous relationships, but not single males (Scheele et al., 2012). Moreover, OT enhances activation within brain reward systems while viewing the romantic partner's face in both males and females, with the exception of females using hormonal contraceptives, indicating that gonadal steroids may alter partner-specific effects (Scheele et al., 2013; Scheele, Plota, & Stoffel-Wagner, 2015). Ditzen and colleagues (Ditzen et al., 2009) demonstrated that 40 IU intranasal OT increases positive communication during a couple conflict in both males and females, and significantly reduces cortisol reactivity. Finally, a single nucleotide polymorphism in the OT receptor gene,

rs7632287, has been associated with pair-bonding behavior in women in multiple samples from twin studies (Walum et al., 2012). Importantly, these effects of OT may differ depending on individual or situational characteristics; there is some evidence that in people with aggressive tendencies, intranasal OT may increase inclination to antisocial behavior (DeWall et al., 2014).

It has been suggested that OT has an evolutionarily ancient role in maternal behavior, and the circuitry promoting this behavior has been co-opted to support paternal, pair-bonding, and possibly more generalized prosocial behavior (Broad, Curley, & Keverne, 2006). Oxytocinergic pathways are essential for maternal behavior; the major physiological effects of OT are uterine contraction during labor and milk ejection. OT also appears to facilitate adaptive maternal as well as paternal behaviors in the central nervous system, and it may accomplish this by making infant stimuli more rewarding (Gregory et al., 2015; Li et al., 2017). One study examined links between OT receptor gene *OXTR* polymorphisms and polymorphisms in the *CD38* gene, critical for OT release, along with parenting behaviors in mothers, fathers, and non-parents. Episodes of parent-infant gaze synchrony were longest among parents with high levels of plasma OT as well as a CD38 allele not connected to risk for disorders of social functioning (Feldman et al., 2012). The effects were similar for mothers as well as fathers, suggesting OT is a mediator of parental behavior. Moreover, OT administration to a parent, which increases key parenting behaviors, can even elicit higher OT levels and engagement behaviors in an infant (Weisman, Zagoory-Sharon, & Feldman, 2012).

There is mixed evidence that OT is associated with another aspect of prosocial human behavior: trust. Kosfeld and colleagues demonstrated that OT affects an individual's willingness to accept social risk in interpersonal actions, suggesting that OT increases trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005). In a study using the iterated Prisoner's Dilemma

game, intranasal OT increased activation of the caudate nucleus in response to reciprocated cooperation, which may facilitate learning that a partner can be trusted (Rilling et al., 2012). Additionally, in another study utilizing an economic game in which an "investor" decides whether to share a sum of money with a "trustee", OT attenuated amygdala reactivity to betrayal and increased the likelihood that investors continued to invest similar sums after experiencing betrayal (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008). However, a recent review claims that the association between OT and trust has not been well-replicated in studies involving endogenous or exogenous OT; moreover, trust does not correlate with any OT-related genetic polymorphisms (Nave, Camerer, & McCullough, 2015).

OT can exert effects on social behavior and cognition by attenuating anxiety and fear responses. OT has known anxiolytic effects in animal studies, in which OT has been shown to be released peripherally and within the brain in response to stress and threat (Neumann, Wigger, Torner, Holsboer, & Landgraf, 2000). Moreover, animal studies have identified a role for OT in inhibiting stress-induced activity of the hypothalamic–pituitary–adrenal axis (Neumann, 2002) as well as activity of the amygdala modulating the autonomic fear response (Huber, Veinante, & Stoop, 2005). Several studies provide support for a similar effect in humans. Kirsch and colleagues (2005) used fear-inducing visual stimuli to assess amygdala activation in healthy men following OT administration or placebo. Intranasal OT reduced amygdala activity and reduced coupling between the amygdala and brainstem regions involved in the autonomic fear response. Another study reported that intranasal OT attenuated the effect of aversive conditions to neutral faces, associated with activity in the anterior cingulate cortex and right medial temporal lobe (Petrovic, Kalisch, Singer, & Dolan, 2008). Moreover, faces with direct gaze induced a specific attenuating effect of oxytocin in the right amygdala and right fusiform gyrus as compared with

averted gaze. OT also attenuates amygdala responses to fearful, angry, and happy faces (Domes, Heinrichs, Gläscher, et al., 2007).

De Dreu argues that the combined effects of OT on increasing social recognition and memory, potentially increasing trust and social attachment, and dampening amygdala reactivity underlying anxiety and fear results in a system promoting cooperation. Moreover, OT does not seem to indiscriminately promote cooperation, but rather may depend on contextual factors. Several studies indicate that the prosocial effects of OT may be limited to members of one's in-group, and OT may even promote derogation, in which members of an outgroup are perceived as threatening to one's in-group (De Dreu, 2012; De Dreu et al., 2010). The effects of OT may also be dependent on characteristics of the individual studied. For example, Bartz and colleagues (2011) found that intranasal OT can hinder trust and cooperation in persons with borderline personality disorder, a disorder characterized by disrupted interpersonal interactions and difficulties with cooperation. It may be that in some cases OT enhances pre-existing tendencies of social cognition and behavior rather than facilitating specific behaviors in everyone.

Vasopressin and Human Behavior

Compared to OT, fewer studies have examined the effects of AVP and AVP-related genes on human social cognition and behavior. However, some general themes have emerged. AVP seems to have some overlapping functions with OT in promoting prosocial behavior and pair-bonding, and it may have opposing or divergent functions in the amygdala response underlying fear and anxiety.

AVP is an essential regulator of pair-bonding in male monogamous prairie voles (Insel & Young, 2001). There is some evidence that AVP is associated with pair-bonding in humans as well. Polymorphisms in the RS3 promoter region of the AVPR1a receptor gene have been found

to predict outcome measures in the Partner Bonding Scale in men, but not women. Additionally, carriers of one specific allele reported lower marital quality as reported by both the men and their wives, and had more often experienced threat of divorce during the last year, though this finding has not been replicated (Walum et al., 2008).

AVP, like OT, may also be involved in more general prosocial interactions. Polymorphisms in the aforementioned RS3 promoter region of the vasopressin V1a receptor gene have been associated with altruistic behavior. One study showed that the amount of money allocated to an anonymous partner in the Dictator Game was greater for participants with long RS3 repeats as opposed to short repeats (Knafo et al., 2008). In the Prisoner's Dilemma, among male participants, intranasal AVP increased cooperative behavior in response to cooperation by a partner in men (Rilling et al., 2012). Intranasal AVP also increases nucleus accumbens and lateral septum response in men viewing pictures of female faces (Rilling et al., 2017). In women, intranasal AVP increases conciliatory behavior in the Prisoner's Dilemma, measured by the amount of times a subject chooses to cooperate after a partner has defected (Rilling et al., 2014). This likely represents an attempt at re-establishing cooperation, perhaps similar to the effect of OT on reestablishing trust (Baumgartner et al., 2008).

AVP appears to enhance reactivity to threat and aggression in males. Thompson and colleagues (Thompson, George, Walton, Orr, & Benson, 2006) investigated the effects of intranasal AVP on cognitive, somatic, and autonomic responses to facial stimuli in healthy males. AVP caused men to react to neutral faces in a manner similar to how placebo men reacted to angry faces in terms of their facial EMG responses. AVP thus may bias individuals to respond to ambiguous stimuli as if they were threatening. Also, there is an association between cerebrospinal fluid levels of AVP and life histories of general aggression in people with

personality disorders (Coccaro, Kavoussi, Hauger, Cooper, & Ferris, 1998). Interestingly, administration of intranasal AVP appears to decrease perception of friendliness in unfamiliar faces for men but increase perceptions of friendliness of unfamiliar faces for females (Thompson et al., 2006).

It is important to note that there appear to be significant sex differences in the effects of OT and AVP on social cognition and behavior. In animals, the expression of neuropeptide receptors in limbic regions is sensitive to the level of gonadal steroids; for example, estrogens upregulate OT receptor expression and stimulate OT release. Testosterone injections in the first weeks of life into female rates or castrated male rates results in AVP fiber density levels similar to control male rates (De Vries & Buijs, 1983). AVP expression is also higher in male rats, while OT peptide expression is higher in females. Interestingly, OT receptor expression is higher in males (Dumais & Veenema, 2015). As mentioned earlier, in humans, intranasal OT can have opposing effects on men and women, enhancing social reward in males while reducing it in females. This effect of OT on reward may follow an inverted U-shaped curve, with females at baseline already experiencing higher reward to same-sex social interaction, while OT makes the neural activity of males look more like females at baseline (Borland, Rilling, Frantz, and Albers, 2019). Investigating whether there are sex differences in receptor distribution in humans (and non-human primates) will be an important step in understanding how these behavioral differences may be mediated within the brain.

OT and AVP each have various effects on social cognition and behavior; some of these, such as pair-bonding and cooperation, may overlap, while others, such as the attenuation or promotion of anxiety, seem to be distinct. Due to their high homology, these neuropeptides can each bind to the other's receptors, though there is evidence that OT is selective for OXTR in

humans (Albers, 2014; Manning et al., 2012, 2008). Further research should explore the extent to which binding to the other neuropeptide's receptor influences behavior, and whether this phenomenon might be responsible for overlapping functions of OT and AVP in the human brain. Finally, most of our knowledge of these effects come from intranasal administration of OT or AVP in a laboratory setting. A greater number of naturalistic studies investigating endogenous OT and AVP changes in response to typical social interactions – more comparable to urinary studies in chimpanzees – are needed to identify potential evolutionary functions.

The Present Study

The importance of OT and AVP in regulating social behavior across a wide variety of animal species is undeniable. Sophisticated techniques have allowed for a detailed understanding of how features of OT/AVP neurobiology and physiology relate to behavior in rodent species (as well as their homologs in animals outside the mammalian class). Behavioral evidence from studies incorporating intranasal OT or AVP have given us a window into the relationship of these peptides to human behavior as well as that of other primate species, such as rhesus macaques and marmosets. In chimpanzees, urinary levels of OT are associated with various behaviors. Genetic variation in the OT/AVP system is also connected to behavioral differences in humans and chimpanzees.

Thus there is reason to believe that OT and AVP are related to social behavior in primates, and variation in the OT and AVP system may be related to variation in species-typical social behaviors. Yet little is known about even the basic neuroanatomical features of the OT and AVP system in most primates, particularly great apes.

This dissertation aims to characterize key aspects of the neuroanatomy of the OT and AVP systems in four primates. With an appreciation of the social behavioral differences among

these species, and a special interest in implications for the evolution of our own species, I focus on humans, chimpanzees and bonobos. I also include rhesus macaques as an outgroup, and because their common use in laboratory settings means that much more is known about their neurophysiology and behavior than most primate species.

In the second chapter, I use immunohistochemistry to localize immunoreactive cell bodies that produce oxytocin and vasopressin across these four species. Additionally, I map out where these cell bodies send their axonal projections, which a particular interest in the density of fibers in areas relevant to social behavior, such as the amygdala. I hypothesize that in accordance with all mammals studied so far, all species will have a relatively conserved distribution, with OT and AVP immunoreactive cell bodies in the supraoptic and paraventricular nuclei of the hypothalamus. I also hypothesize that humans, the only species of the four that can form pair bonds, will exhibit extra-hypothalamic fibers for OT or AVP in the basal ganglia.

In the third chapter, I again use immunohistochemistry to determine the cortical regions to which OT and AVP cells send axonal projections. I take a detailed look at the cortical layers in which OT and AVP fibers are found, as well as the differences among species. This endeavor was not borne out of specific hypotheses, but rather an initial discovery of AVP fibers in the human insula and OT fibers in the human straight gyrus, which had never been reported in previous attempts to map AVP and OT fibers in humans (or any primates). A systematic account of the cortex in each species ensued, with potential implications for mechanisms of signaling in the primate OT and AVP system.

In the fourth chapter, I use receptor autoradiography to determine the distribution of OT and AVP receptors in chimpanzee brains. I then compare my results with research published in humans, rhesus macaques, and other primate species. I hypothesize that chimpanzees would have

a lower density of OT and/or AVP receptors in the reward system (including ventral striatum) as compared with humans due to a lack of pair-bonding behavior. I also hypothesize that chimpanzees would have a greater density of AVP v1a receptors in the amygdala, which may be related to their heightened aggression and territoriality.

Finally, in the fifth chapter, I combine my findings about the distribution of OT and AVP cells and fibers in humans, chimpanzees, macaques, and bonobos with my findings about chimpanzee receptors (and data about macaque and human receptors from other sources). I discuss potential implications for the neurobiology of social behavioral diversity, cross-reactivity of receptors, and the bigger picture of great ape and human evolution.

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Chapter 2: Oxytocin (OT) and arginine-vasopressin (AVP) cell bodies and fibers in rhesus macaques, bonobos, chimpanzees, and humans

This chapter to be submitted as:

Rogers, C.N., Ross, A.P., Sahu, S.P., Siegel, E.R., Stopa, E.G., Hopkins, W.D., Stimpson, C., Sherwood, C.C., Young, L.J., Rilling, J.K., Albers, H.E., Preuss, T.M. Oxytocin (OT) and arginine-vasopressin (AVP) cell bodies and fibers in rhesus macaques, bonobos, chimpanzees, and humans.

Abstract

The neuropeptides oxytocin (OT) and arginine-vasopressin (AVP) are strongly implicated in the regulation of social behavior in mammalian species. While OT- and AVP-producing cells are consistently located in the hypothalamus across species, less is known about variation in the distribution of extra-hypothalamic cell bodies and processes. Moreover, the anatomical distribution of these neuropeptides in great apes, such as chimpanzees, the animals most closely related to humans, has not been studied to date. We used immunohistochemistry to identify cell bodies and fibers containing OT and AVP in fixed, postmortem tissue from humans (n= 3), chimpanzees (n=7), bonobos (n=7), and rhesus macaques (n=5). All four species showed labeling for OT and AVP cell bodies in the hypothalamus as well as fibers in several extrahypothalamic regions including the basal ganglia, amygdala, and thalamus. In multiple amygdala nuclei as well as the lateral septum, a higher density of AVP- fibers was observed in macaques and chimpanzees than in bonobos and humans. Our results suggest that primates differ from many rodent species (e.g., mice, rats, and voles), which have prominent AVP-containing cell bodies in the medial amygdala that send dense fiber projections to several forebrain areas. Our results also suggest that the distribution of AVP fibers may have been reduced over the course of evolution in the human and bonobo amygdala, as well as other regions implicated in social behavior.

Introduction

The neuropeptides oxytocin (OT) and arginine-vasopressin (AVP) are involved in the regulation of social behaviors across mammal species. In rodent species, OT has been associated with social recognition, pair-bonding, and maternal bonding (Hammock & Young, 2006; Donaldson & Young, 2008). AVP is often anxiogenic and has been associated with aggressive and territorial behaviors (Ferris et al., 1997; Goodson, 2005; Donaldson & Young, 2008), but also pair-bonding, paternal behavior, and mate guarding in males (Insel, Wang, & Ferris, 1994; Wang, Ferris, & De Vries, 1994; Insel, 2010). In humans and non-human primates, studies investigating the relationship of OT and AVP with behaviors such as cooperation, conflict, trust, social bonding, and mating have proliferated in recent decades. This research has proceeded without a detailed understanding of the neuroanatomy of these systems in primates.

In all mammalian species studied thus far, OT and AVP are produced in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus, with AVP additionally produced in the suprachiasmatic nucleus (SCN). Outside of these, groups of OT -and AVP-producing cells vary by species, though sites of OT production are generally more restricted (Kelly & Goodson, 2014). From these sites, AVP and OT can exert effects through volume release from the soma or dendrites of magnocellular neurons (Ludwig & Leng, 2006). Additionally, OT- and AVP- producing neurons can send axonal projections into other regions, with OT and AVP likely being released from axonal boutons *en passant*.

An early study concluded that rats and humans had identical OT and AVP extra-hypothalamic projections; however, this study only investigated the brainstem and upper spinal cord in each species (Sofroniew, 1980). In fact, not only is there clear evidence of AVP projections to extra-hypothalamic forebrain regions in rats (Hernández et al., 2015), but also OT-

and AVP-containing fibers have been found in forebrain regions in a few primate species. For example, immunoreactive AVP cells have been found in common marmosets (*Callithrix jacchus*) in the bed nucleus of the stria terminalis (BNST), as well as the lateral hypothalamus and suprachiasmatic nucleus, while OT cells have been found in the BNST and medial amygdala. In addition, immunoreactive OT fibers have been found in these animals in the nucleus accumbens of the basal ganglia (Wang et al., 1997), an area implicated in pair-bonding in rodents as well as primates (Freeman et al., 2014; Walum & Young, 2018). In Japanese macaques (*Macaca fuscata*), AVP- and OT-containing fibers are found in several nuclei of the hypothalamus as well as the BNST (Kawata & Sano, 1982). Long-tailed (crab-eating) macaques (*Macaca fascicularis*) exhibit extra-hypothalamic AVP fibers in forebrain regions including the amygdala, hippocampus, and BNST, but reportedly have no forebrain OT fibers (Caffé et al., 1989). Direct comparison of the results of these previous primate studies is complicated by the fact that each experiment focused on a particular set of brain regions, and thus some regions containing OT- or AVP- projections may not have been reported.

Social-behavioral differences exist among rhesus macaques, chimpanzees, bonobos, and humans which would suggest possible differences in OT- and AVP- neuroanatomy. For example, humans are the only ones among these species able to form pair bonds (Quinlan, 2008; Chapais, 2013). Within this set of species, chimpanzees and bonobos present an interesting contrast, as they are genetically closely related (Prüfer et al., 2012), but chimpanzees are more territorial and aggressive, while bonobos are known to resolve tension with sexual behavior (Goodall, 1986; de Waal, 1987).

Little research has been done connecting differences in OT and AVP cells and fiber projections with species-typical social behavioral differences. Their distribution is typically

considered to be more conserved among mammals, while differences in the distribution of receptors have more commonly been linked to behavior (Albers, 2014). For example, dense OT and/or AVP v1a receptors have been found in reward system areas of monogamous rodents (Insel & Shapiro, 1992) and primates (Freeman et al., 2014) that are not found in non-monogamous species (Freeman et al., 2014; Freeman & Young, 2016). OT and AVP may interact with the dopamine system to facilitate pair-bonding (Walum & Young, 2018). OT and AVP receptors in the amygdala have also been connected with social behavior. The central nucleus of the amygdala is of particular interest, as it receives information about stimuli from the lateral and basal nuclei and sends signals to the hypothalamus and brainstem to trigger the expression of fear (LeDoux, 2000). In rats, optogenetically released OT acts in the central nucleus to suppress anxiety as measured by decreased freezing behavior (Knobloch et al., 2012). While OT decreases the firing rate in the central amygdala, AVP increases it (Huber et al., 2005).

In the present study, we use immunohistochemistry to determine the distribution of OT- and AVP- cell bodies and axonal projections was determined in rhesus macaque, chimpanzee, bonobo, and human brains, from the rostral striatum to the caudal hippocampus (including the midbrain in macaques). We predict that the distribution of cells and fibers will be relatively conserved among the four species, with cells in the PVN and SON of the hypothalamus and fiber projections into several limbic forebrain regions. However, social-behavioral differences may be reflected in differences in fiber density in forebrain region. We predict that humans will have more OT- or AVP- fibers in reward areas as compared with the other species. We also predict that bonobos will have more OT fibers in the amygdala by which OT released during sexual activity can inhibit amygdala activity and quell anxiety, while chimpanzees will have more AVP fibers here due to their higher degree of territoriality. This is the first study to map these cells and

fibers in bonobos and chimpanzees, and the first to include this wide a range of brain regions at a high level of detail in rhesus macaques and humans.

Methods

Specimens

Table 1 provides a list of specimens used. All chimpanzee (3 males, 4 females, ages 23-50 years) and rhesus macaque (3 males, 2 females, ages 4-20 years) tissue came from animals housed at Yerkes National Primate Research Center. Brain tissue was opportunistically collected upon the natural death of the animal, rinsed with phosphate-buffered saline, and chimpanzee brains were separated into hemispheres. In many cases, one hemisphere was wrapped in foil and frozen at -80 degrees Celsius, while the other was fixed in formalin. Bonobo (3 males, 4 females, ages 5-62) tissue came from animals housed at Milwaukee County Zoo, Jacksonville Zoo, and the Ape Cognition and Conservation Initiative. Human (3 males, ages 28-54 years) tissue was sourced from the Warren Alpert Medical School at Brown University.

Table 2.1: Specimens used for immunohistochemistry.

Specimen	**Sex**	**Age**
Rhesus macaque	male	4
Rhesus macaque	male	25
Rhesus macaque	male	25
Rhesus macaque	female	10
Rhesus macaque	female	20
Chimpanzee	male	23
Chimpanzee	male	32
Chimpanzee	male	43
Chimpanzee	female	23
Chimpanzee	female	21
Chimpanzee	female	49
Chimpanzee	female	50
Bonobo	male	5
Bonobo	male	25
Bonobo	male	<30

Bonobo	female	12
Bonobo	female	25
Bonobo	female	52
Bonobo	female	62
Human	male	28
Human	male	31
Human	male	54

Immunohistochemistry

Fixed tissue was sectioned at 40 µm thickness using a microtome (Leica Biosystems). The sections were first washed with phosphate buffered saline (PBS) and then incubated in citrate buffer at 37.5ºC for 30 min for antigen retrieval. Next, a 1:12 series of sections (1:20 for bonobos) were washed with 3% peroxide in methanol for 10 min to inactivate endogenous peroxidase. Sections were incubated in block buffer containing PBS, 2% serum, and 0.2% tween-20 for 1 hr, then incubated overnight at 4ºC in primary antibody at a dilution of 1:20000 (see Table 2 for antibody information). The next morning, sections were washed then incubated in secondary antibody for 1 hr followed by a solution of biotinylated peroxide + avidin (VECTOR ABC reagent). Sections were then stained with diaminobenzidine (DAB) solution using the Vector DAB peroxidase substrate kit (Vector Laboratories, Inc, Burlingame, CA). Tissue was mounted on gelatin-coated slides, air-dried, and coverslipped before slide images were digitized using an Aperio Digital Pathology Slide Scanner (Leica Biosystems) and analyzed qualitatively in ImageScope for localization of cell bodies and fibers.

Table 2.2. Antibody information.

