Neurochemical Insights of Human Origins: A comparative analysis of dopaminergic axon innervation of the ventral striatum among primates

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TABLE OF CONTENTS

CHAPTERS

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CHAPTER 1: INTRODUCTION

What does it mean to be human? Our unique demographic success and our intense sociality, including the evolution of language, empathy, and altruism, are unique characteristics of aspects of modern human behavior. However, it has always been difficult to account for the evolution of these complex social traits through natural selection (Darwin 1859). Most studies of human brain evolution have focused on the cerebral cortex, as it is the most expanded region in the human brain compared to other species. However, the emergence of our lineage occurred before any notable brain expansion in the fossil record, suggesting that "the initial neurochemical changes necessary for prosocial behavior and promotion of social monogamy were facilitated by an evolutionarily older region of the brain" (Raghanti et al. 2018). New investigations are now examining the striatum, a subcortical region in the basal ganglia (Raghanti et al. 2018, Raghanti et al. 2016, Baez-Mendoza & Shultz 2003, Nambu 2011, Haber 2003). Traditionally thought to only regulate motor control, the striatum is now known to play a large role in cognition, learning and memory, and to modulate social behavior through relative neurotransmitter activity in the dorsal and ventral regions (Cools et al. 1989, Van den Bercken & Cools 1980). A unique human neurochemical profile in the dorsal striatum suggests changes in this area may have initiated prosocial behavior in early hominids and that these changes eventually gave way to the evolution of complex social traits in modern humans (Raghanti et al. 2018).

Early hominid success and selection for prosocial behavior

The discovery of *Ardipithecus ramidus*, a predecessor of *Homo* dating back to 4.4 Mya, resolved many questions concerning the nature of the last common ancestor (LCA) humans shared with the clade leading to extant chimpanzees and bonobos (Lovejoy 2009, White et al. 2009). In brief, morphological and ecological research revealed that *Ardipithecus* lived in a woodland habitat in northeastern Ethiopia and retained above-branch quadrupedalism and a relatively primitive postcanine dentition (Lovejoy 2009, White et al. 2009). However, *Ardipithecus* possessed several distinct derived traits, such as postcrania morphology adapted for bipedal locomotion and a significantly reduced sectorial canine complex (SCC) (Lovejoy 2009, White et. al 2009). These traits indicated that early hominids were on their own evolutionary path distinct from those of our ape relatives. In fact, *Ardipithecus* revealed that apes evolved from a more human-like ancestor, nullifying referential models that dominated human evolution in the past (Lovejoy 2009). Even so, *Ardipithecus* raised questions of its own, as the appearance of these derived anatomical traits appeared disadvantageous when taken independently (Lovejoy 2009, White et al. 2009).

Distinct behavioral changes that included provisioning and social monogamy likely played a pivotal role in early hominid demographic success compared to other Miocene hominoid groups. Like other Miocene apes, our clade's ancestors shared intense K-selected life history traits including prolonged lifespan, extensive periods of infant dependency, and greater interbirth intervals (Lovejoy 1981). However, while late Miocene hominoids struggled to maintain stable populations as a result of these increasingly K-selected traits and were consequently restricted to small pockets of Miocene refugia, hominid clades flourished and occupied most of Africa by the end of the Pliocene (Lovejoy 1981, Lovejoy et al. 2009, White et al. 2009). This disparity is

exaggerated even moreso today, as only a few extant examples of our great ape relatives remain while human population numbers continue to grow substantially.

In addition to these life history pressures, our ancestors exhibited mild sexual dimorphism and completely abandoned the sectorial canine complex (SCC), the primate "social tooth" that is typically used to assert dominance and aggression (Lovejoy 1981, Lovejoy 2009). Moreover, early hominid postcranial fossils display major anatomical changes to accommodate bipedal locomotion, an unusual form of habitual locomotion for any animal (Lovejoy 2009). Considered independently, the evolution of these traits would seem to be vastly maladaptive. However, in combination with novel behaviors such as dedicated provisioning and social monogamy, these uniquely human traits may have allowed early hominids to overcome these disadvantages and increase reproductive success (Lovejoy 2009).

This hominid adaptive suite, proposed by C.O. Lovejoy, suggests male provisioning interwoven with a monogamous mating strategy increased fitness for both males and females, as well as strengthened selection for bipedality (Lovejoy 1981). Bipedal locomotion freed the hands to transport food over long distances, and exchanges of food for copulations would have further encouraged mating with provisioning males (Lovejoy 2009). Females that chose to regularly mate with provisioning males would enjoy benefits in the form of rich food sources that would increase their own reproductive fitness. Moreover, the lowered mobility of females and their offspring decreased exposure to predators and accidents while traveling (Lovejoy 2009). In fact, increased female survivorship is a key component to population stability and growth (Meindl et al. 2018). As a result, female choice would shift from a preference for aggressive, large-bodied males to more affiliative males with an increased awareness of the environment. Provisioning males would benefit from reduced resource competition and allow for repeated copulations, the

latter of which are virtually requisite as human females do not possess an externally recognizable estrous (Lovejoy 2009). Lastly, as early hominids were facing advanced K-selected modifications, intensified parenting from both females and males became virtually mandatory to safeguard their investment in reproduction (Lovejoy 2009).

Moreover, modern reproductive correlates between humans and other non-human primates support a pair-bond mating system. Modern human ratios of testes volume to body mass are similar to other monogamous primates such as gibbons, while chimpanzees exhibit ratios three times higher (Lovejoy 2009). Additionally, the absolute rate of sperm production is only about 20% that of the much smaller, polygynous, rhesus macaque (Moller 1998). Lastly, humans are the only catarrhine without an os baculum and have the least complex penis morphology of any primate (Dixson & Anderson 2002, Dixson 1987). These comparisons provide additional support for the proposed socially monogamous reproductive strategy.

While anatomical evidence supports this pair-bonded mating system, resolving the neurochemical changes necessary to promote such a system in early hominids is challenging. Several hypotheses have attempted to account for the extraordinary evolution of the human brain through via expansion of the cerebral cortex (Hare 2017, Richerson et. al 2016). Common to these hypotheses is the idea that major cognitive changes began occurring relatively late in human evolution (at approximately 2 MYA) after major brain expansion. However, it is important to note that personality differences have been categorized throughout the animal kingdom, suggesting a large hominid cortex is unnecessary to create such differences (Budaev et al. 2015, Buske and Gerlai 2012, Nettle 2006, Wolf and Weissing 2012). Sheer volume and other absolute parameters of the brain do not have any causal relationships with form, function, and behavior except on an extremely broad level. Moreover, each animal brain bears a specific

signature of qualitative and quantitative features (brain size, neuron densities, amount of neurotransmitters, etc.) that are acted on by natural selection, and brain volume comparisons merely mask any species-specific adaptations occurring at the neuronal level (Holloway 1972). Therefore, brain volume changes in hominid ancestors are likely to be merely a byproduct, not a requirement, for elevated prosocial behavior.

It is likely that the hominid brain underwent a reorganization as a result of the evolutionary advantages of affiliative behavior (Holloway 1972). In fact, novel transcriptome and histological features support the likelihood of molecular and cellular reorganization of human neural circuits, especially those generated by genes associated with the dopaminergic system in the striatum and neocortex (Sousa et al. 2017). The success of our clade relied on social behavioral adaptations preceding any brain enlargement, suggesting the origins of highly social, and later complex behaviors such as language, empathy, and altruism are facilitated, and probably promoted, by a reorganized, evolutionarily older region of the brain.

The striatum and its role in social behavior

Novel investigations in comparative neurobiology have focused on a subcortical region of the brain known as the striatum (Raghanti et al. 2018, Raghanti et al. 2016, Baez-Mendoza & Shultz 2003, Nambu 2011, Haber 2003). It is the primary input structure of the basal ganglia and communicates extensively with the cerebral cortex, thalamus, and brainstem. The basal ganglia were traditionally thought to be involved in motor control exclusively, but are now understood to participate heavily in learning and memory, perception, and cognition (Middleton & Strick 2000). Moreover, some regions of the basal ganglia are activated before the cortex during cognitively demanding tasks, and lesions of the basal ganglia can mirror lesions of the cortex,

demonstrating the extensive role of the basal ganglia in modulating cognition (Lombardi et al. 2000).