Antibody	**Structure**	**Animal**	**M or P**	**Manufacturer**	**Cat #**	**RRID**	**Concentration**
AVP	Polyclonal	rabbit	P	Peninsula Laboratories International	T-4563	none	1:20
OXT	Monoclonal	mouse	M	Millipore	MAB5263	AB_2157626	1:20

Nissl Stain

Adjacent sections were Nissl stained using the following protocol: 3- 5 min washes in distilled water (dH2O) were followed by a series of dehydrating alcohol incubations, each for 5 min in 50%, 70%, 95% (2x), 100% (2x) ethanol. The tissue was then delipidized in xylene twice for 5 min each, followed by a chloroform/xylene incubation for 20 min. The tissue was then once again placed in xylenes for 5 min and then rehydrated with another series of alcohol immersions, each for 5 min in 100%, 95%, 70%, and 50% ethanol. Slides were then washed in dH2O for 5 min then placed in thionin for 7 min, followed by a dH2O rinse and 70% acetic acid for approximately 3 min, 95% ethanol for 1 min, and then 100% ethanol for 2 min. Tissue was then moved to an ethanol/xylene solution for 2 min, and 2 xylene incubations for 5 min each.

Fiber Quantification

In the central amygdala, average fiber length per area was calculated for each species. The borders of the central amygdala were identified on adjacent Nissl sections and superimposed on OT and AVP sections. Within the borders, fibers were traced in Aperio Imagescope software (Leica Biosystems) and a total length of these tracings was calculated and divided over the area of the central amygdala. These values were averaged in each individual for an estimation of fiber length density. Nonparametric tests were used to compare these means statistically.

Results

See Table 2.3 for a summary of AVP-ir cells and fibers, and Table 2.4 for a summary of OT-ir cells and fibers by brain region and species. Figures 2.1 and 2.2 present a graphical overview of results.

Table 2.3. AVP-ir cells and fibers by brain region. Plus sign (+) indicates cells, asterisk (*) indicates fibers. +/* = sparse, ++/** = moderate, +++/*** = dense

	Rhesus macaques	*Chimpanzees*	*Bonobos*	*Humans*
EXTENDED AMYGDALA				
Central amygdala	**	**	*	*
Medial amygdala	**	**	*	*
Cortical amygdala	***	*	*	*
Basolateral amygdala	**	**	*	*
Accessory basal amygdala	*	*	*	*
Lateral amygdala				
Hippocampus-amygdala transition area		**		
Bed nucleus of stria terminalis	+**	+**	+*	+*
Nucleus basalis of Meynert	*	*	*	*
BASAL GANGLIA				
Caudate				
Putamen		*		
Nucleus accumbens	*	*		*
Internal globus pallidus	+**	+**	+**	+**
SEPTUM				
Lateral septum	**	**	*	*

Diagonal band of Broca	*	*	*	*
HYPOTHALAMUS				
PVN	+++***	+++***	+++***	+++***
SON	+++***	+++***	+++***	+++***
SCN	+++***	+++***	+++***	+++***
Accessory nuclei	+***	+***	+***	+***
AH	*	*	*	*
LH	*	*	*	*
MPOA	*	*	*	*
Posterior hypothalamic area	*	*	*	*
Infundibulum	***	***	***	***
Hippocampus	*	*		
THALAMUS				
Paraventricular thalamic nucleus	*	*	*	*
Rhomboid thalamus	*	*	*	*
Lateral habenula	*	*	*	*
MIDBRAIN and BRAINSTEM				
Substantia nigra	**	**	*	*

Table 2.4. OT-ir cells and fibers by brain region. Plus sign (+) indicates cells, asterisk (*) indicates fibers. +/* = sparse, ++/** = moderate, +++/*** = dense

	Rhesus macaques	*Chimpanzees*	*Bonobos*	*Humans*
AMYGDALA				
Central Amygdala	*	**	*	*
Medial Amygdala	*	**	*	*
Cortical Amygdala	***	*	*	*
Basolateral Amygdala	*	**	*	*
Accessory Basal Amygdala	*	*	*	*
Lateral Amygdala				
Hippocampus-Amygdala Transition Area		**		
Bed Nucleus of Stria Terminalis	+**	+**	+**	+**
Nucleus Basalis of Meynert	*	*	*	*
BASAL GANGLIA				
Caudate				
Putamen		*		
Nucleus accumbens		*	*	*
Internal Globus Pallidus	+**	*	*	*
SEPTUM				
Lateral septum	**	**	*	*
Diagonal Band of Broca	*	*	*	*
HYPOTHALAMUS				
PVN	+++***	+++***	+++***	+++***
SON	+++***	+++***	+++***	+++***
SCN				

Accessory Nuclei	+***	+***	+***	+***
AH	*	*	*	*
LH	*	*	*	*
MPOA	*	*	*	*
Posterior hypothalamic area	*	*	*	*
Infundibulum	***	***	***	***
Hippocampus		*		
THALAMUS				
Paraventricular thalamic nucleus	*	*	*	*
Rhomboid thalamus	*	*	*	*
Lateral habenula	*	*	*	*
MIDBRAIN and BRAINSTEM				
Substantia nigra				

Figure 2.1. Graphical representation of brain regions in each species with AVP-ir cells and fibers. Results taken from the present study as well as (Rogers et al., 2018). AC = anterior cingulate cortex, LS = lateral septum, DB = diagonal band, SG = straight gyrus, OlfN = olfactory nucleus, OlfCtx = olfactory cortex, NAcc = nucleus accumbens, NBM = nucleus basalis of Meynert, GPe = external globus pallidus, GPi= internal globus pallidus, LHab = lateral habenula, PVThal=paraventricular thalamus, RThal= rhomboid nucleus of the thalamus, PVN = paraventricular nucleus of the hypothalamus, AN = anterior nucleus of the hypothalamus, SON = supraoptic nucleus of the hypothalamus, SCN = suprachiasmatic nucleus of the hypothalamus, MeA = medial amygdala, CeA = central amygdala, CoA = cortical amygdala, ABA = accessory basal amygdala, BLA = basolateral amygdala, SNi = substantia nigra.

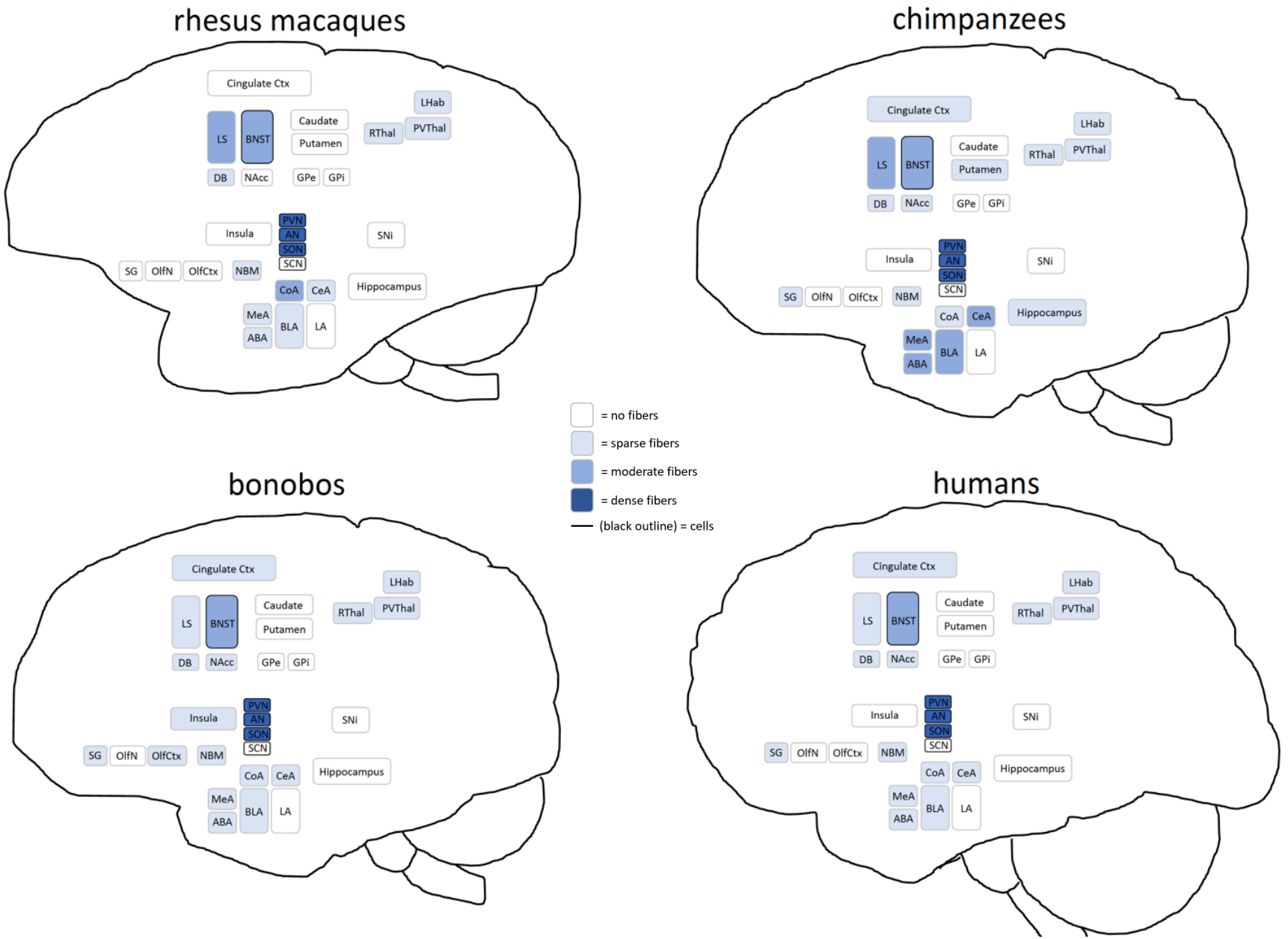
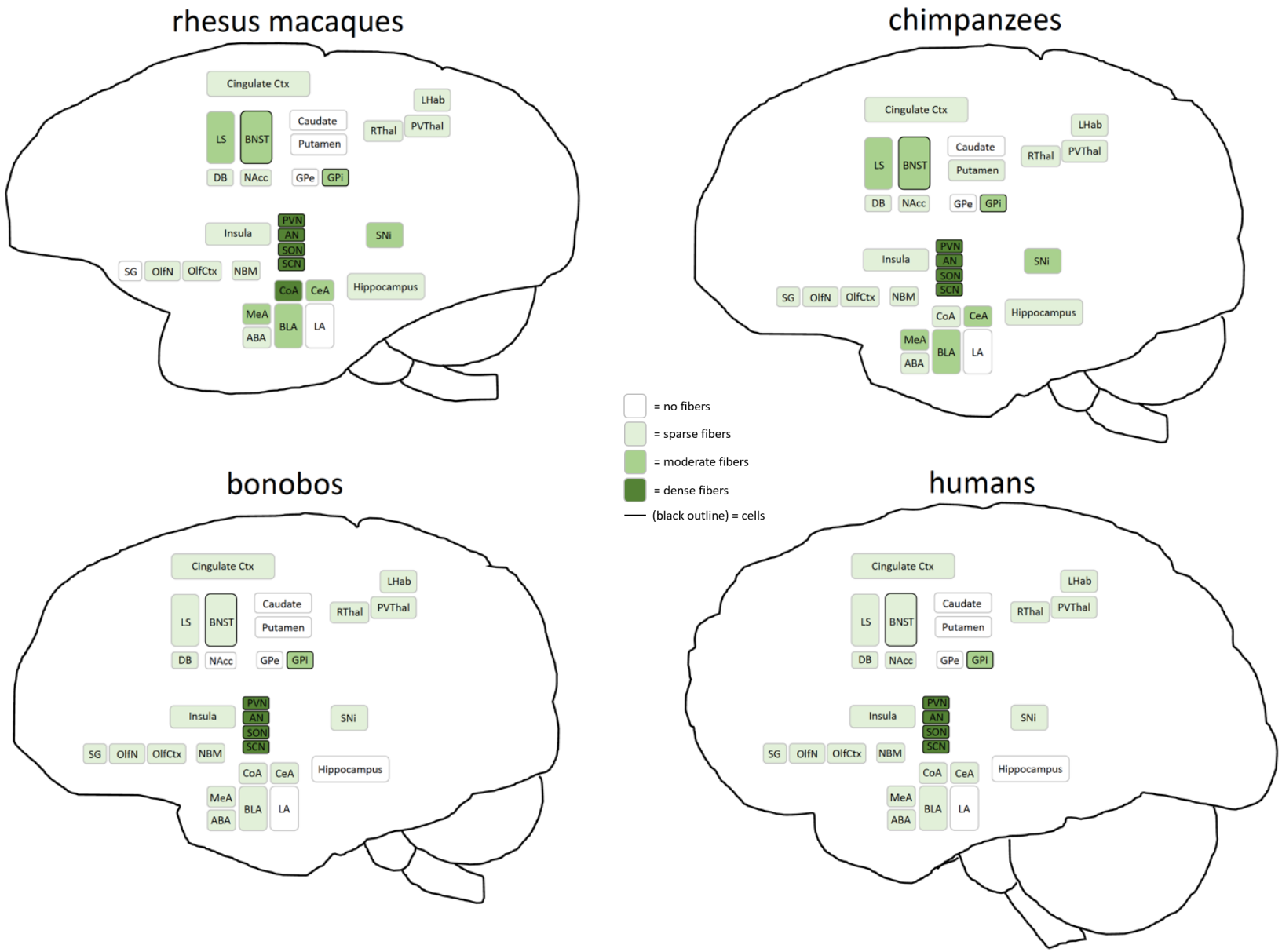

rhesus macaques
Cingulate Ctx
LHab
LS
BNST
Caudate
Putamen
RThal
PVThal
DB
NAcc
GPe
GPi
Insula
PVN
AN
SON
SCN
SNi
SG
OlfN
OlfCtx
NBM
CoA
CeA
Hippocampus
MeA
ABA
BLA
LA
chimpanzees
= no fibers
= sparse fibers
= moderate fibers
= dense fibers
(black outline) = cells
bonobos
humans

Figure 2.2. Graphical representation of brain regions in each species with OT-ir cells and fibers. Results taken from the present study as well as (Rogers et al., 2018). AC = anterior cingulate cortex, LS = lateral septum, DB = diagonal band, SG = straight gyrus, OlfN = olfactory nucleus, OlfCtx = olfactory cortex, NAcc = nucleus accumbens, NBM = nucleus basalis of Meynert, GPe = external globus pallidus, GPi= internal globus pallidus, LHab = lateral habenula, PVThal=paraventricular thalamus, RThal= rhomboid nucleus of the thalamus, PVN = paraventricular nucleus of the hypothalamus, AN = anterior nucleus of the hypothalamus, SON = supraoptic nucleus of the hypothalamus, SCN = suprachiasmatic nucleus of the hypothalamus, MeA = medial amygdala, CeA = central amygdala, CoA = cortical amygdala, ABA = accessory basal amygdala, BLA = basolateral amygdala, SNi = substantia nigra.


rhesus macaques
Cingulate Ctx
LHab
LS
BNST
Caudate
Putamen
RThal
PVThal
DB
NAcc
GPe
GPi
Insula
PVN
AN
SON
SCN
SNi
SG
OlfN
OlfCtx
NBM
CoA
CeA
Hippocampus
MeA
BLA
LA
ABA
chimpanzees
Cingulate Ctx
LHab
LS
BNST
Caudate
Putamen
RThal
PVThal
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NAcc
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GPi
Insula
PVN
AN
SON
SCN
SNi
SG
OlfN
OlfCtx
NBM
CoA
CeA
Hippocampus
MeA
BLA
LA
ABA
= no fibers
= sparse fibers
= moderate fibers
= dense fibers
(black outline) = cells
bonobos
Cingulate Ctx
LHab
LS
BNST
Caudate
Putamen
RThal
PVThal
DB
NAcc
GPe
GPi
Insula
PVN
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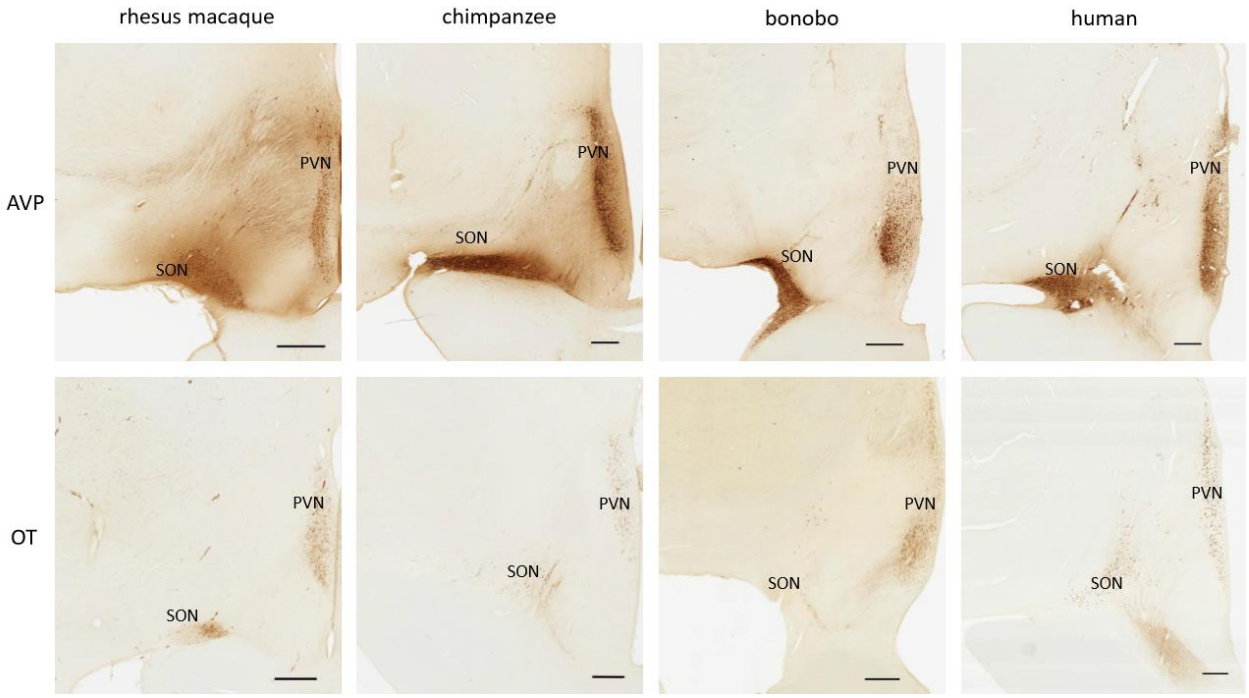
SNi
SG
OlfN
OlfCtx
NBM
CoA
CeA
Hippocampus
MeA
BLA
LA
ABA
humans
Cingulate Ctx
LHab
LS
BNST
Caudate
Putamen
RThal
PVThal
DB
NAcc
GPe
GPi
Insula
PVN
AN
SON
SCN
SNi
SG
OlfN
OlfCtx
NBM
CoA
CeA
Hippocampus
MeA
BLA
LA
ABA

Hypothalamus.

In all four species, dense oxytocin immunoreactive (OT-ir) and vasopressin immunoreactive (AVP-ir) cell bodies were found in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus, as well as scattered cells in the accessory nucleus of the hypothalamus (Figure 2.3). Additionally, AVP-ir cell bodies were found in the suprachiasmatic nucleus in all species. OT-ir and AVP-ir fibers in the medial pre-optic area, lateral, anterior, and posterior hypothalamus were also observed in all species, along with a dense accumulation of fibers projecting from the PVN and SON into the infundibular stalk.

Figure 2.3. OT and AVP producing cells in the hypothalamus of rhesus macaques, chimpanzees, bonobos, and humans. PVN = paraventricular nucleus, SON = supraoptic nucleus.



Septum and Diagonal Band

OT-ir fibers were observed in all species in both the medial and lateral septum as well as the diagonal band, though these fibers were particularly sparse in humans. In all four species, AVP-ir fibers were observed in both the medial and lateral septum as well as the diagonal band. Septal fibers were sparse in bonobo and human tissue.

Extended Amygdala

In all four species, OT-ir fibers were found consistently in the medial and central nuclei of the amygdala, with sparse fibers in the ventral part of the basolateral nucleus as well as the accessory basal nucleus. OT-ir fibers were absent from the lateral nucleus in all species. Additionally, chimpanzees, bonobos, and humans had solitary or sparse fibers in the cortical amygdala, while macaques had a dense accumulation of fibers here (Figure 2.6). Chimpanzees had dense OT-ir fibers in the amygdala-hippocampus transition area.

AVP-ir fibers were found in the medial and central amygdaloid nuclei of all four species, though they were denser in macaque and chimpanzee brains than in the other species. As with oxytocin, AVP-ir fibers were found in the cortical, basolateral, and accessory basal nuclei of the all species, but a dense patch of fibers in the cortical amygdala was only found in macaques (2.6), and chimpanzees had dense AVP-ir fibers in the amygdala hippocampus transition area.

All four species had an accumulation of OT-ir and AVP-ir fibers in the central amygdala. The average total length of AVP-ir and OT-ir fibers within the volume of the central amygdala was estimated for each species. A Kruskall-Wallis test identified a significant group difference among the means of AVP fiber density ($p=.003$), but not OT. The difference between the chimpanzee and bonobo AVP means was significant (Mann-Whitney U test, $p<.01$) but did not survive Bonferroni correction for multiple comparison.

All four species showed OT-ir and AVP-ir cell bodies and fibers in the bed nucleus of the stria terminalis (BNST), with macaques and chimpanzees showing a more dense accumulation of AVP fibers compared to humans and bonobos. These cell bodies were found in both the medial and lateral BNST of all species, both dorsally and lateral to the anterior commissure. Fibers originating from these neurons could be observed in the stria terminalis as well.

*Figure 2.4***.** Oxytocin fiber density in the central amygdala by species.