The striatum is involved extensively in the nigrostriatal and mesolimbic pathways, which facilitate motor production and reward regulation, respectively. The nigrostriatal pathway connects the substantia nigra pars compacta with the dorsal striatum (caudate nucleus and putamen). Volitional movements are facilitated through the direct loop, while the indirect loop prevents unwanted movement (Middleton and Strick 2002, Haber 2003). Loss of function in the nigrostriatal pathway is well characterized in Parkinson's disease, and manifests in severe motor problems such as hypokinesia, tremors, and muscle rigidity, as well as personality changes (Savitt et al. 2006, Kaasinen et al. 2001). The mesolimbic pathway connects the ventral tegmental area (VTA) to the ventral striatum and mediates pleasurable experiences (Haber 2003, Baez-Mendoza & Shultz 2003). These pathways are highlighted in **Figure 1**. The connectivity of these two regions is the brain's primary mechanism for encoding reward and have been extensively studied in clinical experiments as it is "hijacked" during the use of addictive drugs in humans (Kalivas et al. 2005). However, evolutionarily, the reward system perpetuates behaviors that increase fitness by linking them to rewarding effects. It in part does this through dopamine (DA), a monoamine neurotransmitter that signals reward (Haber 2003). The release of dopamine from the VTA into the nucleus accumbens (NAcc) regulates incentive salience, the motivation to behave in a particular way in response to certain stimuli (Baez-Mendoza & Shultz 2013). Evaluating different aspects of reward, including value versus risk, while minimizing maladaptive outcomes, is an essential component to developing goal-directed behavior, especially in the context of group living (Haber 2014).

The striatum can modulate social behavior in a dichotomous manner through the distinctive connectivity and function of its dorsal and ventral regions (Baez-Mendoza & Shultz 2003, van den Bos 2015). The dorsal region is involved in internally motivated, goal-directed behaviors. In contrast, the ventral region is associated with various limbic structures of the brain and regulates emotions and externally-guided behaviors (van den Bos 2015). Moreover, the ventral striatum is thought to facilitate behavioral flexibility through sensitivity to social and environmental cues, and mediate social conformity in humans (Stallen & Sanfey 2015, Klucharev et al. 2009, Zaki et al. 2011).

Early studies on cynomolgous macaques show that individual personality style is dependent on the relative activity levels of these two regions and underlies differences in social behavior (Van Den Bercken & Cools 1980). In such studies, individuals with high dorsal striatal activity were internally driven and displayed a high level of autonomy, while those with high ventral activity were externally driven and were sensitive to the actions of others. Moreover, dorsal-dominated individuals were less responsive to their environment and exhibited more aggressive behaviors (Van den Bercken & Cools 1980). In contrast, individuals with ventral striatum-dominant personalities exhibited elevated curiosity of their environment and less aggression overall (Van Den Bercken & Cools 1980). This suggests personality style, in part, can be predicted by individual striatal profiles, and could be a potential region for selection given evolutionary significant benefits of particular social behaviors.

Additionally, individual-specific features of Wistar Rats as a result of differential neurotransmitter transmission in the dorsal and ventral striatum has been documented (Cools et al. 1989). Rats that primarily fled during social defeat experiments possessed low noradrenergic activity in the ventral striatum and low dopaminergic activity in the dorsal striatum, while rats

that froze showed high noradrenergic in the ventral striatum also showed high activity in the dorsal striatum (Cools et al. 1989). This evidence supports the notion that individual specific behavior can be modulated by neurotransmitter activity level variation in the dorsal and ventral striatum.

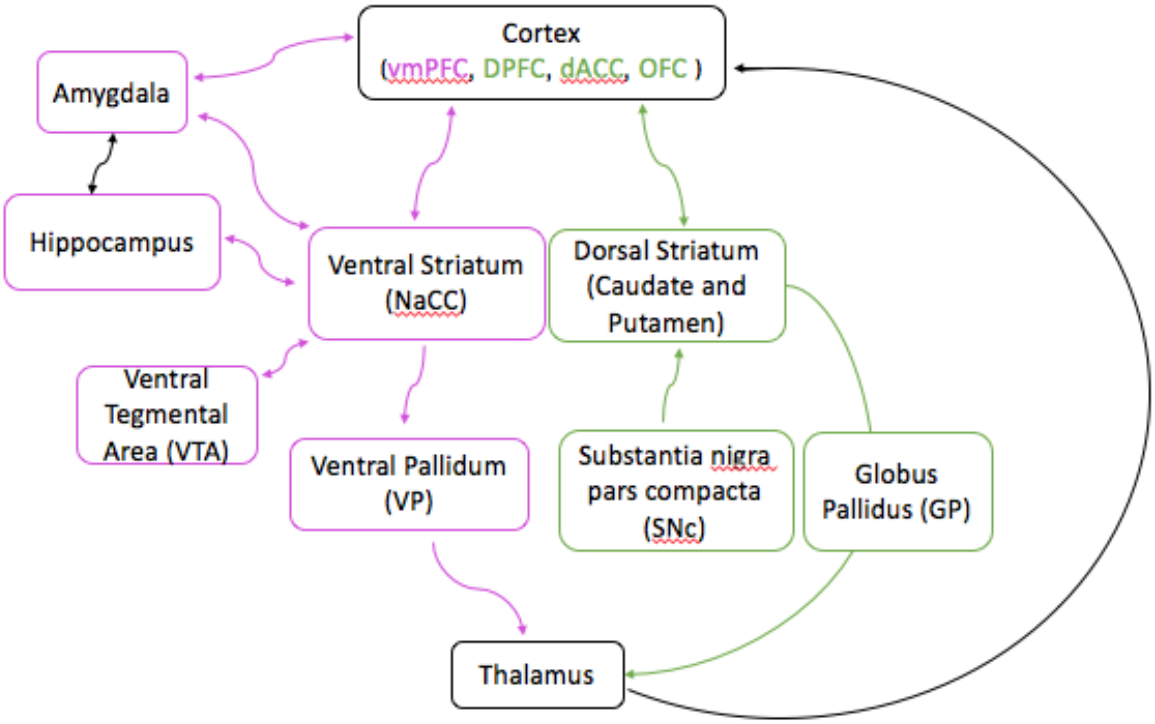

Figure 1: Simplified diagram of mesolimbic (pink) and nigrostriatal (green) dopaminergic pathways with emphasis on ventral striatum connections.

The role of dopamine in species-specific behavior

In addition to regional activity levels, relative concentrations of neurotransmitters within the striatum influence personality and behavior (Bergey et al. 2015, Jolly et al.2008, Van den Bercken & Cools 1980, Aragona et al. 2004). Specifically, variation in the dopaminergic reward pathway underlie different social behaviors. These behaviors can be further subjected to evolutionary forces and as a result, produce life history consequences. Comparative research involving closely related taxa with differing social systems provides insights on dopamine's role in the evolution of species-specific complex behaviors. For example, olive (*Papio anubis*) and hamadryas (*Papio hamadryas*) baboons have distinct social structures despite being interfertile and diverging less than 1 MYA (Bergey et al. 2015). Male olive baboons disperse from their natal group, and access to fertile females that is dependent on alliances with other males and agonistic competition. In contrast, male hamadryas baboons are philopatric and aggressively pursue and defend females in one-male groups (Bergey et al. 2015, Jolly et al. 2008). The divergent behavior between the two groups is a result of differences in reward structures for either impulsive or restrained behavior. Studies investigating the underlying neurochemical basis of social system variation show significant hormonal and molecular differences associated with dopaminergic pathways (Bergey et al. 2015, Jolly et al. 2008). Higher levels of homovanillic acid, a dopamine metabolite, were found in the cerebrospinal fluid of hamadryas males (Jolly et al. 2008). Moreover, a transcriptome wide sequencing of 212 individuals reveals three single nucleotide polymorphisms in genes (DAT1, COMT, PPP1CC) that are associated with dopamine transmission, suggesting that dopamine genes play a significant role in behavioral differences (Bergey et al. 2015). Variation in these genes may manifest as differences in personality traits (ie; boldness) that, in part, determine both intragroup dynamic and alternative life-history

strategies (Bergey et al. 2015). For example, slower breakdown of dopamine in male olive baboons may manifest as affiliative behavior or increased behavioral inhibition necessary for cooperation with other males. In contrast, higher HVA levels in males hamadryas baboons may be the basis for adaptive impulsivity to secure mating opportunities (Jolly et al.2008). In general, these studies show genetic variation and their endocrine correlates are associated with dopamine and are likely subject to population-level evolutionary forces. Variation in male baboon temperament is only one example demonstrating the essential role that dopamine transmission plays in the selection of species-typical behavior in all social animals, including humans and their ancestors.

It has long been postulated that our early hominid ancestors exhibited unique social and reproductive strategies that contributed to elevated fitness (Lovejoy 1994). In fact, social attachments play a central role in modern human society, and the inability to form meaningful bonds is associated with various psychological illnesses (Curtis et al. 2006). The understanding of social ties between adults and, particularly, the mechanisms underlying monogamous life strategies, are only now beginning to be understood. While it is impossible to study the neurochemistry of extinct species, changes were almost certainly occurring in *Ardipithecus ramidus* and its descendants that facilitated pair-bond behavior. Based on extensive studies of other species (especially voles), the neural mechanisms that supports the formation of a pair-bond likely co-opted the ancient pathway that supports the mother-infant bond. This same pathway probably supports other affiliative relations such as friendships as well (Curtis et al. 2006).