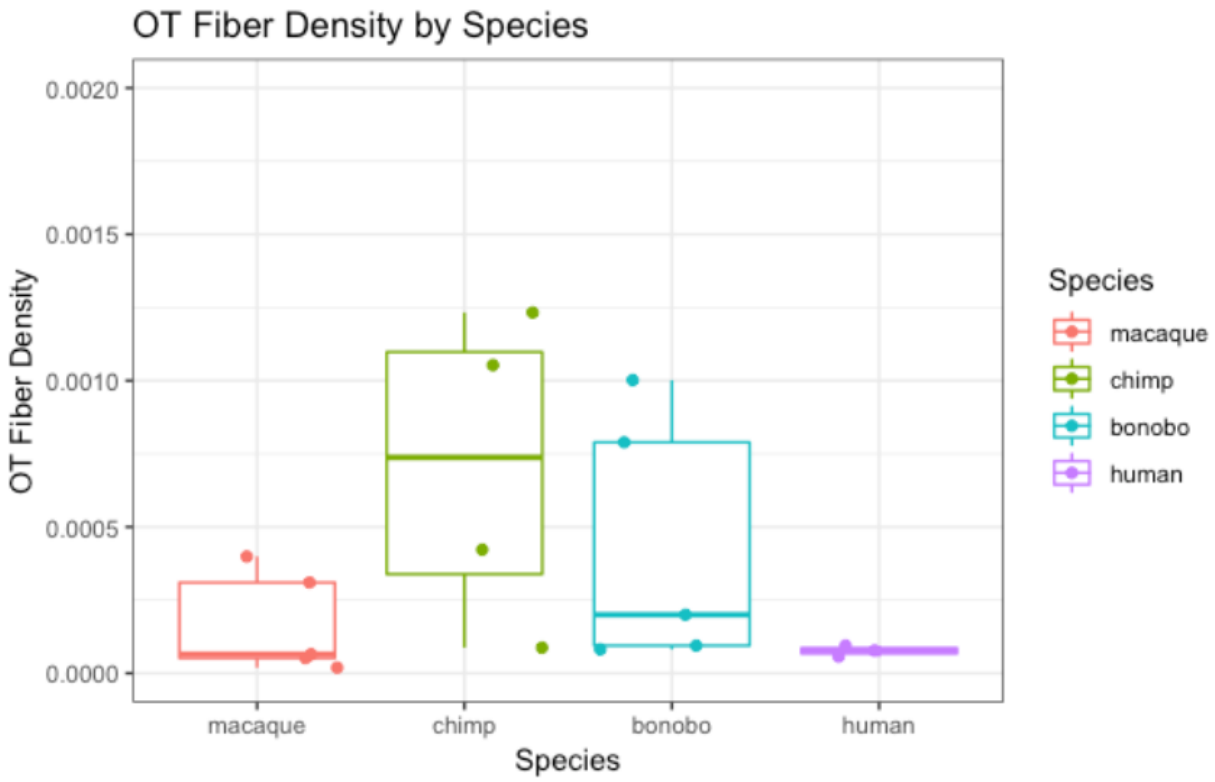


Figure 2.5. Vasopressin fiber density in the central amygdala by species.

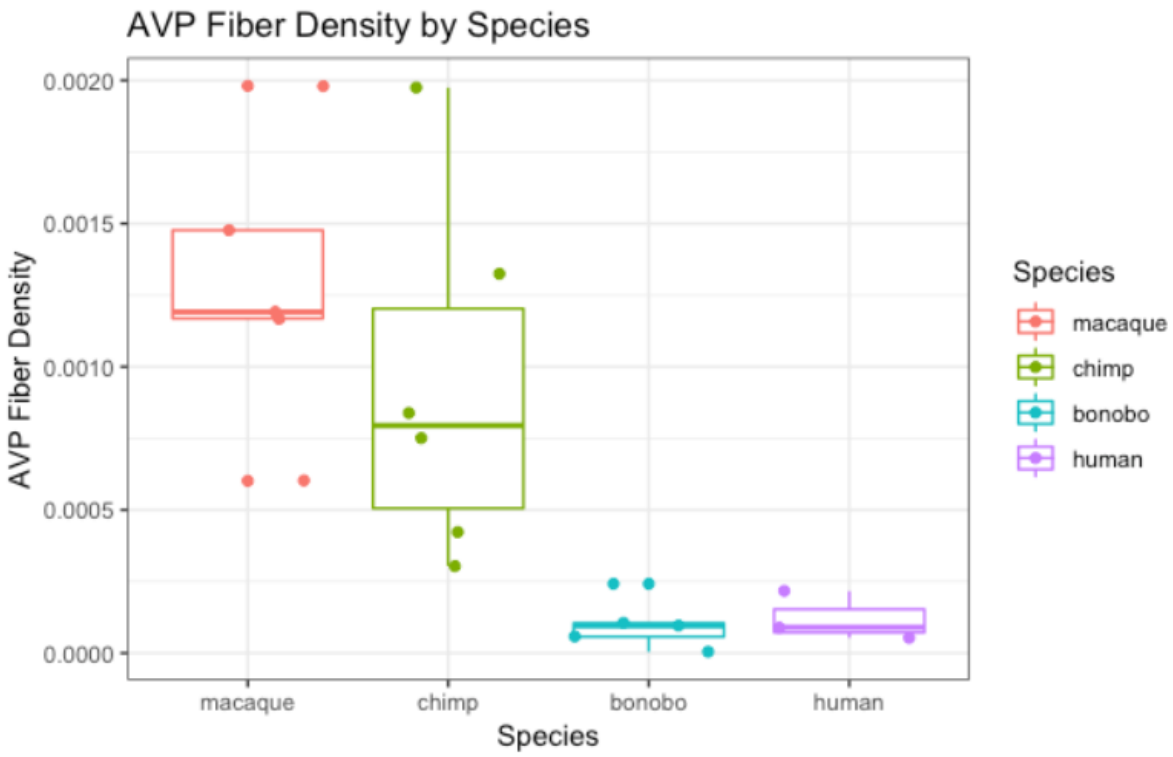
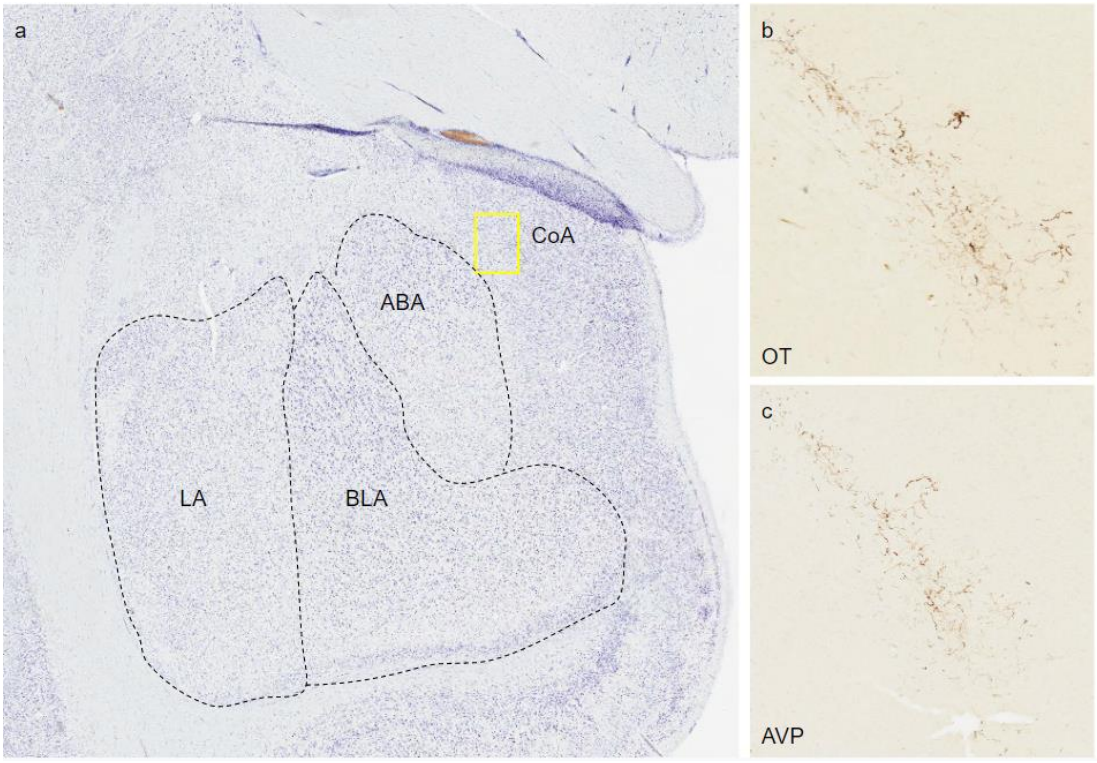


Figure 2.6. OT-ir and AVP-ir fibers in the macaque cortical amygdala. a) The nuclei of a macaque amygdala are shown on an adjacent Nissl section. The yellow box corresponds to the fiber location in the cortical amygdala. b) Dense accumulation of oxytocin fibers in the macaque cortical amygdala. c) Dense accumulation of vasopressin fibers in the macaque cortical amygdala. LA = lateral amygdala, BLA = basolateral amygdala, ABA = accessory basal amygdala, CoA = cortical amygdala.



Basal Ganglia

OT-ir cell bodies and fibers were found in the internal globus pallidus (GPi) in macaques, but only fibers were found in the GPi of chimpanzees and bonobos, and no OT staining was found in the GPi of humans. In all four species, sparse fibers were observed in the external capsule extending laterally ventral to the striatum. Solitary OT-ir fibers were found in the nucleus accumbens in chimpanzees, bonobos, and humans, but not macaques. OT-ir fibers were also observed in the putamen in chimpanzee brains.

AVP-ir cell bodies and fibers were found in the internal globus pallidus (GPi) in all four species. These cells and fibers were particularly dense in macaques, with a pathway of fibers originating in the paraventricular nucleus extending through the medial medullary lamina. In all species, fibers were observed in white matter ventral to the striatum extending laterally to the external capsule. In macaques, chimpanzees, and humans, solitary fibers were found in the nucleus accumbens. Finally, sparse AVP-ir fibers were found in the putamen in chimpanzee brains.

Thalamus and Epithalamus

In all four species, sparse OT-ir and AVP-ir fibers were found in the midline thalamic nuclei, specifically the paraventricular and rhomboid nucleus of the thalamus. In macaques, chimpanzees, and humans, solitary OT-ir and AVP-ir fibers were found in the lateral habenula. In macaques, AVP-ir fibers were also found in the stria medullaris.

Hippocampus

OT-ir fibers were found in the hippocampus of chimpanzees, but not macaques, bonobos, or humans. AVP-ir fibers were found inconsistently in the hippocampus of macaques (3 out of 5 individuals) but were not observed in chimpanzees, bonobos, or humans.

Midbrain

Most of the midbrain was not present in chimpanzee, bonobo, and human samples we examined, most midbrain regions regions could not be compared across species. Table 5 presents a summary of OT-ir and AVP-ir fiber locations in midbrain regions in macaques. In this species, sparse OT-ir fibers were found in the periaqueductal gray.

AVP-ir fibers were found in the pars compacta of the substantia nigra of all four species. In the macaque brains, fibers were also observed in the interpeduncular nucleus, the

periaqueductal gray, and the medial and dorsal raphe. Two macaque brains contained sections caudal enough to contain the parabrachial nucleus, locus coeruleus, and Koelliker-Fuse nucleus, all of which contained dense AVP-ir fibers.

Table 2.5. AVP-ir and OT-ir fibers in the midbrain of rhesus macaques.

Brain region	**Rhesus macaques**	
	AVP	OT
Substantia nigra pars compacta	**	
Ventral tegmental area	*	
Periaqueductal gray	*	*
Ventral tegmental area	*	
Interpeduncular nucleus	*	
Dorsal raphe	*	
Locus coeruleus	*	
Parabrachial nucleus	**	
Kolliker-fuse	**	

Cortex.

Detailed characterization of OT-ir and AVP-ir fibers in the cortex of chimpanzees, humans, and macaques has been published previously (Rogers et al., 2018). Here, we add our observations from bonobo brains. In human and chimpanzee brains, OT-ir cortical fibers were found in the straight gyrus and anterior cingulate cortex. In bonobos, oxytocin fibers were observed in the anterior cingulate cortex, the straight gyrus, and agranular insula.

As published previously (Rogers et al., 2018), AVP-ir fibers were observed in the cingulate cortex in macaques, chimpanzees, and humans, and in the insula in humans and chimpanzees. In bonobos, AVP-ir fibers were observed in the subcallosal and anterior cingulate cortex.

Discussion

As expected, OT- and AVP-ir cell bodies and fibers were found in the hypothalamus of all four species. Both OT and AVP cell bodies were found in the paraventricular and supraoptic nuclei, with AVP cell bodies also present in the suprachiasmatic nucleus. Dense fiber projections extended from the cells in these nuclei to the posterior pituitary through the infundibular stalk. This is consistent with other mammalian species that have been studied (Sofroniew, 1983).

The results for the four primate species investigated here differ in some ways from previous reports in rodents. Several rodent species have numerous AVP cell bodies in the BNST and medial amygdala which send dense fiber projections to the lateral septum (Knobloch et al., 2012). In contrast, in our study, macaques, chimpanzees, bonobos, and human individuals did not have cell bodies in the medial amygdala or had very few cell bodies continuous with the SON, and only a moderate amount of fibers in the lateral septum. Other rodent species such as Syrian hamsters share this AVP phenotype (Albers & Bamshad, 1998). All four primate species did have AVP and OT cell bodies in the medial BNST, dorsal to the anterior commissure. In contrast, OT neurons in the BNST of mice are located ventrally to the anterior commissure; future research on the comparative neurochemistry of the BNST is needed to determine if these cells occupy homologous subnuclei (Fox et al., 2015). AVP fiber density in the BNST was denser in rhesus macaques and chimpanzees than humans and bonobos. The BNST is considered to be a part of the “extended amygdala” (Heimer & Van Hoesen, 2006; Alheid & Heimer, 1988) is developmentally related to the medial and central amygdala (Fox et al., 2015; Stoop et al., 2015). The BNST is thought to be involved in extended-duration fear states, in contrast to the immediate fear response mediated by the amygdala (Lebow & Chen, 2016).

Among the four species, notable differences emerged in amygdala innervation for both OT and AVP. In all four species, OT and AVP fibers were observed in the medial and central

nuclei of the amygdala. However, AVP fibers in the central amygdala were denser in macaques and chimpanzees than in humans and bonobos, while OT in the central amygdala was slightly denser in chimpanzees and bonobos than humans and macaques. Moreover, macaque and chimpanzee brains showed moderate innervation of the basolateral amygdalar nuclei, which was sparse in bonobos and humans. Axonal release of OT and AVP exhibits known effects (anxiolytic and anxiogenic, respectively) on the central amygdala (Huber, Veinante, & Stoop, 2005; Knobloch et al., 2012). It is possible that AVP in the macaque and chimpanzee central amygdala increases threat detection during social interactions. Chimpanzees and macaques have higher threat responses to unfamiliar conspecifics than humans or bonobos (Wrangham, 1999); it may be that neurobiological differences in the OT and AVP innervation of the amygdala contribute to such behavioral propensities. It is less clear what the functional effect of axonal release in the basolateral amygdala might be. Previous studies investigating human OT receptor distribution using different methods have provided opposing claims on the presence of OT receptors in human basolateral amygdala (Loup et al., 1991; Boccia et al., 2013). A recent study showed humans with selective basolateral amygdala damage due to Urbach-Wiethe disease, a genetic disorder that can affect the medial temporal lobes, showed increased hypervigilant responses to fearful faces, suggesting a role for this region in the brain's threat vigilance system (Terberg et al., 2012).

We predicted possible differences in the basal ganglia; namely, that humans would have denser OT-ir or AVP-ir fibers within reward areas. However, in all four species, fibers within the nucleus accumbens were very sparse or absent. Interestingly, all four species had large AVP-ir neurons in the internal globus pallidus. AVP cells in this area have been previously reported in one primate, the Japanese macaque (Kawata & Sano, 1982). However, this has not been reported

in previous studies of extrahypothalamic AVP neurons in rodents or humans (Fliers et al., 1986). The internal globus pallidus is involved in the regulation of voluntary movement, and serves as output center of the basal ganglia along with the substantia nigra, which contained AVP-ir fibers in the species examined here. It is possible that in primates, AVP may be exerting effects on the GABAergic neurons of the internal globus pallidus which send inhibitory signals to the lateral habenula and brainstem (Parent, Lévesque, & Parent, 1999; Hong & Hikosaka, 2008).

All four species had sparse OT and AVP fibers in the lateral habenula. This brain region is thought to be involved in behavioral choice (Hikosaka, 2010), specifically as a source for "negative reward" or punishment signaling in dopamine neurons (Matsumoto & Hikosaka, 2007). The interactions of OT and AVP with dopamine in the reward system is well documented, particularly in the case of pair-bonding. It may be that OT and AVP play a role in modulating brain networks for social punishment as well as reward. It is also possible that the fibers here are projecting to the nearby pineal gland; previous studies have described OT and AVP fibers in the pineal gland of macaques (Ronnekleiv, 1988) as well as rats (Buijs & Pévet, 1980), and AVP may be involved in melatonin synthesis (Stehle et al., 1991). Our tissue sampling did not include the pineal gland, so it remains to be discovered whether similar projections to this area are found in great apes and humans.

Of the species examined in this study, only the samples from macaques included tissue from the midbrain. Therefore it is uncertain what aspects of OT and AVP midbrain neuroanatomy may differ among the three primate species. Nevertheless, we report that OT and AVP fibers were found in the central gray in macaque brains, as well as vasopressin in several other midbrain regions. Notably, AVP fibers were detected in the interpeduncular nucleus in macaques, a region with extensive connections to the lateral habenula (Herkenham & Nauta,

1979; Kobayashi et al., 2013); again, this may suggest a role for AVP in sensitivity to social punishment.

AVP and OT axonal projections into the cortex were reported previously for macaques, chimpanzees, and humans (Rogers et al., 2018). In the present study, solitary bonobo AVP and OT fibers were observed in the insula, a region implicated in interoception, self-awareness, and empathy in humans (Craig, 2009), while in chimpanzees and humans only AVP fibers were found here. Given the sparsity of these fibers and the limitations on sample size in ape neurobiology, more research is needed to determine if this is specific to bonobos or if OT-ir fibers were not captured in the sample of the previous study. Given the cross-reactivity of the OT and AVP v1a receptor in primates, it is possible that OT or AVP released in the insula in bonobos could act on the same receptor population.

There is considerable evidence that the OT and AVP systems have species-specific neuroanatomy that contributes to species-typical social cognition and behavior. Differences in the distribution of receptors are known to correlate with behavioral differences in both rodents (Insel, Wang, & Ferris, 1994; Johnson & Young, 2015) and primates (Freeman et al., 2014); however, these receptors are often far from the hypothalamus. A previous assumption in the field was that dendritic release in the hypothalamus affected such faraway receptors through diffusion. If true, this would severely limit the specificity and speed with which brain regions known to be affected by OT and AVP could receive their inputs. However, an increasing number of studies, including this one, are showing axonal projections from OT and AVP neurons into distant brain regions, allowing for targeted release and rapid modulation of these regions.

In the present study, we found evidence for extensive extrahypothalamic OT and AVP neurons and fibers in macaques, chimpanzees, bonobos, and humans. Our results suggest that

over the course of human evolution, as well as over the course of bonobo evolution, the central amygdala may have become less innervated by AVP and possibly OT, as well as the lateral septum and BNST.

These results will need to be compared to data on OT and AVP V1a receptor distribution in chimpanzees, bonobos, and humans. Previous attempts to localize these receptors in humans have yielded conflicting results (Loup et al., 1991; Boccia et al., 2013) due to the structural similarity of the two receptors in primate brains. Studies on the two species using a competitive binding protocol (Smith et al., 2012; Freeman & Young., 2016) are needed to complete the picture of the brain regions modulated by OT and AVP and the method of transmission involved. Future research is also needed to establish a complete characterization of within-species variation (both for neurons and fibers as well as receptors) and effects on individual variation in behavior. This will become more feasible with the development of appropriate PET ligands and may have important implications for disorders of social cognition such as autism, in which aspects of OT or AVP neuroanatomy may be altered (Freeman et al., 2018).

Acknowledgments

This research was supported by funding from The Leakey Foundation (#38217) to CR and The Templeton Foundation (#40463) to HEA, LJY, JKR, and TMP. Additional support was provided by NIH grants P50MH100023 to LJY and by NIH OD P51OD11132 to YNPRC.

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Chapter 3: Oxytocin- and arginine vasopressin-containing fibers in the cortex of humans, chimpanzees, and rhesus macaques

This chapter has been published as:

Rogers, C.N., Ross, A.P., Sahu, S.P., Siegel, E.R., Dooyema, J.M., Cree, M., Stopa, E.G., Young, L.J., Rilling, J.K., Albers, H.E., Preuss, T.M. (2018). Oxytocin- and arginine vasopressin-containing fibers in the cortex of humans, chimpanzees, and rhesus macaques. *American Journal of Primatology*, 80:e22875. https://doi.org/10.1002/ajp.22875

Abstract

Oxytocin (OT) and arginine-vasopressin (AVP) are involved in the regulation of complex social behaviors across a wide range of taxa. Despite this, little is known about the neuroanatomy of the OT and AVP systems in most non-human primates, and less in humans. The effects of OT and AVP on social behavior, including aggression, mating, and parental behavior, may be mediated primarily by the extensive connections of OT- and AVP-producing neurons located in the hypothalamus with the basal forebrain and amygdala, as well as with the hypothalamus itself. However, OT and AVP also influence social cognition, including effects on social recognition, cooperation, communication, and in-group altruism, which suggests connectivity with cortical structures. While OT and AVP V1a receptors have been demonstrated in the cortex of rodents and primates, and intranasal administration of OT and AVP has been shown to modulate cortical activity, there is to date little evidence that OT-and AVP-containing neurons project into the cortex. Here, we demonstrate the existence of OT- and AVP-containing fibers in cortical regions relevant to social cognition using immunohistochemistry in humans, chimpanzees, and rhesus macaques. OT-immunoreactive fibers were found in the straight gyrus of the orbitofrontal cortex as well as the anterior cingulate gyrus in human and chimpanzee brains, while no OT-immunoreactive fibers were found in macaque cortex. AVP-immunoreactive fibers were observed in the anterior cingulate gyrus in all species, as well as in the insular cortex in humans, and in a more restricted distribution in chimpanzees. This is the first report of OT and AVP fibers in the cortex in human and non-human primates. Our findings provide a potential mechanism by which OT and AVP might exert effects on brain regions far from their production site in the hypothalamus, as well as potential species differences in the behavioral functions of these target regions.