While the definition of a pair-bond varies throughout the literature, it is typically described, across species, as an enduring preference formed between two sexually mature adults (Young et

al. 2011). It is characterized by selective contact, affiliation, and partner preference during copulation (Young et al. 2011). Moreover, other behaviors, such as territoriality, mate-guarding and bi-parental care of young are regularly associated with pair-bonds (Young et al. 2011). For example, rodents from the genera *Microtus* have been extensively used in the study of social and mating behavior. Prairie vole (*Microtus ochrogaster)* exhibit monogamous mating behavior, as well as intense parental investment compared to their polygamous meadow vole *(M. pennsylvanicus)* relatives. Comparative data between these two species have shown that the interplay between oxytocin and vasopression receptor distribution throughout within the mesolimbic reward pathway facilitates the formation of a pair-bond (Carter et al. 1995, Donaldson and Young 2008, Pitkow et al. 2001, Curtis et al. 2006).

Dopamine plays an essential role in the formation of a pair-bond through its ability to associate rewarding aspects of sex with a specific partner and significant species differences associated with dopamine receptor distribution in the reward pathway are present in the two vole species (Donaldson and Young 2008, Pitkow et al. 2001, Curtis et al. 2006). Specifically, although D1 and D2 receptors are present in the NAcc of both species, meadow voles have greater D2 binding than do prairie voles (Aragona et al. 2004). Moreover, sex differences indicate polygamous male voles display greater D1 binding than do males of monogamous species, while D1 binding in females is non-significant (Aragona et al. 2004). Briefly, the dopamine receptor subtypes are associated with their own functions, adding another layer of complexity to pair-bonding modulation. The D2 receptor subtype is involved with the formation of pair bonds, as administration of the D2 antagonist eticlopride inhibits pair bond formation while administration of quinpirole, a D2 agonist, induces partner preferences in the absence of mating (Gingrich et al. 2000). The D1 dopamine receptor subtype is involved with pair bond

maintenance, with D1 receptor binding increasing significantly in male prairie voles after having lived with their mate for 2 weeks compared to sexually naïve males or sexually experienced males with only short-term exposure to a mate (Curtis et al. 2006). Additionally, dopamine transmission in the NAcc regulates partner preference formation in prairie voles. Peripheral injections of a nonspecific DA receptor agonist induced partner preference formation in the absence of mating, while antagonist blocked mating-induced preferences (Young 2011).

Human genetic studies examining neuropeptides and their role in sociality indicate endorphins and dopamine have a widespread impact on dispositions, relationships, and social networks in addition to other traditionally studied neurotransmitters such as oxytocin, vasopressin, and testosterone. (Pearce et al. 2017). Further, it is worth noting that is it likely an interaction of multiple neurotransmitter systems that is responsible for pair bond mating behavior. Studies examining the monogamous titi monkey *(Callicebus moloch*) indicate that administration of a kappa opioid receptor antagonist buffers social stress during mate separation (Ragen et al. 2015). Further, the presence of a pair-mate modulates the results of pharmacological manipulation of the opioid system (Ragen et al. 2013). However, titi monkey pairs are territorial and often mate-guard, two indicators of pair-bond aggression that humans do not exhibit, and more research is necessary to understand the effects neurotransmitter system interactions can have on pair-bond behavior (Norconk 2007).

Overall, dopamine transmission and distribution, in addition to likely interactions with other neuropeptides, can create novel social behavioral traits which contribute to larger species-specific characteristics, such as dispersal and mating systems, that can be subject to evolutionary forces.

Dopaminergic systems: targets for evolution

It is important note that the characterization and causation of behavioral and personality differences are fascinating ventures alone, the role they play in ecological and evolutionary processes (and their interactions), such as demography, dispersal patterns, speciation, and social evolution is equally as significant (Wolf and Weissing 2012). The effects of evolutionary forces on neural networks, especially dopaminergic systems, are critical species-specific behavior. They are thus fertile areas for exploration.