Introduction

Research in a wide range of taxa has revealed a significant role for the neuropeptides oxytocin (OT) and arginine-vasopressin (AVP) in social cognition and behavior (Caldwell & Albers, 2015; Johnson & Young, 2017). Many of these behaviors are species-specific and reflect differences in the OT and AVP systems; the clearest example is the presence of OT and AVP receptors in the reward system of pair-bonding vole species, and their absence in these regions in non-pair-bonding but closely related species (Johnson & Young, 2017; Young, Murphy Young & Hammock, 2005). There is increasing evidence that OT and AVP modulate social cognition and behavior in humans and non-human primates (Crockford, Deschner, Ziegler & Wittig, 2014; Feldman, Monakhov, Freeman & Young, 2016) and that disruptions of the OT and AVP systems in humans may be associated with disorders such as autism, Williams syndrome, and schizophrenia (Dai et al., 2012; Guastella & Hickie, 2016; Meyer-Lindenberg, Domes, Kirsch & Heinrichs, 2011; Zhang, Zhang, Han & Han, 2017). However, there is limited knowledge of the neurobiology underlying the OT and AVP systems in humans, or how these systems may vary among primates. Here, we used immunohistochemistry to examine OT- and AVP-immunoreactive (ir) innervation of regions of the cerebral cortex relevant to social cognition in humans, chimpanzees, and rhesus macaques.

Intranasal administration of OT and AVP has been the primary source of information about the effects of these peptides in humans. Although effects on behavior using this procedure are varied and complex, several themes emerge. OT appears to facilitate social cognition and social approach, depending on the context (Preckel, Scheele, Kendrick, Maier & Hurlemann, 2014; Scheele et al., 2012) and may play a role in social attachment (King, Walum, Inoue, Eyrich & Young, 2016). OT also modulates amygdala reactivity to social stimuli, with potential

for anxiety reduction (Chen et al., 2016; Petrovic, Kalisch, Singer & Dolan, 2008). The prosocial effects of OT may be context dependent and may depend on individual characteristics as well as the group status (ingroup vs. outgroup) of an interaction partner (De Dreu, 2012; Marsh et al., 2017). AVP effects are also context-dependent, as it can promote pair-bonding behavior and cooperation (Brunnlieb et al., 2016; Rilling et al., 2014), but can also increase reactivity to threat and enhance anxiety (Morales-Medina, Witchey & Caldwell, 2016).

Several of these behavioral and cognitive traits associated with OT and AVP—bonding, disposition toward outgroup individuals, and anxiety—show remarkable diversity among primate species. Humans differ from chimpanzees and rhesus macaques in their tendency to form pair-bonds. Chimpanzees and rhesus macaques live in multi-male, multi-female societies in which individual animals mate with multiple members of the opposite sex. Although the human mating system is characterized by flexibility and varies across cultures, stable mating relationships are widespread across human societies (Quinlan & Quinlan, 2008), suggesting humans possess the biological substrates to facilitate pair-bonding to a greater degree than chimpanzees or macaques. Chimpanzees are pronounced in their xenophobia and territoriality (Furuichi & Thompson, 2007), and they commonly engage in lethal aggression, while human societies show high variation in rates of intergroup and lethal aggression (Fry & Soderberg, 2013).

Despite this behavioral variation, the neurobiology of the OT and AVP systems in human and non-human primates is not well characterized. OT and AVP are produced in the paraventricular, supraoptic, and suprachiasmatic (AVP only) hypothalamic nuclei in all mammalian species studied, as well as a limited number of cell groups outside the hypothalamus, which vary according to species (Kelly & Goodson, 2014; Ragen & Bales, 2013; Sofroniew, 1980). OT- and AVP-producing cells in the hypothalamus send dense fiber projections through

the median eminence into the posterior pituitary to be released into the bloodstream. These hypothalamic cells can also affect the brain by volume transmission, or by sending axonal fibers to distant regions for fast, targeted release (for reviews see Albers, 2015; Johnson & Young, 2017).

There is evidence that OT and AVP affect the activity of cortical regions in humans. For example, OT can modulate insula and inferior frontal gyrus responses to infant crying in women (Riem et al., 2011). Both OT and AVP have also been shown to modulate insula activation of human subjects during a dyadic social interaction task (Feng et al., 2015). Finally, OT can attenuate the effect of aversive conditioned responses to neutral faces, associated with activity in the anterior cingulate gyrus and right medial temporal lobe (Petrovic et al., 2008). It is unknown whether these effects are exerted through direct modulation of cortical regions by OT and AVP, or indirectly through potential connections within a larger brain network (Chini, Verhage & Grinevich 2017). To resolve this, it is important to know whether OT- and AVP-producing cell groups send fiber projections to cortical regions and whether receptors for the peptides are found there.

A few studies have demonstrated the presence of OT and AVP V1a receptors in non-human primate cortex. AVP V1a receptors are present in various cortical regions in rhesus macaques (Young, Toloczko, & Insel, 1999), titi monkeys (Freeman et al., 2014), and common marmosets (Freeman & Young, 2016; Schorscher-Petcu, Dupre, & Tribollet, 2009). OT receptors have been found in primary visual cortex in titi monkeys (Freeman et al., 2014). Two studies suggest that OT and AVP receptors may be present in cortical regions in humans: Loup, Tribollet, Dubois-Dauphin and Dreifuss (1991) reported AVP V1a receptors in entorhinal cortex, and Boccia, Petrusz, Suzuki, Marson and Pedersen (2013) reported OT receptors in anterior

cingulate gyrus. Notably, these studies were conducted using disparate methodologies and examined very few cortical regions. The use of pharmacologically optimized methods to demonstrate OT and AVP V1a receptors in areas of the human brainstem (Freeman, Smith, Goodman & Bales, 2016) offers potential for the full elucidation of these receptors in the cortex and other forebrain regions.

The presence of OT and AVP V1a receptors in primate cortex suggests that there are fiber projections to the cortex from peptide-containing cell bodies. There is mixed evidence for fiber projections within the cortex across mammalian species. OT fibers have been found in the insula of rats (Knobloch et al., 2012) and AVP in the insula of mice (Rood & De Vries, 2011). However, no OT or AVP cortical fibers were found in the tree shrew, the closest living relative of primates (Ni et al., 2014). In non-human primates, AVP fibers have been found in the limbic cortical regions of crab-eating macaques, specifically the primary olfactory (piriform) and entorhinal cortex, but not in neocortex, and no OT or AVP fibers have been reported in the other species examined, which include rhesus macaques, Japanese macaques, marmosets, and squirrel monkeys (see Ragen & Bales, 2013 for review). Previous reports of OT and AVP fibers in humans have focused on a limited set of subcortical limbic (Fliers, Guldenaar, van de Wal & Swaab, 1986) and brainstem (Sofroniew, 1980) regions, so it is unknown whether any cortical regions are innervated by OT and AVP in humans. Moreover, no previous study has attempted to identify OT or AVP fibers in great apes. In the present study, we address these gaps by examining the distribution of OT- and AVP-immunoreactive fibers in select cortical (including neocortical) regions of human, chimpanzee, and rhesus macaque cortex.

Methods

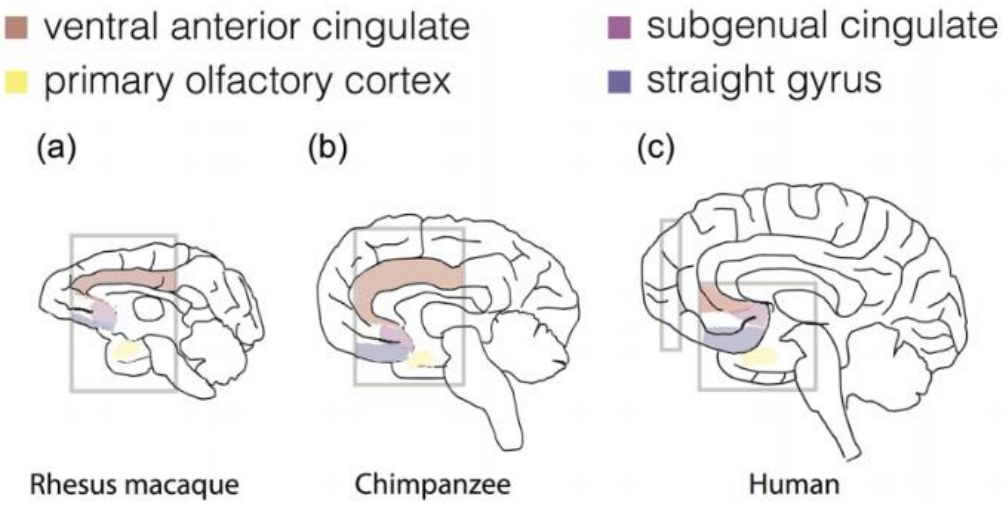
Specimens

All human tissue (three males, ages 28, 31, 54 years) was sourced from the Warren Alpert Medical School at Brown University and the Lifespan Consortium, and research with this material is approved under FAW00001230 by Lifespan's IRB and 836–2005 by Emory's IRB. Brains were collected post-mortem and fixed by immersion in 10% formalin. All chimpanzee (one male, age 43; 2 females, ages 23, 50 years) and rhesus macaque (three males, ages 4, 25, 25; two females, ages 4, 20 years) tissue came from animals housed at Yerkes National Primate Research Center (YNPRC), the brains being opportunistically collected after animals died of natural causes or after euthanasia per veterinary order. All procedures with macaques and chimpanzees were carried out in accordance with protocols approved by the YNPRC and the Emory University Institutional Animal Care and Use Committee (IACUC, approval # YER-2001206). Brain tissue was rinsed with phosphate-buffered saline. Chimpanzee brains were separated into hemispheres, with one hemisphere stored frozen at −80 °C, while the other was fixed; only the fixed tissue, prepared by immersion in 10% neutral-buffered formalin, was used in this study. The macaque brains were processed whole. Tissue was perfusion or immersion fixed in 10% neutral-buffered formalin. After fixation, the human, chimpanzee, and macaque tissue was stored in an ethylene-glycol-based cryopreservative solution at −20 °C until use. Post-mortem intervals for macaques and chimpanzees were <3 hr, and <48 hr for humans. Previous studies using similarly prepared tissue have shown binding specificity using antibodies for nonphosphorylated neurofilament protein (SMI-32), calbindin, and VGLUT2 (Bryant et al., 2012; Preuss & Coleman, 2002).

Regions investigated

Figure 3.1 depicts a lateral view of the regions sampled in each species. In every macaque, both hemispheres were examined from the most rostral part of the striatum through the posterior hippocampus, corresponding to Paxinos, Huang, and Toga (2000) atlas levels 24 through 96. Due to the much larger size of chimpanzee and, especially, human brains, select cortical regions were examined in tissue blocks of one hemisphere spanning the most rostral part of the striatum through the anterior hippocampus. These blocks included posterior orbital, cingulate, and insular cortex, as well as portions of fronto-parietal and anterior temporal cortex, and correspond to level 15 through 56 in the Ding et al. (2016) human atlas. An additional, more anterior sample of human frontal cortex (level 10) was also examined. In all three species, sections from the hypothalamus were also used as positive controls.

Figure 3.1. Mid-sagittal view of anatomical regions included in this analysis for (a), rhesus macaques, (b), chimpanzees, and (c), humans. Regions inside the rectangles represent the blocks examined in each species. Important target regions are labeled. See Figures 3.4–3.6 for a coronal view including insular regions not visible in a mid-sagittal view.



Tissue sectioning, antibodies, and immunohistochemistry

Fixed tissue was sectioned in the coronal plane at 40 μm thickness using a freezing microtome (Leica Biosystems, Wetzlar, Germany); blockface images were collected for every second section using a camera mounted over the freezing stage. The sections were first washed with phosphate-buffered saline (PBS) and then incubated in citrate buffer at 37.5 °C for 30 min for antigen retrieval. Next, sections were washed with 3% peroxide in methanol for 10 min to inactivate endogenous peroxidase. Sections were incubated for 1 hr in blocking buffer containing PBS, 2% serum, and 0.2% Tween-20, then incubated overnight at 4 °C in primary antibody at a dilution of 1:20,000. The next morning, sections were washed, then incubated in secondary antibody for 1 hr followed by a solution of biotinylated peroxide plus avidin (Vector ABC reagent). Sections were then reacted with diaminobenzidine (DAB) solution using the Vector DAB peroxidase substrate kit (Vector Laboratories, Inc, Burlingame, CA).

The OT antibody (Millipore, Burlington, MA, MAB5296) was a mouse monoclonal antibody made against OT conjugated to thyroglobulin. Specificity was tested by competitive ELISA and no reactivity to AVP or vasotocin was found (Millipore technical information datasheet). The AVP antibody (Peninsula Laboratories International, San Carlos, CA, T-4563) was a rabbit polyclonal antibody made against a synthetic AVP peptide. Specificity was tested with a no-primary control. Based on dilution tests, a concentration of 1:20,000 was found to be optimal for both the OT and AVP antibodies. In all three species, a 1:12 series of sections was labeled for OT, and an adjacent 1:12 series of sections labeled for AVP. In addition, a 1:12 series of sections adjacent to AVP was stained for Nissl substance. Stained sections were mounted on gelatin-coated slides, air-dried, and coverslipped.

Digital image capture and examination

Digital images of the stained sections were captured using an Aperio Digital Pathology Slide Scanner (Leica Biosystems) and analyzed qualitatively for localization of cell bodies and fibers. Imagery for each stained section was comprehensively examined at high magnification on a computer screen by at least two investigators. Possible immunoreactive (ir) fibers were flagged using Aperio's ImageScope software and each flagged site evaluated by multiple investigators. The images used in Figures 3.2–3.6 were extracted from the original scans using ImageScope software.

Results

Fiber morphology and orientation

OT and/or vasopressin fibers were found in a set of cortical regions in each species (see Table 3.1). In all three species, axonal fibers in the cortex showed morphology typical of those found in the hypothalamus and other subcortical structures, characterized by large varicosities that likely include en passant boutons (Figure 3.2). They are distinguishable from blood vessels by their thin and beaded appearance. OT and AVP fibers were observed in all cortical layers and showed no laminar preference. In deep cortical layers, a majority of fibers were oriented radially (parallel to the columns of cerebral cortex), with some oriented tangentially (perpendicular to the columns of cerebral cortex) or twisted in no obvious configuration. In layer I, fibers were mainly oriented tangentially (Figure 3.3). OT and AVP fibers were very sparse in every region in which they were observed.

Figure 3.2. Morphology of AVP-ir fibers in the cortex closely resemble those in the hypothalamus. (a) Labeled hypothalamic cell bodies and fibers in the human supraoptic nucleus. (b) Cortical fibers in human insula. (c) Cortical fibers in chimpanzee insula. (d) Cortical fibers in macaque subgenual cingulate gyrus. OT and AVP fibers in all species showed the same morphological features. Roman numerals indicate cortical layers. Scale bar = 100 μm

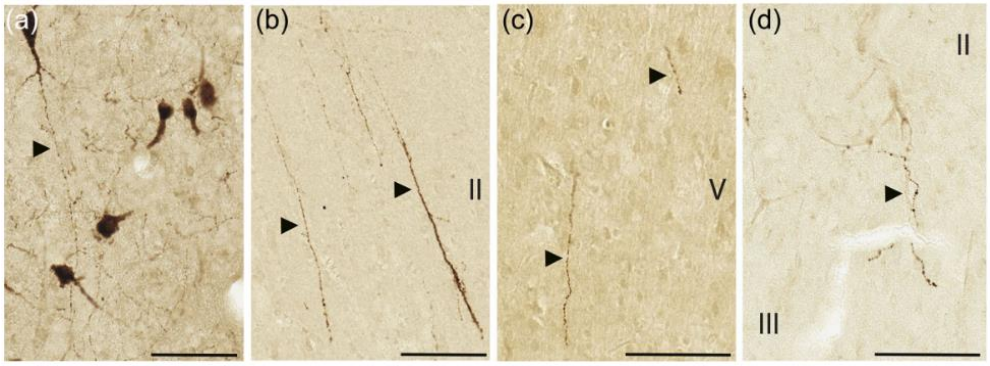
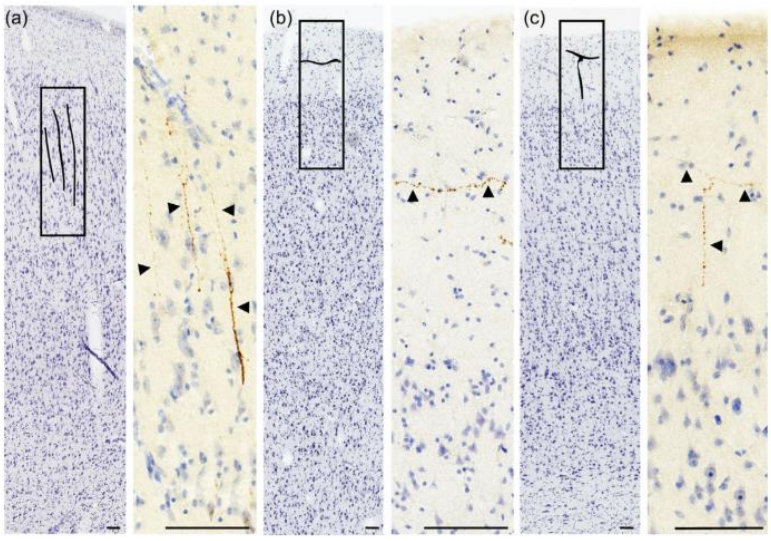


Figure 3.3. AVP-ir fibers in the human insula. Nissl sections are shown at lower magnification. AVP fibers are shown at higher magnification, enhanced for contrast, and brightness with Aperio ImageScope software. (a) Radially oriented fibers in cortical layer II. (b) Fibers in the human insula oriented tangentially in cortical layer I. (c) A radial fiber that appears to end with tangential branches in cortical layer I. Scale bar = 100 μm



Fiber distribution

Table 3.1 provides a summary of results.

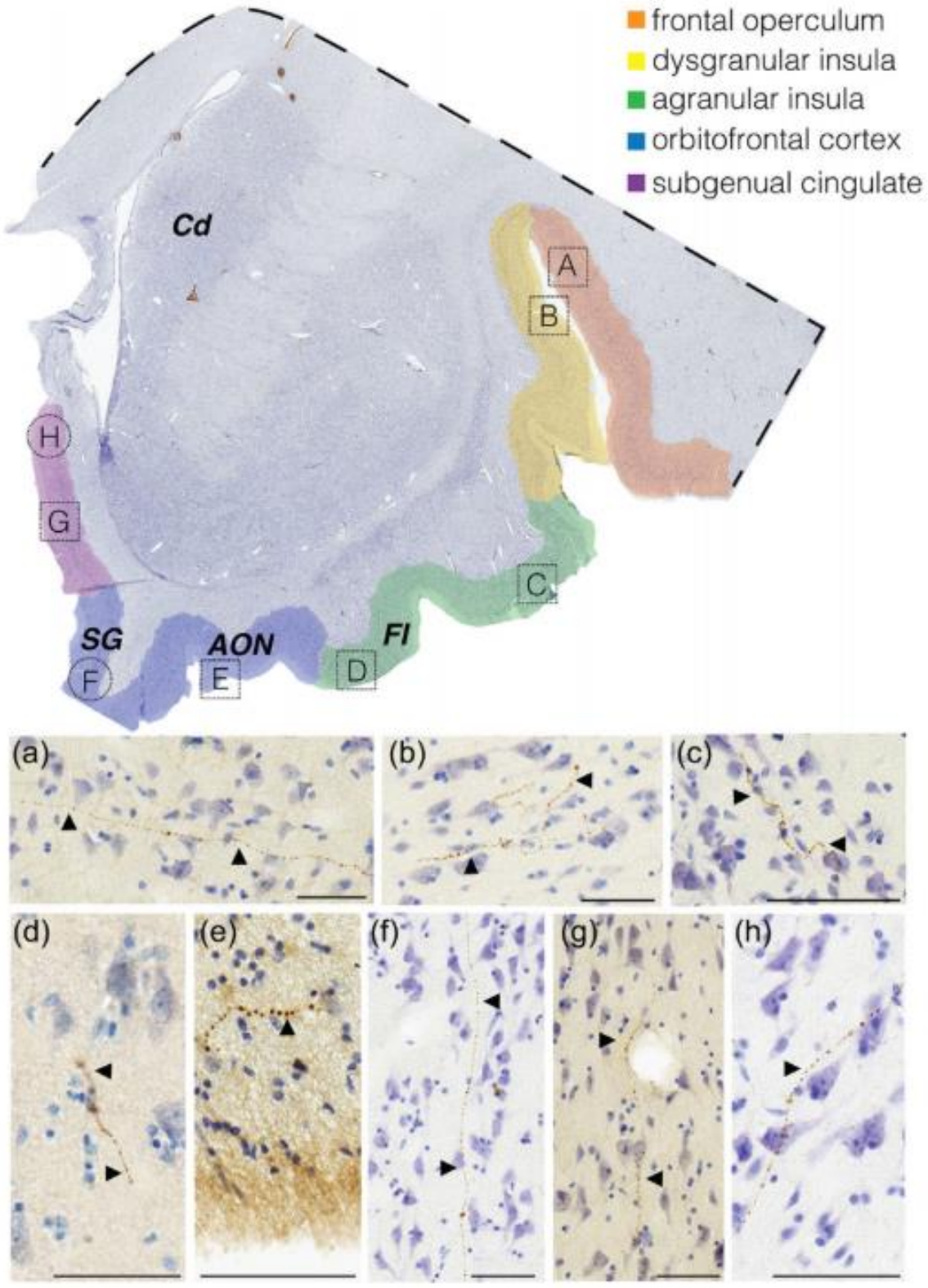
Table 3.1. Summary of results. A plus sign indicates fibers observed in that region. A Ø indicates no fibers were found in our sampling. NE indicates that the region was not examined in that species.

	Rhesus macaque	Chimpanzee	Human
OXYTOCIN			
Paleocortex			
Anterior olfactory nucleus	Ø	Ø	Ø
Primary olfactory cortex	Ø	Ø	Ø
Periallocortex			
Agranular insula	Ø	Ø	Ø
Proisocortex			
Dysgranular insula	Ø	Ø	Ø
Straight gyrus of orbitofrontal cortex	Ø	+	+
Ventral anterior cingulate gyrus (Area 24)	Ø	+	+
Subgenual anterior cingulate gyrus (Area 25)	Ø	+	+
Isocortex			
Frontal operculum	Ø	Ø	Ø
Frontal pole	NE	NE	Ø
VASOPRESSIN			
Paleocortex			
Anterior olfactory nucleus	+	+	+
Primary olfactory cortex	+	+	+
Periallocortex			
Agranular insula	Ø	+	+
Proisocortex			
Dysgranular insula	Ø	Ø	+
Straight gyrus of orbitofrontal cortex	Ø	+	+
Ventral anterior cingulate gyrus (Area 24)	Ø	Ø	Ø
Subgenual anterior cingulate gyrus (Area 25)	+	+	+
Isocortex			
Frontal operculum	Ø	Ø	+
Frontal pole	NE	NE	Ø

Humans

OT-immunoreactive (OT-ir) fibers were found in the straight gyrus (also known as gyrus rectus) and the ventral and subgenual anterior cingulate gyrus (Brodmann's areas 24 and 25) in human cortex (Figure 3.4). Notably, due to the much larger size of the human brain, it was not feasible to examine the entire anterior cingulate gyrus, as we did in macaques and chimpanzees. OT-ir fibers were not observed in the insula or primary olfactory cortex. In all three human brains, immunoreactive AVP fibers were found in the insular cortex. These fibers were found in granular insula (regions of insula lacking the granule cells of layer IV), including frontoinsular cortex, as well as dysgranular insula (transition area between agranular and more posterior granular insula). Fibers were observed in all layers of human insular cortex. Sparse AVP-ir fibers were also found in the frontal operculum, primary olfactory cortex, and the subgenual cingulate gyrus (Figure 3.4).

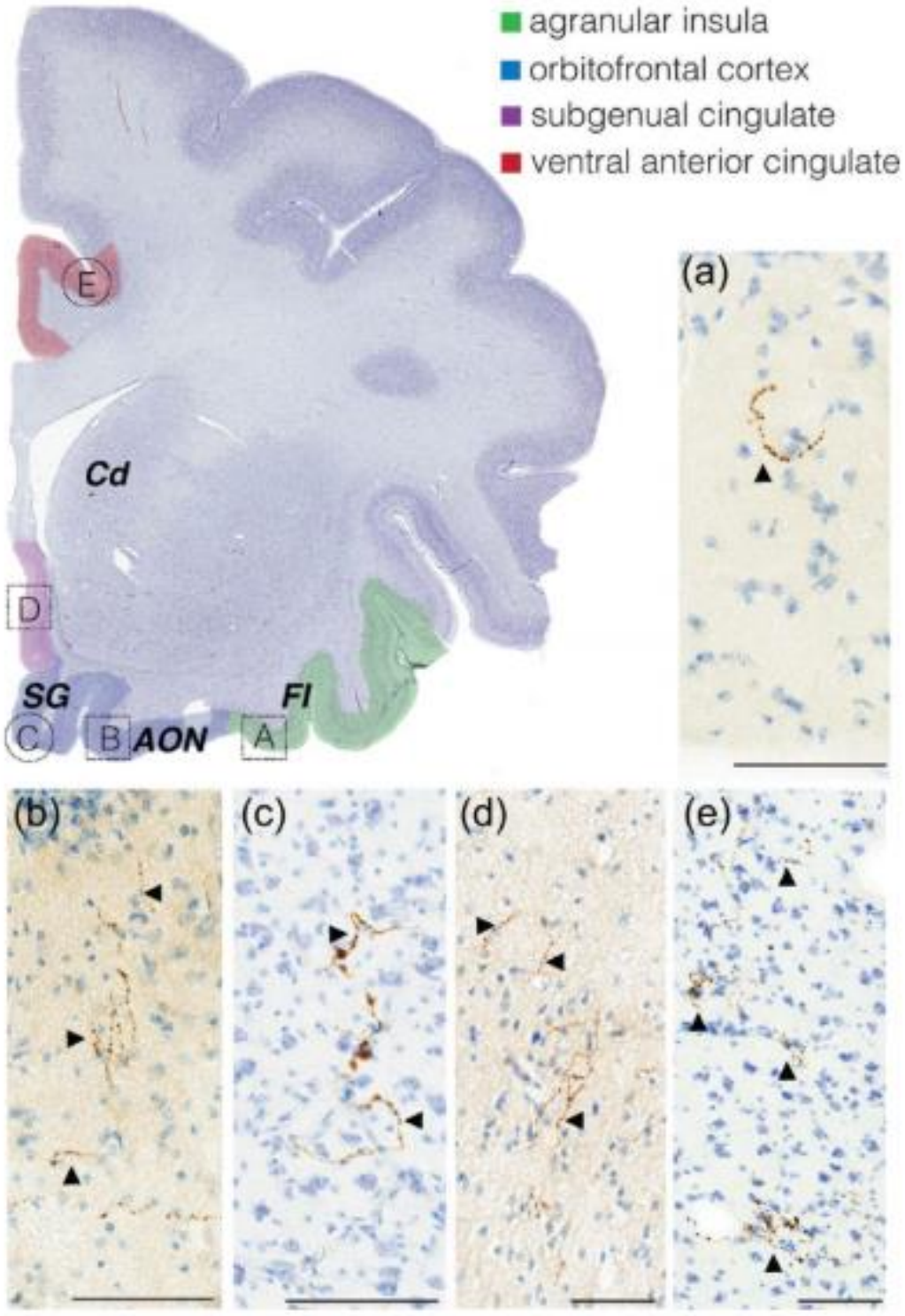
Figure 3.4. Nissl section illustrating a selection of regions where OT and AVP fibers were found in human cortex. The midline is to the left; the dashed line at the dorsal edge indicates where the section was cut through the corpus callosum, internal capsule, and superior parts of frontal cortex. Tissue with OT fibers are marked by letters within circles; letters within rectangles represent AVP fibers. a–h) High magnification view of OT and AVP fibers. Letters on section view correspond to histology panels. Fiber images are from nearby sections (within 1 mm). FI, frontoinsular cortex; AON, olfactory nucleus; SG, straight gyrus; Cd, caudate. Scale bar = 100 μm.


frontal operculum
dysgranular insula
agranular insula
orbitofrontal cortex
subgenual cingulate
Cd
A
B
C
D
E
F
G
H
SG
AON
FI
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

Chimpanzees

OT-ir cortical fibers were found in the straight gyrus in chimpanzee brains, as in human brains. OT-ir fibers were also found in the chimpanzee ventral anterior cingulate gyrus, subgenual cingulate gyrus, and superior frontal gyrus. There were no OT-ir fibers in the insula in chimpanzee brains. AVP-ir fibers were found in the primary olfactory cortex, as well as the subgenual cingulate gyrus, of chimpanzees. Additionally, solitary AVP-ir fibers were found in agranular insular cortex in the chimpanzee (Figure 3.5). AVP-ir fibers were not observed in granular insula, dysgranular insula, or the ventral anterior cingulate gyrus.

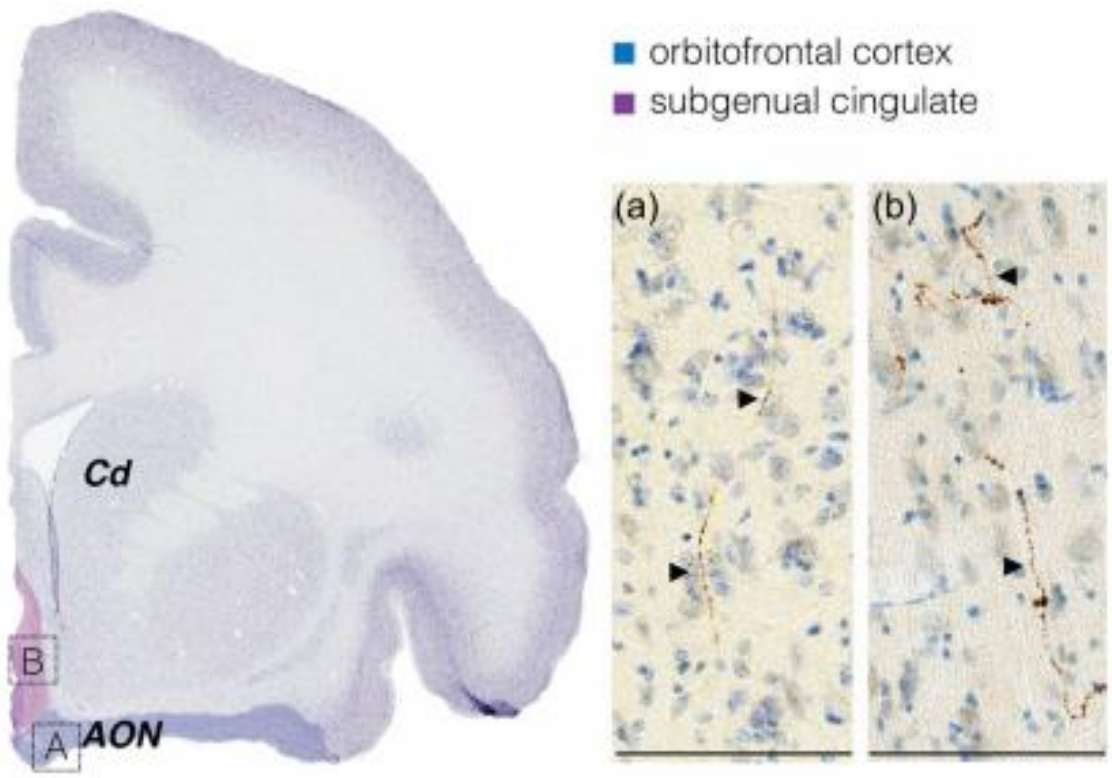
Figure 3.5. Nissl section illustrating a selection of regions where OT and AVP fibers were found in chimpanzee cortex. Tissue with OT fibers are marked by letters within circles; letters within rectangles represent AVP fibers. a–e) High magnification view of OT and AVP fibers. Letters on section view correspond to histology panels. Fiber images are from nearby sections (within 1 mm). FI, frontoinsular cortex; AON, olfactory nucleus; SG, straight gyrus; Cd, caudate. Scale bar = 100 μm


agranular insula
orbitofrontal cortex
subgenual cingulate
ventral anterior cingulate
E
Cd
D
SG
C
B
AON
FI
A
(a)
(b)
(c)
(d)
(e)

Rhesus macaques

OT-ir fibers were not found in any cortical region in rhesus macaques. AVP-ir fibers were found in the subgenual cingulate gyrus and primary olfactory cortex of rhesus macaques (Figure 6). AVP-ir fibers were not observed in the insular cortex or ventral anterior cingulate gyrus in this species.

Figure 3.6. Nissl section illustrating a selection of regions where AVP fibers were found in macaque cortex. Letters within rectangles represent AVP fibers. a,b) High magnification view of AVP fibers. Letters on section view correspond to histology panels. Fiber images are from nearby sections (within 1 mm). AON, olfactory nucleus; Cd, caudate. Scale bar = 100 μm.



Discussion

To our knowledge, this is the first report of OT- and AVP-ir fibers in the cortex in humans and the first report of OT-ir fibers in the cortex in non-human primates. Previous studies of human OT and AVP distribution of extra-hypothalamic projections have focused on non-cortical limbic and brainstem regions. It is not clear whether those studies examined the cortex and found no OT- or AVP-containing fibers or did not examine enough of the cortex to observe them. Both are plausible, given that the fibers are very sparse in all species examined here, consistent with the sparsity of OT innervation of rodent cortex (Knobloch et al., 2012). Here, OT and/or AVP axonal projections into the cortex were found in multiple non-isocortical regions in human, chimpanzee, and macaque brains. In addition, AVP fibers were observed in isocortical (neocortical) areas of human cortex (Table 1). Isocortical regions are comprised of six layers and are the most evolutionarily recent form of cortex.

OT- and AVP-ir cortical fibers in all three species were observed to have the same morphological features as hypothalamic fibers. These axonal fibers appear beaded due to varicosities (sites of neurotransmitter release). Release from varicosities of these fibers with *en passant* boutons, rather than classic axo-dendritic synapses, could be the mechanism by which OT and AVP modulate cortical activity (Grinevich & Charlet, 2017; Putnam, Young, & Gothard, 2018), similar to serotonin and catecholamine innervation of the cortex. Axonal release of a single vesicle of OT is estimated to be sufficient for 50% occupancy of OT receptors within a 55 μm radius (Chini et al., 2017). Thus even a limited number of projections may be able to influence cortical activity.

In our samples, OT and AVP fibers were most commonly oriented radially (parallel to the columns of cerebral cortex) in deep cortical layers and tangentially (perpendicular to cortical

columns) in layer I, but both orientations as well as fibers twisted in no particular configuration were observed in all layers. While OT and AVP are produced in the hypothalamus, in several species, receptors with known functional effects have been found in brain regions that are likely too far from the hypothalamus to receive peptide through passive diffusion (Chini et al., 2017; Freeman & Young, 2016). Our results suggest that OT- and AVP-producing cells may be able to modulate cortical activity through targeted axonal release. Due to the columnar organization of the cerebral cortex, the tangential orientation of fibers traveling across these columns, particularly in layer I, could make peptide available to the apical dendrites of pyramidal cells to an extent out of proportion to the relatively small number of these peptidergic fibers. Further studies can be done tracing projections through adjacent sections to clarify their exact path. Additionally, OT and AVP fibers could modulate cortical activity via receptors not detectable in studies using autoradiography, such as those expressed in presynaptic terminals, distal to their site of synthesis in the cell body (Dölen, Darvishzadeh, Huang & Malenka, 2013). For example, OT receptors are abundantly expressed in the nucleus basalis of Meynert of nonhuman primates and humans, and this region is known to send cholinergic projections to many cortical structures (Freeman & Young, 2016). It is possible that OT receptors are transported to these distal projections and expressed in axon terminals. Further research is needed to determine if this is a mechanism by which OT and AVP innervation can modulate cortical activity. Regardless, the presence of axonal fibers may suggest the presence of receptors that are difficult to localize in postmortem samples.

With respect to localization, OT-ir fibers were found in the straight gyrus (also called gyrus rectus) within the medial orbitofrontal cortex in human and chimpanzee brains. The medial orbitofrontal cortex generally is associated with decision-making and emotional regulation

(Bechara, Damasio & Damasio, 2000; Hsu & Price, 2007). The function of the straight gyrus is not well understood, but it has been proposed as a site of sensory integration (Passingham & Wise, 2012), and is known to have extensive connections to areas important for motivation, reward, and emotion, including the amygdala, hippocampus, cingulate gyrus, and insular cortex (Morecraft, Geula & Mesulam, 1992). Chimpanzees and rhesus macaques show some differences in the reward value of social stimuli; for example, rhesus macaques avoid direct eye gaze (Mendelson, Haith & Goldman-Rakic, 1982) while chimpanzees do not (Leavens & Hopkins, 1998). It is possible that OT acts in this region to affect social reward, though this interpretation is at the moment speculative.

OT-ir fibers were also found in regions of the anterior cingulate gyrus of chimpanzees and humans. In both species, OT-ir fibers were present in the subgenual cingulate gyrus as well as the ventral anterior cingulate. The ventral anterior cingulate gyrus is involved in assessing emotional salience (Apps, Rushworth & Chang, 2016). Interestingly, OT receptors in the anterior cingulate gyrus have been shown to mediate empathy-related consoling behavior in prairie voles (Burkett et al., 2016). It may be that OT has a conserved role in this region in detecting and responding to the emotional state of others.

AVP-ir fibers were observed in the paleocortical primary olfactory cortex and the subgenual cingulate gyrus in all three species. While primates have evolved to depend highly on their visual system for social communication, olfaction is the main social sense for most mammals (Barton, Purvis, & Harvey, 1995). Nevertheless, within primates, aspects of olfactory-related neuroanatomy correlate with social group size (Barton, 2006). The presence of vasopressin fibers in the primary olfactory cortex of all three species is possibly a conserved mammalian trait. AVP-ir fibers were also found in the subgenual cingulate gyrus (Brodmann's

area 25), a non-isocortical region with high connectivity with the hypothalamus, insula, and amygdala, all of which are involved in emotion processing (Phan, Wager, Taylor & Liberzon, 2002).

The most numerous AVP-containing fibers were observed in the agranular and dysgranular insula (including frontoinsular cortex) in humans. AVP fibers were also present in agranular insula in chimpanzees. In humans, the insula has been implicated in empathy and interoception (Craig, 2002; Singer & Lamm, 2009), and it has been proposed that the agranular cortex of the anterior insula is uniquely enlarged in humans (Bauernfeind et al., 2013; Nieuwenhuys, 2012). In one study, intranasal AVP increased empathic concern among individuals who had experienced parental warmth (Tabak et al., 2015). It may be that targeted release of AVP in the human insula contributes to the role of AVP in empathy.

Additionally, the frontoinsular cortex, as well as the anterior cingulate gyrus, contains Von Economo neurons, large bipolar neurons present in humans, anthropoid primates, and other large-brained mammals, including cetaceans and farm animals (Allman et al., 2011; Raghanti et al., 2015). Though the functional role of this class of neurons is not well understood, they are known to be altered in some individuals with neuropsychiatric conditions affecting social skills and emotional function (Butti, Santos, Uppal & Hof, 2013). Although we did not observe direct contact between OT or AVP fibers and Von Economo neurons, further research is needed to determine whether local OT or AVP release modulates the activity of these neurons.

Among primates, there is evidence for human-specific modifications to the localization of cortical axonal fibers in several neuromodulatory systems (Sousa, Meyer, Santpere, Gulden, & Sestan, 2017); the OT and AVP systems appear to be no exception. However, while our sample included individuals of both sexes in chimpanzees and rhesus macaques, our human brain

tissue was from males only. Sex differences in neurobiology and behavior have been demonstrated for OT and AVP in rodents (Albers, 2015; Dumais & Veenema, 2016) and in behavior after intranasal administration in humans (Feng et al., 2015; Rilling et al., 2014). Further study is needed to determine whether target regions of OT and AVP projections in humans may be different in females. Finally, due to the large size of the human brain, our study focused on selected regions of cortex and did not include the entirety of sizable regions such as the anterior cingulate gyrus. Future studies should investigate a greater number of areas, such as the fusiform gyrus, superior temporal sulcus, and right somatosensory cortex, which are involved in processing social information (Adolphs, 2001), as well as more posterior and dorsal regions of anterior cingulate gyrus in humans. Our results suggest that in primates, OT and AVP effects on cortical function and social cognition may be mediated by direct cortical projections from the hypothalamus, and the targets of these projections may be species-specific.

Acknowledgments

This research was supported by funding from The Leakey Foundation (#38217) to CR and The Templeton Foundation (#40463) to HEA, LJY, JKR, and TMP. Additional support was provided by NIH grants P50MH100023 to LJY and by NIH OD P51OD11132 to YNPRC.

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Chapter 4: Neuroanatomical distribution of oxytocin and vasopressin v1a receptors in chimpanzees

This chapter to be submitted as:

Rogers, C.N., Coppeto, D.J., Inoue, K., Rilling, J.K., Preuss, T.M., Young, L.J. Neuroanatomical distribution of oxytocin and vasopressin v1a receptors in chimpanzees (*Pan troglodytes*).

Abstract

Despite our close genetic relationship with chimpanzees, there are notable differences between chimpanzee and human social behavior. Two neuropeptides known to regulate social behavior across mammalian species are oxytocin and vasopressin. Yet little is known about the neuroanatomy of these systems in primates, and virtually nothing in great apes. Here, we used receptor autoradiography with a competitive binding protocol to localize oxytocin and vasopressin v1a receptors in seven chimpanzee brains. Oxytocin receptors were detected in the lateral septum, hypothalamus, medial amygdala, nucleus basalis, and substantia nigra. Vasopressin v1a receptors were observed in the cortex, lateral septum, hypothalamus, entire amygdala, dentate gyrus, and substantia nigra. These findings suggest evolutionary conservation in receptor distribution among other primate species in several areas, including those important for social visual attention, as well as in the interaction with other neuromodulatory systems such as dopamine and acetylcholine. They also suggest potential differences between humans and chimpanzees in neuropeptide receptor expression in the reward system as well as areas important for threat.

Introduction

Chimpanzees (*Pan troglodytes*), along with their sister species, bonobos (*P. paniscus*), are our closest living relatives (Prüfer et al., 2012). Accordingly, we share many features of social behavior, yet differ in key ways as well. Like humans, chimpanzees form strong social bonds, and will show cooperation even among non-kin, particularly in hunting or defense contexts (Langergraber, Mitani, & Vigilant, 2007; Mitani, 2009; Langergraber, Mitani, & Vigilant, 2009). However, humans often form pair-bonds, while chimpanzees mate promiscuously and opportunistically, though there may be some stable male-female relationships (Langergraber, Mitani, Watts, & Vigilant, 2013). Moreover, unlike humans, chimpanzees rarely engage in paternal care (Lehmann, Finkenscher, & Boesch, 2006). Finally, lethal aggression is common among chimpanzees, both within (Gilby et al., 2013; Pusey et al., 2008) and between groups (Wilson et al., 2014). Human groups also occasionally engage in lethal intergroup aggression, though there is great variability between societies based on subsistence mode, population density, economic defensibility of resources, and other factors (Dyson-Hudson & Smith, 1978; Fry & Soderberg, 2013; Kaplan, Hooper, & Gurven, 2009).

The neuropeptides oxytocin (OT) and vasopressin (AVP) may contribute to social-behavioral differences among primate species, including chimpanzees and humans. These neuropeptides are known to regulate social behavior across a wide range of mammalian species (Donaldson & Young, 2008). Produced by separate cells in the supraoptic and paraventricular nuclei of the hypothalamus (Dierickx & Vandesande, 1977), the two peptides each consist of nine amino acids, differing at only two positions in the sequence. OT and AVP are evolutionarily ancient; derived from a common ancestral peptide vasotocin, the two separate peptides resulted from a gene-duplication event early in the mammalian lineage (Knobloch & Grinevich, 2014).

OT and AVP have known physiological effects when released from the pituitary gland into the bloodstream: OT acts at mammary glands to facilitate milk ejection, stimulates uterine contraction during labor (Burbach, Young, & Russell, 2006), and is released during sexual intercourse, while AVP acts mainly to regulate the body's retention of water (Jaenike & Waterhouse, 1961; Knepper, Kwon, & Nielsen, 2015).

Both OT and AVP can also be secreted into the brain, where they can modulate social behavior. In rodents, OT has been associated with social recognition, pair-bonding, and maternal bonding (Donaldson & Young, 2008; Hammock & Young, 2006; Johnson & Young, 2017). OT is thought to modulate complex social behavior by enhancing the salience and reinforcing value of social stimuli (Young 2015, Walum & Young, 2018). OT is generally anxiolytic and has been found to facilitate trust, cooperation, and in-group altruism in humans (De Dreu, 2012; Israel, Weisel, Ebstein, & Bornstein, 2012; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011; Jureck & Neumann, 2018). AVP is often anxiogenic, enhancing vigilance, and has been associated with aggressive and territorial behaviors (Donaldson & Young, 2008; Ferris et al., 1997; Goodson, 2005), but also pair-bonding, paternal behavior, and mate guarding in males (Insel, Wang, & Ferris, 1994; Wang, Ferris, & De Vries, 1994; Nair & Young, 2006).

Evidence from voles demonstrates that species-specific receptor distributions for OT and AVP can contribute to species-typical social behaviors. In the monogamous prairie vole (*Microtus ochrogaster*), OT receptor (OXTR) density is high in several regions, including the prelimbic cortex, nucleus accumbens, and lateral amygdala, all of which show less or no binding for OXTR in the polygynous montane vole (*Microtus montanus*). By contrast, in the polygynous species, OXTR are localized to the lateral septum, ventromedial hypothalamus, and cortical

nucleus of the amygdala. AVP v1a receptors (AVPR1a) follow a species-specific distribution in these same species as well (Insel et al., 1994). Specifically, AVP receptors in the ventral pallidum and lateral septum of the male prairie vole, and OT receptors in the nucleus accumbens and medial prefrontal cortex of the female prairie vole, facilitate pair-bonding (Johnson & Young, 2015; Lim, Hammock, & Young, 2004; Young & Wang, 2004; Young, Lim, Gingrich, & Insel, 2001). Moreover, receptor antagonists in these areas block partner-preference formation (Lim et al., 2004; Liu, Curtis, & Wang, 2001; Young et al., 2001), while manipulating receptor gene expression has the predicted effect on social behavior (Barrett et al., 2013; Lim & Young, 2004; Keebaugh et al., 2015; Ross et al., 2009). Thus, the effects of OT and AVP on social behavior are highly dependent on the distribution of their receptors. Further individual variation in receptor densities contribute to individual variation in social behavior among prairie voles (Hammock and Young 2005, King et al, 2016).

There is reason to suspect that OT and AVP are involved in the regulation of social behavior in chimpanzees as well. Urinary measures of OT in chimpanzees have been associated with various aspects of behavior. OT may be involved in mediating cooperative relationships among both related and unrelated chimpanzees. In one study of wild chimpanzees, urinary OT levels were higher in strongly socially bonded dyads (whether kin or non-kin) after a grooming interaction as compared with dyads without a strong social bond (Crockford et al., 2013). Elevated urinary OT levels have also been observed in chimpanzees after a food sharing event in both the food donor and food receiver (Wittig et al., 2014). Food sharing provided an even higher increase in OT than grooming, though it did not vary based on strength of social bond. Urinary OT in chimpanzees has also been associated with both the anticipation of and cohesion during intergroup conflict (Samuni et al., 2017), reconciliation after conflict (Preis et al., 2018),

and coordinated hunting (Samuni et al., 2018). Thus OT may be acting in chimpanzees to strengthen and promote long-term social bonds as well as short-term social group, which may be a component of the coordination needed to defend against an outgroup.

While to our knowledge there are no studies linking urinary AVP to chimpanzee behavior, some have explored variation in the V1a receptor gene (*AVPR1a)*. In contrast to humans and bonobos, chimpanzees are polymorphic for a deletion of 360 base pairs in the promoter region of *AVPR1a* (+/- DupB), which contains a microsatellite RS3 (Donaldson et al, 2008). In humans, a longer form of the RS3 microsatellite is associated with greater prosocial behavior (Knafo et al., 2008) and increased amygdala activation in face recognition (A. Meyer-Lindenberg et al., 2009). The deletion of this DupB region in chimpanzees is associated with reduced sociality, particularly in males (Staes et al., 2014), as well as lower dominance and higher conscientiousness (Hopkins, Donaldson, & Young, 2012), and reduced performance on a task of joint attention (Hopkins et al., 2014) as well as mirror self-recognition (Mahovetz, Young, & Hopkins, 2016).

Given the evidence that OT and AVP are related to chimpanzee social behavior, a crucial step is understanding where in the brain these neuropeptides act. A handful of studies have investigated the neuroanatomical distribution of OXTR and AVPR1a in a small number of primate species, revealing some unifying features as well as species differences. Rhesus macaques (*Macaca mulatta*), a sexually promiscuous and rigidly hierarchical species of catarrhine monkey, show no binding for either OXTR or AVPR1a in the basal ganglia. Interestingly, this brain region encompasses the reward system pathways implicated in prairie vole pair-bonding. Rhesus macaques show dense binding for AVPR1a in the BNST, and the medial and central amygdala. They also have significant binding for both AVPR1a in the cortex

(Freeman, Inoue, Smith, Goodman, & Young, 2014; Young, Toloczko, & Insel, 1999). The distribution of OXTR and AVPR1a has also been investigated in two platyrrhine species. The monogamous, biparental coppery titi monkey (*Callicebus cupreus*) shows widespread AVPR1a receptor binding within the basal ganglia, AVPR1a in the central amygdala, and OXTR in the hippocampus (Freeman et al., 2014). Finally, cooperatively-breeding marmosets (*Callithrix jacchus*) have a significant presence of OXTR and AVPR1a in the basal ganglia, dense AVPR1a in the medial amygdala and bed nucleus of the stria terminalis (BNST), and moderate OXTR in layer 6 of the cerebral cortex (Schorscher-Petcu, Dupré, & Tribollet, 2009; Wang et al., 1997)

A few studies have explored the distribution of OXTR and AVPR1a in humans. Receptor autoradiography has revealed OXTR binding in regions including the substantia nigra pars compacta, nucleus basalis of Meynert, ventral lateral septum, BNST, ventral pallidum, and globus pallidus (Loup et al., 1991; Freeman et al., 2018). Moreover, transcriptomics analysis has revealed OXTR expression in the nucleus accumbens (Bethlehem et al., 2017). AVPR1a binding has been observed in humans in the lateral septum, midline and intralaminar nuclei of the thalamus, the dentate gyrus, and the dorsolateral basal amygdaloid nucleus, among other regions (Loup et al., 1991). Interestingly, AVPR1a binding is more limited in humans compared with OXTR binding, while the reverse has been found in rhesus macaques and titi monkeys (Freeman et al., 2014a; Freeman et al., 2014b).

No studies have described the neuroanatomical distribution of chimpanzee OXTR or AVPR1a. Here, we characterize the distribution of OXTR and AVPR1a in chimpanzee brains in a set of regions spanning from the rostral part of the basal ganglia through the posterior hippocampus and midbrain. We had several a priori predictions regarding receptor distribution in chimpanzee brain. First, given that we have detected AVP immunoreactive fibers in the

agranular insula and OT immunoreactive fibers in the straight gyrus (Rogers et al., 2018), we expected to find AVPR1a and OXTR binding in cortical regions as well. We also hypothesized that in contrast to humans, chimpanzees would not have dense OXTR or AVPR1a in reward areas such as the nucleus accumbens and ventral pallidum, due to their lack of pair-bonding behavior. Finally, we would expect OXTR or AVPR1a in the nucleus basalis of Meynert in chimpanzees in accordance with previous primate research.

Methods

Specimens

Blocks of fresh brain tissue from 7 chimpanzees were recovered at necropsy and flash frozen opportunistically after natural death. Blocks were sectioned on a cryostat into slides at 20 μm thickness and stored in a freezer at -80 degrees Celsius. See Table 4.1 for a list of specimens by age and sex.

Table 4.1. Specimens.

Species	**Age**	**Sex**
Pan troglodytes	21	F
Pan troglodytes	23	F
Pan troglodytes	34	F
Pan troglodytes	35	F
Pan troglodytes	47	F
Pan troglodytes	57.7	F
Pan troglodytes	43	M

Receptor autoradiography

For receptor autoradiography, slides were thawed in racks at room temperature for 1 hour and then placed in a vacuum desiccator for 1 hour. Once thawed, slides were dipped in 0.1% paraformaldehyde in 7.4 pH phosphate buffered saline (PBS) and rinsed in 50 mM Tris buffer to remove endogenous ligand. To reveal OXTR binding, adjacent sections were placed in one of three conditions, adapted from Freeman et al. (2014): 1) 50 nM ^{125}I-ornithine vasotocin analog (^{125}I-OVTA) alone, 2) 50 nM ^{125}I-OVTA plus 1 nM unlabeled AVPR1a-selective compound SR49059 (Tocris), or 3) ^{125}I-OVTA plus 1 nM SR49059 plus 50 nM unlabeled OXTR-selective T4G7. To reveal AVPR1a binding, adjacent sections were placed in one of two conditions, following Young et al. (1999): 1) 50 nM nM ^{125}I-v1a antagonist (Phenylacetyl1, 0-Me-D-Tyr2,

[^{125}I-Arg6]-) (Perkin Elmer), or 2) 50 nM ^{125}I-v1a antagonist plus 50 nM unlabeled [1-(*β*-mercapto-*β*, *β*-cyclo-pentamethylene propionic acid), 2-(O-methyl)tyrosine]-arg8- vasopressin (Manning compound). After ligand incubation, sections were washed in 50 mM Tris buffer plus 2% MgCl2, pH 7.4 to remove excess radioligand, and then dipped in deionized water and air-dried. Slides were then exposed to BioMax MR film for 14 days (Kodak, Rochester, NY). Images were obtained from the films using a light box and adjusted for equal brightness and contrast using Adobe Photoshop (San Jose, CA).

Acetylcholinesterase

After receptor autoradiography, sections previously used for receptor autoradiography were stained for acetylcholinesterase to provide an anatomical guide (Lim et al., 2004). Slides were placed in a solution of ethopropazine, glycine, cupric sulfate, acetylthiocholine iodide, and sodium acetate for 6 hours. Next, they were rinsed in deionized water and then placed in sodium sulfide solution for 30 minutes. Sections were rinsed in water again and then exposed to silver nitrate for intensification. Slides were air dried overnight and coverslipped.

Results

Selectivity of Ligands

To ensure selectivity of our binding, we incubated sections in multiple conditions - either with ligand alone or with some combination of unlabeled competitor. For OXTR, the selective AVPR1a compond, SR49059 did not affect binding density, while T4G7 drastically reduced binding, indicating that ^{125}I-OVTA was indeed binding to OXTR and not AVPR1a (Figure 4.1). For AVPR1a, the Manning compound moderately reduced binding (Figure 4.2).

Figure 4.1. ^{125}I-OVTA binding with and without competitors. a) Acetylcholinesterase stain for anatomical guide. b) ^{125}I-OVTA alone produces binding in the LS and MPOA. c) ^{125}I-OVTA plus SR49059, an unlabeled vasopressin antagonist, produces binding in the LS and MPOA. d) ^{125}I-OVTA plus SR49059 plus unlabeled oxytocin antagonist drastically reduces binding. LS = lateral septum, MPOA = medial preoptic area

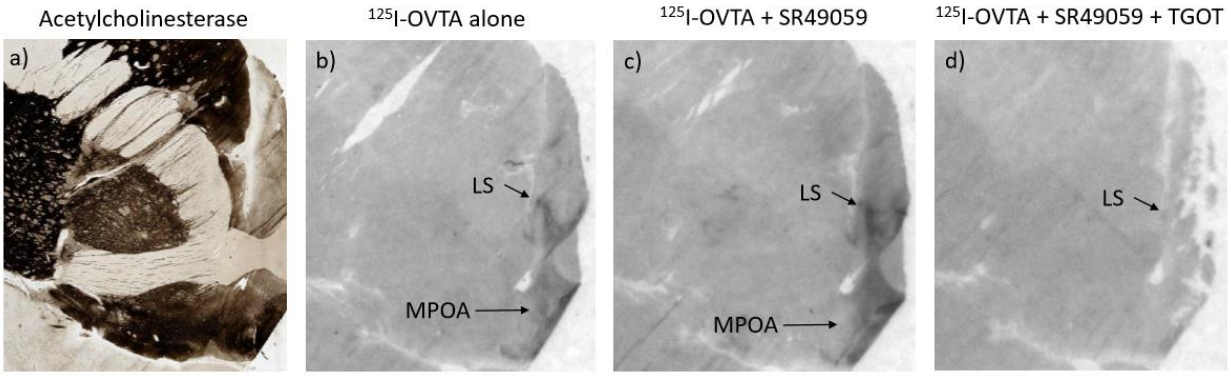
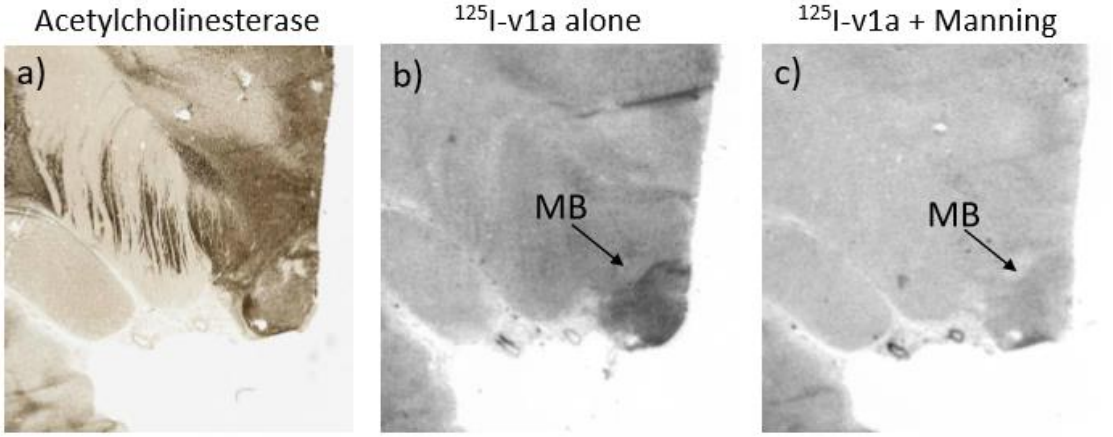


Figure 4.2. ^{125}I-V-1a binding with and without competitors. a) Acetylcholinesterase stain for anatomical guide. b) ^{125}I-V-1a alone produces dense binding in the MB and moderate binding in the amygdala. c) ^{125}I-V-1a plus the Manning compound reduces binding in the MB and amygdala. MB = mammillary body.



OXTR distribution

In chimpanzees, OXTR distribution was relatively limited when compared with AVPRR1a. Dense OXTR was observed in the ventral lateral septum (Figure 4.3) as well as the diagonal band of Broca. OXTR also showed dense binding in several regions of the hypothalamus, including the medial preoptic area (Figure 4.3), supraoptic nucleus, and paraventricular nucleus. In the midbrain, dense OXTR was observed in the substantia nigra (Figure 4.4). Moderate OXTR binding was found in the zona incerta, nucleus basalis of Meynert, and medial amygdala (Figure 4.5), while very light binding was observed in the cortex and dentate gyrus (Figure 4.6).

AVPR1a distribution

Dense AVPR1a binding was found in the cortex, particularly the temporal and insular cortex. AVPR1a binding was also observed in the frontal cortex in Brodmann's area 8. As with OXTR, dense AVPR1a binding was observed in the ventral lateral septum (Figure 4.3). Dense AVPR1a binding was also found in the dorsal lateral septum (Figure 4.3). Moderate AVPR1a binding was found in the medial preoptic area, supraoptic nucleus, and paraventricular nucleus as well as the substantia nigra (Figure 4.4). Moderate AVPR1a binding was also found in all amygdalar nuclei (Figure 4.5), whereas OXTR was only found in the medial amygdaloid nucleus. Finally, dense AVPR1a binding was observed in the dentate gyrus of the hippocampus (Figure 4.6).

Figure 4.3. OXTR and AVPR1a in the lateral septum (LS). a) Acetylcholinesterase stain for anatomical guide. b) OXTR in the ventral lateral septum and medial preoptic area (MPOA). c) Dense AVPR1a in the dorsal and ventral lateral septum.

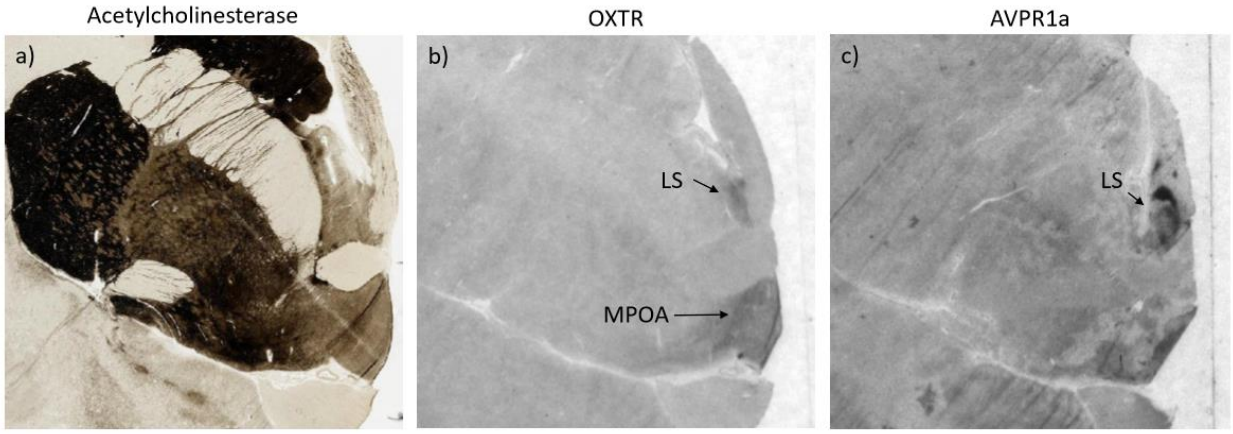


Figure 4.4. OXTR and AVPR1a in the substantia nigra (SNi). a) Acetylcholinesterase stain for anatomical guide. b) OXTR in the substantia nigra pars compacta. c) AVPR1a in the substantia nigra pars compacta and pars reticulata.

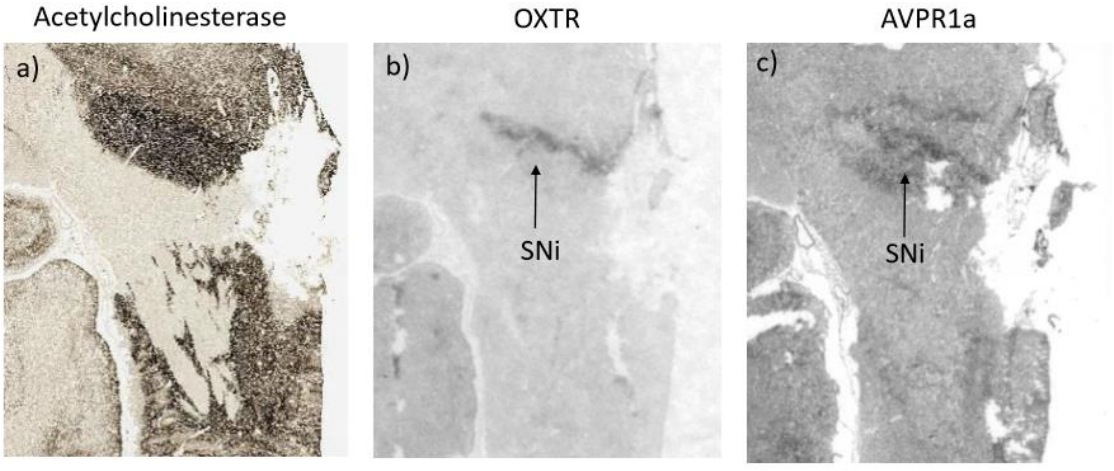


Figure 4.5. OXTR and AVPR1a in the amygdala. a) Acetylcholinesterase stain for anatomical guide. b) OXTR in the medial amygdala. c) AVPR1a in all nuclei of the amygdala, as well as entorhinal cortex. MeA = medial amygdala, Amyg = amygdala, Ent. Ctx = entorhinal cortex

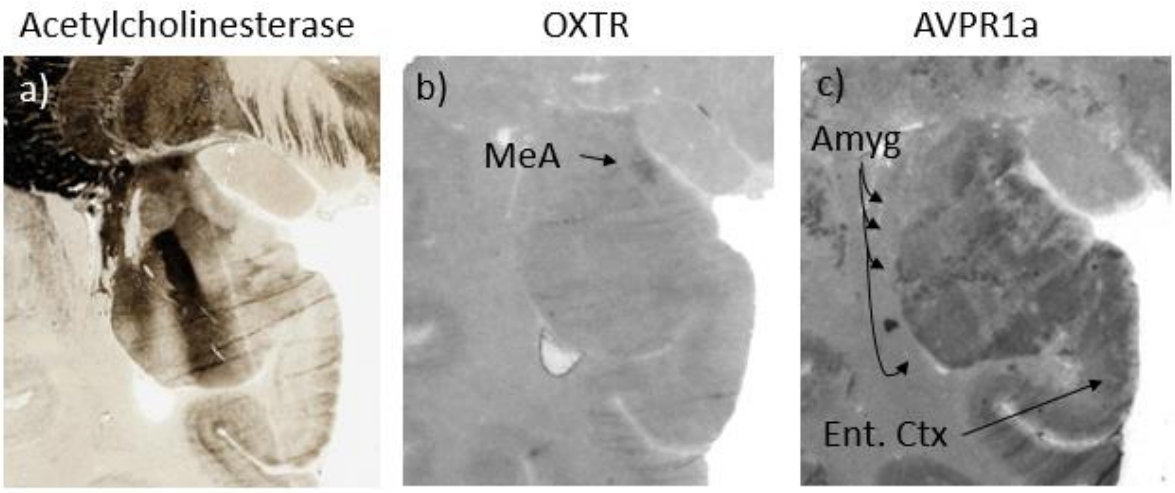
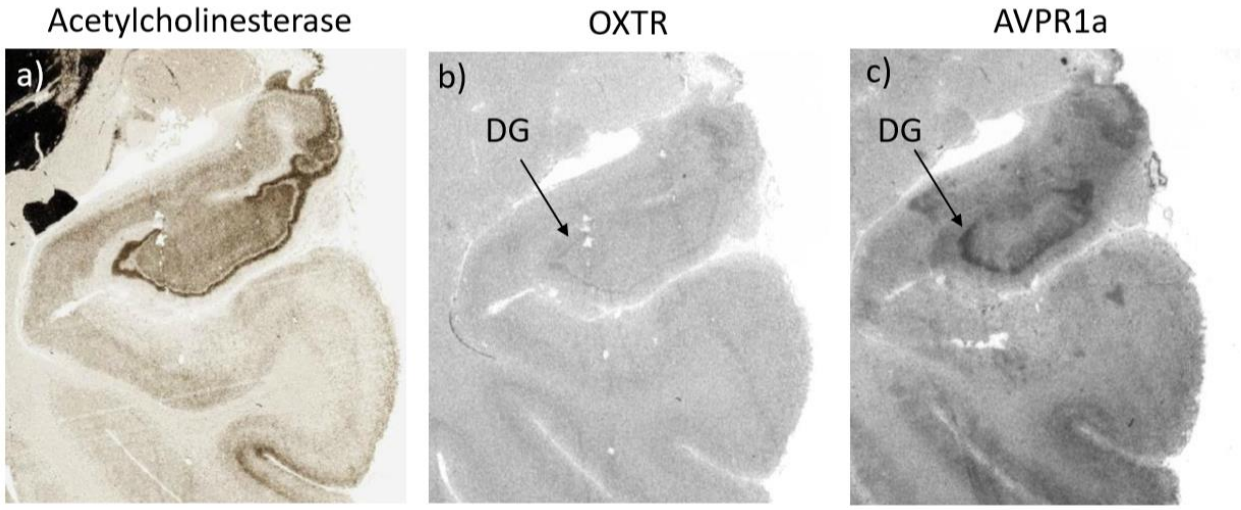


Figure 4.6. OXTR and AVPR1a in the dentate gyrus (DG). a) Acetylcholinesterase stain for anatomical guide. b) Light OXTR in the dentate gyrus. c) Dense AVPR1a in the dentate gyrus.



Discussion

In the present study, we characterized the distribution of OXTR and AVPR1a in seven chimpanzee brains. We used a competitive binding procedure to characterize the distribution of OXTR and AVPR1a in these seven chimpanzees. For oxytocin, sections were either incubated in the oxytocin ligand I-125 OVTA alone, the oxytocin ligand plus an AVPR1a antagonist, or the oxytocin ligand plus both an AVPR1a and an OXTR antagonist. In comparison to the OVTA alone condition, the OVTA plus AVPR1a antagonist did not result in a reduction of binding. However, the OVTA ligand plus both antagonists lowered all binding. This may suggest that the issue of ligands promiscuously binding to both the OXTR and AVPR1a receptor may not be as much of an issue in chimpanzees (or possibly great apes as a whole) as has been demonstrated in rhesus macaques.

We hypothesized that chimpanzees would fall in line with the evolutionary primate trend of having OXTR or AVPR1a in areas important for orienting visual attention to social stimuli. Consistent with this, we found moderate OXTR binding in the nucleus basalis of Meynert, an area important for visual attention, which has been observed in all primate species examined for OXTR distribution. We also hypothesized that chimpanzees would have a lower density of OXTR and/or AVPR1a in the reward system as compared with what has been reported in humans. Two studies have provided evidence for dense OXTR in the human ventral pallidum (Loup et al., 1991; Freeman et al., 2018). We did not observe OXTR in the ventral pallidum of chimpanzees. We hypothesized this difference could be due to greater pair-bonding behavior in humans as compared with chimpanzees. As discussed above, in the monogamous titi monkey, AVPR1a is found in the ventral pallidum, whereas it is absent in this region in non-monogamous rhesus macaques. Moreover, OXTR is found here in monogamous marmosets (Schorscher-Petcu

et al., 2009). If OXTR in the ventral pallidum is indeed related to pair-bonding in apes, we would expect to see this in gibbons, which form strong pair bonds and have low sexual dimorphism (Palombit, 1994). To our knowledge, no research has been done on the OT and AVP systems in gibbons. Additionally, in humans, OXTR levels in the ventral pallidum are lower in individuals with autism (Freeman et al., 2018), who may experience difficulties in social attachment (Dölen, 2015).

Previous research has revealed that immunoreactive cells that produce OT and AVP can send long-range axonal fibers from the hypothalamus into the cortex in primates (Rogers et al., 2018). AVP-immunoreactive fibers have been found in the cingulate cortex, insula, and olfactory cortex in chimpanzees. OT-immunoreactive fibers have been found in the straight gyrus and cingulate cortex in chimpanzees. Accordingly, we found widespread AVPR1a binding in the cortex, including the cingulate cortex, insula, and olfactory cortex, as well as entorhinal cortex, cingulate cortex, and frontal cortex (specifically Brodmann's area 8). We did not have sections rostral enough to examine the straight gyrus for OXTR in chimpanzees. The presence of AVPR1a in these regions provides further evidence that cortical areas important for many "higher" aspects of social cognition such as empathy (e.g., insula and anterior cingulate cortex) are sensitive to OT and AVP in great apes. Previous studies (Loup et al., 1991) did not characterize OXTR or AVPR1a distribution in the cortex of humans. Because OT- and AVP-immunoreactive fibers were found in the same cortical areas in humans, with AVP fibers additionally in the dysgranular insula in humans, future research can determine if these cortical areas have a similar pattern of OXTR and AVPR1a in humans as in chimpanzees.

AVPR1a and OXTR binding were also observed in the amygdala in chimpanzees. This was much more widespread for AVPR1a than OXTR. AVPR1a binding was found across the

entire amygdala in chimpanzees, whereas OXTR was highly restricted to the medial amygdala, at the border with the posterior cortical nucleus. Evidence from receptor autoradiography suggests there is AVPR1a binding in the basal nucleus of the amygdala and no OXTR binding in the amygdala of humans (Loup et al., 1991). However, another study (Boccia et al., 2013) did report OXTR in the cortical nucleus of humans using immunohistochemistry. Few resources exist for delineating the border between the medial and cortical nucleus in chimpanzees (or any great ape). Further research is needed to determine if there are true differences in neuropeptide receptor distribution in the amygdala between humans and chimpanzees. Moreover, it would be useful to compare chimpanzee and bonobo receptor distribution in amygdalae, given the fact that bonobos are closely related to chimpanzees but much lower in aggression and higher in tolerance of strangers (Hare et al., 2012).

Importantly, this study did not examine all brain regions, but rather a selection from the most anterior part of the basal ganglia through the substantia nigra. Future research is needed to determine the extent of OXTR and AVPR1a binding in the brainstem of chimpanzees. All other primate species examined (humans, titi monkeys, marmosets, rhesus macaques) have OXTR in the spinal trigeminal nucleus and superior colliculus (Freeman et al., 2016; Freeman et al., 2014; Freeman et al., 2014; Schorscher-Petcu et al., 2009). We would expect chimpanzees to have OXTR here as well. Additionally, humans have AVPR1a binding in the nucleus prepositus of the brainstem, important for eye gaze stabilization (Freeman et al., 2016). Given that humans are especially reliant on eye gaze as social cues (Tomasello et al., 2007), it would be interesting to determine whether chimpanzees and humans differ in the distribution of AVPR1a in this region. Finally, six of the seven chimpanzee brains examined for OXTR and AVPR1a in this study were female. While we did not observe any obvious differences in the one male chimpanzee, a more

systematic approach to sampling by sex is needed to make any definitive conclusions. Overall, our findings suggest chimpanzees have similarities to other primates in the distribution of OXTR and AVPR1a, but may have differences with humans in areas relevant to reward and threat that may contribute to divergent social behavior.

Acknowledgements

This research was supported by funding from The Leakey Foundation (#38217) to CR and NIH grants 1P50MH100023 to LJY and P51OD11132 to YNPRC.

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Chapter 5: Discussion

Introduction

Long before the theory of evolution was put forward by Charles Darwin and Alfred Russel Wallace, scientists and philosophers have appreciated the incredible variation in social behavior among the animal kingdom. In his 350 B.C. work, *History of Animals*, Aristotle noted, "[T]he following differences are manifest in [animals'] modes of living and in their actions. Some are gregarious, some are solitary, whether they be furnished with feet or wings or be fitted for a life in the water; and some partake of both characters, the solitary and the gregarious. And of the gregarious, some are disposed to combine for social purposes, others to live each for its own self." He would not have suspected these behaviors could be linked to characteristics of the brain. Aristotle believed the heart was the seat of thought, reason, and emotion; notably, this was a departure from his mentor Plato, who did suspect these processes arose from the brain.

Two millenia later, we are well-positioned to understand both why and how the variation Aristotle noted may have arisen. With the explosion of new technologies in the 20th and 21st centuries, we can ask fine-tuned questions about the mechanisms underlying animal behavior. Within mammals, this is especially true for rodent species which are not endangered, have relatively short lifespans, and can be bred in laboratory settings. Thus much of our understanding of animal neuroscience and behavior is really an understanding of rats and mice, and sometimes hamsters and voles. Nevertheless, the advent of non-invasive technologies for brain research such as neuroimaging and the refinement of post-mortem histological techniques has allowed for progress in the understanding of human and non-human primate neurobiology.

A promising area of research incorporating both human and animal studies and sitting at the crux of biological anthropology and social neuroscience is the study of the oxytocin (OT) and

vasopressin (AVP) neuropeptide systems. The work presented in this dissertation joins a growing literature extending the detailed understanding of these neuropeptides from rodents to primates. I examined the distribution of immunoreactive cell bodies and fibers containing oxytocin and vasopressin in rhesus macaques, chimpanzees, bonobos, and humans. This had not been done before in great apes outside of the hypothalamus, and only in a couple other primate species in such detail. I also characterized the distribution of oxytocin and vasopressin v1a receptors in chimpanzees, which has not been done before in any great ape. The findings of this dissertation work confirm some similarities uniting the neuroanatomy of the OT and AVP systems across primates, but also indicate differences that may be related to species-specific behaviors.

Neuroanatomy of the OT and AVP Systems across Primates Species

Chapter 2 of this dissertation mapped the distribution of OT- and AVP- immunoreactive cell bodies and fibers in rhesus macaques, chimpanzees, bonobos, and humans. Table 5.1 (OT) and Table 5.2 (AVP) add these findings to what is known in all primate species studied so far, including common marmosets, long-tailed macaques (Caffé et al., 1989), Japanese macaques (Kawata and Sano 1982), and squirrel monkeys (Sofroniew et al., 1981). Chapter 4 characterized the distribution of OXTR and AVPR1a in chimpanzees. Table 5.3 and Table 5.4 compare these findings to those from marmosets (Schorscher-Petcu, Dupré, & Tribollet, 2009), rhesus macaques (Young, Toloczko, & Insel, 1999; Freeman, Inoue, Smith, Goodman, & Young, 2014), titi monkeys (Freeman et al., 2014), and humans (Loup, Tribollet, Dubois-Douphin, & Dreifuss, 1991; Loup, Tribollet, Doubois-Dauphin, Pizzolato, & Dreifuss, 1989).

Cells and Fibers in All Species

Table 5.1. Immunoreactive OT-containing cells and fibers by primate species and brain region. An asterisk (*) indicates fibers, while a plus sign (+) indicates cell bodies. ND indicates "Not Described". For the original research (rhesus macaques, chimpanzees, bonobos, humans) indications of density were left in; however, this was not always reported in the other studies or may have been judged differently.

OXYTOCIN								
	Common marmosets	*Squirrel monkeys*	*Japanese macaques*	*Long-tailed macaques*	*Rhesus macaques*	*Chimpan-zees*	*Bonobos*	*Humans*
EXTENDED AMYGDALA								
Central Amygdala		*		*	**	**	*	*
Medial Amygdala	+*			*	**	**	*	*
Cortical Amygdala	*			*	***	*	*	*
Basolateral Amygdala	ND	ND	ND	ND	**	**	*	*
Accessory Basal Amygdala	ND	ND	ND	ND	*	*	*	*
Lateral Amygdala	ND	ND	ND	ND				
Hippocampus-Amygdala Transition Area	ND	ND	ND	ND		**		
Bed Nucleus of Stria Terminalis				+	++**	++**	++**	++**
Nucleus Basalis of Meynert	ND	ND	ND	ND	*	*	*	*
BASAL GANGLIA								
Putamen						*		
Nucleus accumbens					*	*		*
Internal globus pallidus			+*	*	++**	+**	+**	+**
SEPTUM								
Lateral septum					**	**	*	*
Diagonal Band of Broca				*	*	*	*	*
HYPOTHALAMUS								
PVN	+*	+*	+*	+*	+++***	+++***	+++***	+++***
SON	+*	+*	+*	+*	+++***	+++***	+++***	+++***
SCN								
Accessory Nuclei	+*	+*	+*	+*	+***	+***	+***	+***
AH	*				*	*	*	*

LH	+			+	*	*	*	*
MPOA				*	*	*	*	*
Posterior hypothalamic area					*	*	*	*
HIPPOCAMPUS					*	*		
THALAMUS and EPITHALAMUS								
Paraventricular thalamic nucleus	ND	ND	ND	ND	*	*	*	*
Rhomboid thalamus	ND	ND	ND	ND	*	*	*	*
Lateral habenula				*	*	*	*	*
MIDBRAIN and BRAINSTEM								
Substantia nigra					**	**	*	*
CORTEX								
Straight gyrus	ND	ND	ND	ND	ND	*	*	*
Agranular insula	ND	ND	ND	ND	ND		*	
Dysgranular insula	ND	ND	ND	ND	ND			
Cingulate cortex						*	*	*
Visual cortex	ND	ND	ND	ND	ND	ND	ND	ND
Piriform cortex								

Table 5.2. Immunoreactive AVP-containing cells and fibers by primate species and brain region. An asterisk (*) indicates fibers, while a plus sign (+) indicates cell bodies. ND indicates "Not Described". For the original research from Chapter 2 of this dissertation (rhesus macaques, chimpanzees, bonobos, humans) indications of density were left in; however, this was not always reported in the other studies or may have been judged differently.

VASOPRESSIN								
	Common marmosets	*Squirrel monkeys*	*Japanese macaques*	*Long-tailed macaques*	*Rhesus macaques*	*Chimpan-zees*	*Bonobos*	*Humans*
EXTENDED AMYGDALA								
Central Amygdala		*		*	**	**	*	*
Medial Amygdala		*		+*	**	**	*	*
Cortical Amygdala				*	***	*	*	*
Basolateral Amygdala	ND	ND	ND	ND	**	**	*	*
Accessory Basal Amygdala	ND	ND	ND	ND	*	*	*	*
Lateral Amygdala	ND	ND	ND	ND				
Hippocampus-Amygdala Transition Area	ND	ND	ND	ND		**		
Bed Nucleus of Stria Terminalis	+*			+*	++**	++**	++**	++**
Nucleus Basalis of Meynert	ND	ND	ND	ND	*	*	*	*
BASAL GANGLIA								
Putamen						*		
Nucleus accumbens	*				*	*		*
Internal globus pallidus			+*	+	++**	+**	+**	+**
SEPTUM								
Lateral septum	*	*			**	**	*	*
Diagonal Band of Broca	*			*	*	*	*	*
HYPOTHALAMUS								
PVN	+*	+*	+*	+*	+++***	+++***	+++***	+++***
SON	+*	+*	+*	+*	+++***	+++***	+++***	+++***
SCN	+	+*	+*	+*	+++***	+++***	+++***	+++***
Accessory Nuclei		+*	+*	+*	+***	+***	+***	+***
AH					*	*	*	*
LH	+			+	*	*	*	*
MPOA				*	*	*	*	*

Posterior hypothalamic area					*	*	*	*
HIPPOCAMPUS		*		*	*	*		
THALAMUS and EPITHALAMUS								
Paraventricular thalamic nucleus	ND	ND	ND	ND	*	*	*	*
Rhomboid thalamus	ND	ND	ND	ND	*	*	*	*
Lateral habenula		*		*	*	ND		ND
MIDBRAIN and BRAINSTEM								
Substantia nigra				*	**	**	*	*
CORTEX								
Straight gyrus	ND	ND	ND	ND		*		*
Agranular insula	ND	ND	ND	ND		*		*
Dysgranular insula	ND	ND	ND	ND				*
Cingulate cortex			*	*	*	*	*	*
Visual cortex	ND	ND	ND	ND	ND	ND	ND	ND
Piriform cortex			*		*	*		*

Cells and fibers: commonalities and divergence

Tables 5.1 and 5.2 present an overview of OT and AVP cell bodies and fibers in the primate species that have been studied so far, including rhesus macaques, chimpanzees, bonobos, and humans from chapter 2 of this dissertation. Importantly, data from the marmoset, long-tailed macaque, Japanese macaque, and squirrel monkey were all collected from different research groups, and the reports are inconsistent in their indications of whether a particular brain region was studied and found to have zero cells/fibers, or whether it was not included in the sampling.

The clearest consistency in the OT and AVP systems across primate species is in the hypothalamus. In all species, OT- and AVP-immunoreactive cells were found in the paraventricular and supraoptic nuclei, as well as accessory nuclei. AVP-ir cells were also observed in the suprachiasmatic nucleus. This is in accordance with the extensive rodent literature, as well as other mammals.

In chimpanzees, bonobos, humans, and rhesus macaques, AVP-ir and OT-ir fibers were found in most nuclei of the amygdala, though differences appeared in density. Across all four species, the lateral amygdala had virtually no AVP/OT fibers. In the cortical amygdala, rhesus macaques had very dense accumulations of both AVP-ir and OT-ir fibers, while the fibers in this region were sparse in chimpanzees, bonobos, and humans. In other monkey species, evidence for fibers here is inconsistent; only the long-tailed macaque and marmoset (OT only) are reported to have them. However, the border between the medial amygdala and cortical amygdala is not always obvious, and different research teams may have interpreted it differently.

The bed nucleus of the stria terminalis, important in anxiety and long-term fear states (Lebow & Chen, 2016), had both AVP and OT cells and fibers in rhesus macaques, humans, chimpanzees, and bonobos. Again, evidence for these elements in other primates is mixed. The position of OT-ir and AVP-ir BNST cells in relation to the anterior commissure is different in rodents compared to macaques, so it is again possible that the neuroanatomy was interpreted differently by different researchers (Fox et al., 2015).

Previous reports in primates did not report of OT-ir and AVP-ir fibers in the thalamus. In contrast, we found both in midline thalamic nuclei, particularly the paraventricular nucleus of the thalamus, in all four species. The paraventricular nucleus projects to the extended amygdala (Li & Kirouac, 2008) and nucleus accumbens (Cheng et al., 2018) and is thought to connect brainstem and hypothalamic signals representing internal states with limbic forebrain regions. Moreover, it may regulate fear processing in the central amygdala (Penzo et al., 2015). The fibers in the thalamus of humans, chimpanzees, bonobos, and rhesus macaques were moderately sparse and may have been missed in previous analyses on other species.

Receptors in all species

Table 5.3. OXTR by primate species and brain region. "ND" indicates that a region was not described in the study.

OXYTOCIN RECEPTORS					
	Marmosets	*Titi monkeys*	*Rhesus macaques*	*Humans*	*Chimpanzees*
EXTENDED AMYGDALA					
Central amygdala					
Medial amygdala					**
Cortical amygdala					
Basolateral amygdala					
Accessory basal amygdala					
Lateral amygdala					
Hippocampus-amygdala transition area					
Bed nucleus of stria terminalis					
Nucleus basalis of Meynert		**	*	**	
BASAL GANGLIA					
Caudate	**				
Putamen	**				
Nucleus accumbens	***				
Globus pallidus				*	
Ventral pallidum				*	
Major island of Calleja				*	
SEPTUM					
Lateral septum		**		**	**
Diagonal band of Broca	**			*	**
HYPOTHALAMUS					
PVN					*
SON					*
MPOA				*	*
HIPPOCAMPUS					
Dentate gyrus		***			
CA1		***			
CA2					
Presubiculum		***			
Subiculum					

THALAMUS					
Paraventricular thalamic nucleus				*	
Rhomboid thalamic nucleus					
MIDBRAIN and BRAINSTEM					
Substantia nigra				**	**
Superior colliculus	**	**	ND	ND	ND
CORTEX					
Cingulate cortex					
Insula					
Visual cortex		**			ND
Olfactory cortex					
Entorhinal cortex					

Table 5.4. AVPR1a by primate species and brain region. "ND" indicates that a region was not described in the study.

VASOPRESSIN v1a RECEPTORS					
	Marmosets	*Titi monkeys*	*Rhesus macaques*	*Humans*	*Chimpanzees*
EXTENDED AMYGDALA					
Central Amygdala		***	***		*
Medial Amygdala	***		**		*
Cortical Amygdala					*
Basal Amygdala				*	*
Accessory Basal Amygdala					*
Lateral Amygdala					*
Bed Nucleus of Stria Terminalis	***		***	*	
Nucleus Basalis of Meynert	**				
BASAL GANGLIA					
Caudate		**			
Putamen		**			
Nucleus Accumbens	**	**			
Globus Pallidus	**	**			
Ventral Pallidum		*			
Islands of Calleja	***			*	
SEPTUM					
Lateral Septum	**		**	*	**
Diagonal Band of Broca	**		*		
HYPOTHALAMUS					
PVN			**		*
SON					*
MPOA	**		*		*
HIPPOCAMPUS					
Dentate Gyrus		*		*	**
CA1					
CA2					
Presubiculum		*	***		
Subiculum					
THALAMUS					
Paraventricular thalamic nucleus				*	*
Rhomboid thalamic nucleus				*	*

MIDBRAIN and BRAINSTEM					
Substantia nigra		**		*	*
Superior colliculus		**			ND
CORTEX					
Cingulate cortex	**	***	***	ND	*
Insula		**	**	ND	*
Visual cortex		***	ND	ND	ND
Olfactory cortex		***	***		*
Entorhinal cortex		ND	***		*
Prefrontal cortex	**	ND	***		*

Receptors: commonalities and divergence

In examining the distribution of OXTR and AVPR1a across all species that have been studied, certain common themes as well as species differences emerge. Overall, the distribution of receptors is much more species-variable than the distribution of OT- and AVP-producing cells, which mirrors the evidence from rodent species (Albers, 2014). All monkey species studied so far have AVPR1a at least somewhere in the cortex (interestingly, in marmosets, AVPR1a is expressed in prefrontal cortex after fatherhood). Across the monkey species studied, AVPR1a is generally be more widespread than OXTR, which is more restricted. This was true for chimpanzees as well. However, this pattern may be reversed in humans, or they may be similarly restricted (Loup et al., 1991)

All primate species, including chimpanzees in the current study, have OXTR in the nucleus basalis of Meynert, which may contribute to orienting gaze to social stimuli (Freeman & Young, 2016). Primates also tend to have OXTR in the superior colliculus, also related to visual attention. This region was not included in our chimpanzee tissue samples; future research with the available tissue will reveal whether other primates have this feature.

In the basal ganglia, only pair-bonding species (marmosets and titi monkeys) had OXTR and/or AVPR1a in the nucleus accumbens, consistent with differences in monogamous and non-monogamous vole species (Walum & Young, 2018). Accordingly, chimpanzees, which do not form pair bonds, did not have either of these receptors in the nucleus accumbens. Transcriptomics evidence suggests OXTR are expressed in humans (Bethlehem et al., 2017). The ventral pallidum is another brain region involved in pair-bonding in rodents (Walum & Young, 2018). OXTR are expressed in the ventral pallidum in humans as well (Freeman et al., 2018) but not in chimpanzees. The expression of OXTR and AVPR1a in certain regions of the dopamine reward system in pair-bonding rodents versus not pair-bonding rodents may be a pattern that extends not only to monkeys, but also apes.

Humans and chimpanzees both displayed OXTR and AVPR1a binding in other regions important for reward-related dopamine signaling: the lateral septum and subsstantia nigra pars compacta. The lateral septum is thought to play a role in reward and reinforcement learning (Olds & Milner, 1954; Cooper, Black, & Paolino, 1971), while the substantia nigra pars compacta provides the striatum with dopamine (Faull & Laverty, 1969). While the lateral septum is involved in pair-bonding in male prairie voles (Liu, Curtis, & Wang, 2001), dense AVPR1a binding in the lateral septum is also observed in non-monogamous voles (Insel & Shapiro, 1992). OXTR and AVPR1a in this region have been connected to social recognition and memory (Everts & Koolhaas, 1997; Lukas, Toth, Veenema, & Neumann, 2013). Humans and chimpanzees are both very social species, and the lateral septum and substantia nigra may be regions where OT and AVP can interact with the dopamine system to promote social reward in general. In contrast, OT and AVP in the ventral striatum may specifically enhance the signal-to-

noise ratio for a mating partner to promote pair-bonding (Johnson, Walum, Xiao, Riefkohl, & Young, 2018).

Expression of neuropeptide receptors was inconsistent in primate species in the hypothalamus, hippocampus, and amygdala. These observations do not lend themselves to any obvious behavioral interpretation. All primate species had AVPR1a in at least one nucleus of the amygdala, while chimpanzees were the only species to express OXTR in the amygdala, specifically the medial nucleus. In the central nucleus of the amygdala in rats, OT and AVP have known anxiolytic and anxiogenic effects, respectively (Huber, Veinante, & Stoop, 2005; Knobloch et al., 2012). If OT and AVP exert similar effects in primates, it will be important to determine whether this is supported by the same neuroanatomical mechanism. If so, either there are receptors present in this nucleus that are not detectable by receptor autoradiography, or a very low density of receptors is sufficient to produce these behavioral effects. If not, then perhaps OT and AVP may exert divergent effects on anxiety by acting on other regions within brain network for responding to threat, such as the hypothalamus, basolateral nucleus of the amygdala, BNST, or midline thalamic nuclei. OT or AVP may also modulate amygdala activity by acting on the nucleus basalis of Meynert, which sends cholinergic projections to the amygdala (Putnam, Young, & Gothard, 2018).

Cells/fibers and receptors: correspondence and non-correspondence

In many species, OXT and AVP may act on the other's receptors because they are highly structurally similar (Manning et al., 2008). Therefore, if OT fibers but only AVP V1a receptors, or vice versa, are found in a particular brain region, it may imply that cross signaling can happen between the two systems in that region.

A recent review examines the extent to which rats have OT-ir and AVP-ir fibers and receptors within nodes of the social brain neural network (Smith et al., 2019). Rats have a low density of oxytocin fibers but a high density of OXTR in the medial amygdala, lateral septum, MPOA, and ventromedial hypothalamus. It is possible that AVP acts on OXTR in these regions. Conversely, rats have a high density of oxytocin fibers but low OXTR binding in the periaqueductal gray, while they have high AVPR1a receptors in this region.

Can potential sites for cross-talk be identified in primates? In this project we have identified the fiber distribution for OT and AVP in rhesus macaques, chimpanzees, bonobos, and humans. We can compare these results with the chimpanzee receptor results from Chapter 4. In chimpanzees, both OT- and AVP-immunoreactive fibers were observed in multiple nuclei of the amygdala, with dense OT and AVP fibers in the central, medial, and basal nuclei, as well as the hippocampal transition area. However, the distribution of AVPR1a in the amygdala was denser and more widespread than OXTR, which was localized to the medial nucleus. Therefore, it is possible OT may act on AVPR1a in areas of the amygdala outside of the medial nucleus in chimpanzees. Another potential area for cross-talk is the substantia nigra, which had dense OXTR and AVPR1a but only AVP fibers. Finally, OT and AVP fibers were found in the cortex in chimpanzees, but only AVPR1a were dense in the cortex, while OXTR were very light and may reflect AVPR1a binding. It is possible that in the regions where OT fibers were found in chimpanzees, such as the cingulate gyrus and straight gyrus, OT may be acting on AVPR1a.

The action radius of one vesicle containing OT or AVP peptide released *en passant* from axonal boutons is estimated to be about 20 μm; release of 10 vesicles may be able to cover a volume with a 120 μm radius (Chini et al., 2017). This suggests that the effects of OT (and, given the structural similarity, likely AVP) are relatively local. However, it is possible that

receiving neurons may be placed in a strategic position relative to the release site (Chini et al., 2017), for example, the fibers we observed in Chapter 2 branching in layer 1 of cortex may make OT and/or AVP available to the pyramidal cells that have dendrites that ramify in layer 1. Overall, the effects are likely to be too local for release in one brain region to reach receptors in a neighboring region, suggesting receptor cross-talk is a possibility that must be investigated.

Importantly, absence of OXTR or AVPR1a binding in receptor autoradiography does not rule out the presence of receptors in a particular area. Receptor autoradiography is capable of revealing the presence of neuropeptide receptors in areas where they are produced.
These cells may receptors along their axonal projections to be expressed at axon terminals, which would not be detectable by receptor autoradiography (Dölen et al., 2013). Moreover, in primates, OTR is expressed in the nucleus basalis of Meynert, which provides cholinergic input to the cortex (Freeman & Young, 2016). Therefore, while asymmetry between areas with immunoreactive fibers and areas with receptors suggests that cross-talk should be evaluated, it is by no means definitive evidence of cross-talk. Finally, there is evidence that in humans, OT is selective for OTR, unlike in rats and mice where is binds promiscuously to both OTR and AVP receptors (Manning et al., 2008; Manning et al., 2012). Given the close genetic relationship between humans and chimpanzees, it would be useful to investigate whether this is the case in chimpanzees as well.

Evolutionary Perspectives

What do we know about the neuroanatomy of OT/AVP from an evolutionary perspective? OT/AVP or their homologs are found across the animal kingdom, but in mammals only, neuropeptide axonal projections expand into forebrain regions (Knobloch and Grinevich,

2014). Because the distribution of OT and AVP neurons and fiber projections appears relatively conserved among rodents and primates, we would expect it to look similar in intermediary species. Rodents, rabbits, tree shrews, flying lemurs, and primates are all members of a mammalian supergroup, the Euarchotonglires. The tree shrew (*Tupaia belangeri,* order Scandentia) is the closest living relative of primates (Janečka et al., 2007). Like all other mammals, OT and AVP cell bodies are found in tree shrews in the PVN and SON of the hypothalamus, and AVP cells in the SCN and medial amygdala. OT fibers are observed in nucleus accumbens, bed nucleus of the stria terminalis, medial amygdala, and the cortical amygdala, while AVP fibers were found in stria terminalis, nucleus of the diagonal band of Broca, hippocampus, and lateral habenula. Male tree shrews had dense AVP fibers in the lateral septum, while only one of the five female tree shrews had any AVP fibers at all in this region.

The characteristics of OT/AVP in tree shrew brains are consistent with what has been observed in primates as well as other mammals. Interestingly, oxytocin fibers were found in the cortical amygdala of tree shrews. Very dense OT and AVP fibers are found in this region in rhesus macaques (see Chapter 2), but are much sparser in chimpanzees, bonobos, and humans. Additionally, no cortical fibers were reported, though they are known to exist in rodents as well as primates (Hernández et al., 2015; Rogers et al., 2018), so this may be due to sampling.

The results of this dissertation as a whole suggest that aspects of OT and AVP neuroanatomy, including the localization of cells, fibers, and receptors, may affect behavior in comparable ways in rodents and primates – not only monkeys, but in great apes, including humans. These cells, fibers, and receptors are localized in brain systems for both threat reactivity and reward in patterns that may be related to species-typical behavior. How does this information

interact with what is known (and what is theorized) about social-behavioral evolution in humans and our closest relatives?

OT/AVP and Sociality in Humans, Chimpanzees, and Bonobos

Features of the Last Common Ancestor(s)

Given the comparative nature of this dissertation, it is possible to make inferences about the OT and AVP systems in human, chimpanzee, and bonobo evolution. What neuroanatomical changes likely took place in each species over the last ~6 million years? More broadly, what changes took place since the last common ancestor of humans, chimpanzees, and bonobos?

Because chimpanzees and bonobos diverged so recently but show stark differences in aggression, tolerance, and sexual behavior, it is interesting to consider the role OT and AVP might have played in the evolution of these two distinct species. Alterations in the OT and AVP system may be one piece of the puzzle of social behavioral contrasts observed in chimpanzees and bonobos. A recent analysis identified which features of each are likely to be derived, and which are likely to be shared with the last common ancestor of the genus *Pan*. Bonobo brains likely became smaller in whole brain and white matter volume since their split with chimpanzees 2-3 million years ago (Staes et al., 2018). Chimpanzees also likely underwent an increase in the size of the central nucleus of the amygdala and granular insular cortex, while bonobos underwent a decrease in these two regions. However, chimpanzees likely have decreased dysgranular and agranular insular volumes since the *Pan* split, while bonobos have increased them. The central nucleus of the amygdala and insular cortex are implicated in the OT/AVP systems. Bonobos, as well as humans, seem to have a lower density of AVP fibers in the central amygdala than chimpanzees, which may impact reactivity to threat. Humans and chimpanzees have more AVP

fibers in the insula, while bonobos have OT fibers there. The insula is implicated in empathy and interoception, and it may be that OT and AVP modulate these processes differentially; however, these fibers are very sparse and were described in a small sample (Rogers et al., 2018; Chapter 2); more research is needed to determine whether this is a true difference or if OT fibers project to the insula in humans and chimpanzees but were missed in this sampling paradigm.

Anthropological Theories of Human Social-Behavioral Evolution

This project characterized neurobiological features of humans, chimpanzees, and bonobos which may contribute to social-behavioral differences. Similarities and differences observed among these species can be placed within the framework of anthropological theories concerning the evolution of human social behavior. Two of the most prominent are the Human Self-Domestication Hypothesis and the Cooperative Breeding Hypothesis.

The Human Self-Domestication hypothesis draws parallels between traits observed in domesticated species and humans and infers similarities in the mechanisms and processes underlying the emergence of these traits. Across domesticated species, selection for "tameness" leads to a suite of (unintended) morphological traits such as reduced brain size, depigmentation, shortened muzzle, floppy ears, decreased tooth size, and more frequent estrus cycles. Domesticated animals have at least two, but typically more, of these traits (Sánchez-Villagra & van Schaik, 2019). These traits are thought to be developmentally related via changes in the neural crest (Theofanopoulou et al., 2018) and associated with hormonal and neurotransmitter changes (Hare, 2017).

Humans exhibit many of the traits of the domestication syndrome, including high intra-group tolerance (Henrich, 2015), reduced cranial capacity and shortened skull shape (Cieri et al., 2014), and depigmentation of the sclera. Fossil records indicate that humans may have

undergone a reduction in prenatal androgens and pubertal testosterone records from the Lower Paleolithic to the Holocene, based on cranial morphology (Cieri et al., 2014).

The idea of self-domestication has also been applied to bonobo evolution (Hare et al., 2012). Behaviorally, bonobos are less aggressive both between and within groups than chimpanzees. They also show some morphological characteristics consistent with the predictions of self-domestication. Bonobos have depigmentation of the lips and white tufts near the tailbone, and they show a reduced cranial capacity compared with chimpanzees.

Current formulations of the Human Self-Domestication hypothesis (and its application to bonobo evolution) posit that changes in the oxytocin system may accompany the other hormonal, physiological, and behavioral traits that have arisen as a part of this evolutionary process (Hare, 2017). Essentially, it is predicted that greater OT availability, whether in the amount of peptide or number of receptors in the brain, may have accompanied human self-domestication. The results of this project suggest two things. First, changes in the AVP system are as important to consider, or possibly more. Bonobos have a lower density of AVP fibers in the central amygdala compared with chimpanzees, which may be related to lower levels of territoriality and aggression. Moreover, humans also have low AVP fiber density in the central amygdala. AVP may be part of the hormonal shifts hypothesized to accompany social behavioral evolution in humans and bonobos. Future studies may determine whether this AVP innervation of the amygdala (or other brain regions) appears different in domesticated species versus their wild counterparts. Second, the results of this study suggest that differences in the OT system may be more specific than greater OT availability (whether this means increased global levels of the peptide or of OTR expression). Changes in the OT system over the course of human evolution are more likely to manifest in specific brain regions or networks that may or may not result in an

overall higher level of OT availability. Another possibility is that not OT or AVP changes alone but rather the balance between the two neuropeptides may shift in domestication. Notably, all non-primate species examined so far for OXTR/AVPR1a distribution – marmosets, rhesus macaques, coppery titi monkeys, and chimpanzees – have more widespread distribution of AVPR1a, while OXTR is more limited. Humans appear to have similarly limited AVPR1a and OXTR, or even a more limited distribution of AVPR1a (Loup et al., 1991; Loup et al., 1989). It would be useful to investigate the distribution of OTR and AVPR1a in domesticated species to determine whether the expression levels of the two are similar, as in humans.

Importantly, while the results of this dissertation (specifically, AVP fiber density in the central amygdala) are relevant to the idea of lowered aggression over the course of bonobo and human evolution, much more research on other brain regions, neuromodulatory systems, and behavioral ecology is needed for a full picture of the biological and environmental bases of aggression. If this neurobiological feature is indeed related to aggression on a species level, the implications of this may change given the assumptions. If we do not assume humans are lower in aggression than chimpanzees, then different mechanisms are needed to explain human aggression than bonobo aggression, though they could still share certain components that contribute to aggression, such as lower threat reactivity to conspecifics.

Another prominent theory of human social evolution is the Cooperative Breeding Hypothesis (Burkart, Hrdy, & van Schaik, 2009; Burkart & van Schaik, 2016). This theory draws upon similarities between psychological abilities of animals that engage in cooperative breeding and the extreme prosocial and cooperative abilities observed in humans. Specifically, it proposes that apes evolved a high level of cognitive ability, which, when combined with the psychological traits associated with cooperative breeding, results in uniquely human social behavioral traits.

The main cognitive ability underlying these human social behavioral traits is thought to be shared intentionality, or the ability (and interest) to share mental states with others (Tomasello et al., 2007; Burkart, Hrdy, & van Schaik, 2009).

This hypothesis draws comparisons between the psychological traits of humans and those of cooperatively breeding primates such as common marmosets and cotton-top tamarins. Like humans, these primates readily engage in spontaneous and proactive helping, while chimpanzees and bonobos do not., or at least not to the same extent. How might a species become more oriented towards the needs of conspecifics, and likely to help? A potentially related insight from comparative research on OXTR/AVPR1a research is that in primates, as a whole, OXTR are found in the nucleus basalis of Meynert and the superior colliculus, which are thought to modulate attention to social stimuli (Freeman et al., 2018). It would be useful to measure whether OXTR receptors or fibers in these regions are denser in humans (and perhaps common marmosets and cotton-top tamarins) than in other primate species.

The propensity for proactive helping in both Callitrichids and humans is presumably related to social reward on a neurobiological level. The results of Chapter 4 of this dissertation suggest that humans may share some aspects of their reward system with marmosets that they do not share with chimpanzees. Chimpanzees do not display dense binding for OXTR in forebrain reward areas such as the ventral pallidum and nucleus accumbens while humans do (Freeman et al., 2018; Bethlehem et al., 2017), and marmosets also have OXTR in these reward areas (Schorscher-Petcu et al., 2009).

OXTR or AVPR1a receptor expression in the ventral pallidum and nucleus accumbens has most popularly been related to pair-bonding, which is relevant here, as marmosets are able to form pair-bonds (Smith, Ågmo, Birnie, & French, 2010). Importantly, this differs from human

mating in that marmosets who are not part of the breeding pair are reproductively suppressed (Barrett, Abbott, & George, 1990). The interaction of OT and/or AVP with the dopamine reward system has also been connected to paternal behavior in humans (Rilling & Young, 2014) and marmosets (Saito & Nakamura, 2011) as well as in other biparental species such as prairie voles (Wang, Ferris, & de Vries, 1994). Critics of the Cooperative Breeding Hypothesis point out that, among other differences, human alloparenting occurs with unrelated individuals, while marmoset alloparents are genetic kin (Bogin, Braggm & Kuzawa, 2014). Nevertheless, it is possible that pair-bonding and alloparenting, however different the details of their expression, require basic components shared among humans and Callitrichids which could ultimately serve a role in the large-scale cooperation among non-kin observed in humans (Boyd, 2009). OT and AVP action in brain regions for reward and social attention are attractive candidates for such basic components of prosocial interaction.

Future Directions

In this dissertation, I have characterized the distribution of OT and AVP cells and fibers in rhesus macaques, chimpanzees, bonobos, and humans. I have also localized OXTR and AVPR1a in chimpanzees. This presents a step forward in our understanding of the human and nonhuman primate evolution of the OT and AVP systems. However, there is much more to learn.

Chapter 2 identified some possible differences between chimpanzees and bonobos in the localization and density of oxytocin and vasopressin fibers. Specifically, chimpanzees appear to have a higher density of vasopressin fibers in the central amygdala as compared with bonobos or humans. One obvious next step is to compare these three species in their amygdala receptor distribution to see if chimpanzees also have a higher density of AVP in the central amygdala than

humans or bonobos. Unfortunately, receptor autoradiography requires fresh-frozen tissue, while all available bonobo tissue in the US has undergone fixation. In the future, development of better antibodies for AVP1a receptors or a change in the brain preservation norms at zoos and research centers will hopefully allow the opportunity to characterize OXTR and AVPR1a in bonobo brains. Another possible avenue is using *in situ* hybridization for OXTR and AVPR1a mRNA in fixed chimpanzee brains to validate the method, then using it in bonobo brains.

Chapter 4 focused on one type of vasopressin receptor, V1a. There exist two other vasopressin receptors, V1b and V2. Both are expressed in the brain of rats. V2 receptors have been found in the lateral septum and periaqueductal gray and may be involved in pain modulation (Landgraf et al., 1991, Yang et al., 2006). In male rats, V1b mRNA is expressed in the hypothalamus, piriform and entorhinal cortices, hippocampus, substantia nigra, olfactory bulb (Vaccari et al., 1998) as well as the medial amygdala, BNST, and MPOA (Arakawa et al., 2010). It is less well characterized than V1a, but there is evidence that it has behavioral effects: In male mice, V1b knockout reduces aggressive behavior (Wersinger et al., 2002) and lowers concentrations of ACTH and corticosterone (Tanoue et al., 2004). V1b receptors may also play a role in maternal behavior (Bayerl et al., 2016). More research is needed to understand the expression and function of the v1b and V2 receptors in both rodents and primates.

Another avenue for potential insight is the comparison of AVPR1a receptor distribution in chimpanzees with and without the DupB deletion in the promoter region of the *AVPR1a* gene. In Chapter 4, only two chimpanzees were heterozygous for the allele while the others had the short version, and no differences in distribution were apparent. However, AVPR1a genotype has been shown to affect chimpanzee personality and behavior in several studies (Staes et al., 2015, Hopkins et al., 2012). A larger sample size, along with tissue from individuals with the long

allele, may be needed to detect whether a particular AVPR1a genotype is associated with altered expression of AVPR1a in chimpanzee brains.

Finally, the neuroanatomical distribution of OT and AVP cells, fibers, and receptors is an essential part of understanding the evolution and functional significance of these systems; however, many more aspects are subject to evolutionary change and open for future studies. Novel forms of the OT peptide in platyrrhine primates (Lee et al., 2011) could potentially lead to differences in signaling or receptor affinity. Peripheral OT and AVP receptors on reproductive organs, the heart, and the gastrointestinal tract are likely to have behavioral consequences (Leng & Ludwig, 2015), and in humans, individual differences in peripheral hormone levels in clinical populations have been observed for disorders of social attachment (Bertsch et al., 2013). Moving forward, neuroanatomical features should be placed within a broader discussion of the evolutionary targets of the OT and AVP systems.

Conclusion

The literature on the oxytocin and vasopressin systems is growing more and more quickly every year. The majority of this research takes place in rodent species, and though a complete understanding is still far off, we are beginning to appreciate the detailed and nuanced effects of these neuropeptides. In the animal work, we see the possibilities of our own neural systems reflected. What makes us who we are, and how did we get this way? As evidence accumulates from intranasal, genetic, and hormonal studies for the influence of oxytocin and vasopressin on human and non-human primate social behavior, the contents of this dissertation provide a grounding for where they act in the brains of a few species of interest. This is a starting point; it provides a snapshot into the structure of two dynamic physiological systems open to social and

environmental influences during a lifetime and shaped by millions of years of evolution and speciation. There is much more to learn about how these forces interact and give rise to behavior.

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