The actions of dopamine are mediated by at least five distinct G protein-coupled receptors subtypes. The underlying roles of some of these are still unknown (Missale et al. 1998). Receptor subtype availability and density have been shown to play roles in cognition, memory, behavior, and personality differences in humans (Pearce et al. 2017, Missale et al. 1998, Sawaguchi & Goldman-Rakic 1991, Zhang et al. 2007). Moreover, polymorphisms in dopamine reuptake genes, notably the dopamine transporter (DAT) have been indicated in humans and nonhuman primates (Jaber et al 2004, Inoue-Murayama et al. 2002, Miller et al. 2001, Doucette-Stamm et al. 1995). Specifically, the DAT gene contains a variable number tandem repeat (VNTR) in several primate species (Inoue-Murayama et al. 2002). The VNTR has been shown to affect the expression of the transporter protein, demonstrating across species differences in the breakdown of dopamine within the synapse, which may give rise to different behavioral phenotypes (Inoue-Murayama et al. 2002). Although the complexity of neural networks, specifically dopaminergic pathways, in modulating cognition, social behavior, and personality in both human and nonhuman primates leaves much to be explored, existing variation creates an ideal scenario for evolutionary forces to act.

Ventral Striatum Specifics: Social Reward and Behavioral Flexibility

Recognizing external cues from other individuals in a group, evaluating these behaviors accurately, and responding to them in a beneficial way requires a carefully orchestrated interaction of dopaminergic circuits, and is therefore evolutionary conserved across the animal kingdom (Budaev et al. 2015, Buske and Gerlai 2012, Nettle 2006, Wolf and Weissing 2012). Social reward is much like any other reward, as it evokes classically conditioned learning, and elicits seeking-behaviors that produce identical pleasurable outcomes (Curtis et al. 2006).

Specifically, the role of the ventral striatum (VS) is to anticipate and detect rewarding stimuli. The VS connected with the amygdala and hippocampus, regions of the brain known to process emotions, encode memories, and provide contextual information necessary for adjusting motivation. Moreover, in humans the VS receives sensory inputs from a large portion of the cortex. The NAcc, the main component of the VS, is divided into two subregions: the core and the shell. The shell determines the value of rewarding stimuli and contains mostly D1-type dopamine receptors (Haber 2011). In contrast, the core is involved in cognitive processing of motor function related to reward and reinforcement. It can encode new motor programs which permit the acquisition of a given reward in the future (Haber 2011). The two subregions work in concert to execute motivated, well planned acts, and evidence shows the NAcc may facilitate flexible approach responses compared to the formation of habits, which may be under the dorsal striatum's control (Ikemoto & Panksepp 1999).

The major output that receives information from the VS is the ventral pallidum (VP) (Baez-Mendoza & Shultz 2013, Haber 2003). The VP's cells receive GABAergic inputs from the VS and respond during learning and performance of reward incentive behaviors. Descending efferent projections are to the thalamic and subthalamic nuclei, lateral hypothalamus, and

substantia nigra pars compacta (Baez-Mendoza & Shultz 2013, Haber 2003). The VP also projects to the lateral habenular nucleus (LHb). The LHb is known to generate negative reward signals in the absence of an anticipated reward (Haber 2011). Growing evidence supports the VP being heavily involved in encoding reward, enhancing rewards learning, pleasurable effects of rewards, and motivation (Smith et al. 2009). Interestingly, VP lesion experiments in mice eliminate "liking" of sucrose and replace it with aversive responses (Smith et al. 2009). Moreover, the VP may encode relative reward value earlier and more robustly than the NAcc, as VP neuron activity was greater and occurred sooner following reward related tasks in rats (Ottenheimer 2018). In conclusion, because the VP plays a central role in reward processing, and is the major output of the VS, the region has also been included in this study.

Specific Hypotheses

With multiple analyses of the dorsal striatum complete, there is evidence of a signature human neurochemical profile that may have either preceded or accompanied the adoption of bipedality and elimination of the SCC (Raghanti et al. 2018). In brief, humans have elevated striatal dopamine, serotonin, and neuropeptide Y, coupled with lowered acetylcholine in the dorsal striatum compared with other non-human primates (Raghanti et al. 2018, Raghanti et al. 2016). The ventral striatum and ventral pallidum, regions that are directly involved in the brain's reward system and which also function to regulate emotion and sensitivity to social and environmental cues, are vet to be analyzed. In the current study we have examined the dopaminergic innervation of the ventral striatum in seven primate species. Specifically, we compared tyrosine hydroxylase-immunoreactive (TH-ir) axons in the NAcc and VP among humans (*Homo sapiens*), chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*), rhesus

macaques (*Macaca mulatta*), pigtailed macaques (*Macaca nemestrina*), tufted capuchin (*Cebus apella*), and cotton-top tamarin (*Saguinus oedipus*).

First, we hypothesize humans will exhibit increased dopaminergic innervation in the ventral striatum and ventral pallidum compared to non-human primates. Support for this hypothesis comes from recent positron emission tomography (PET) studies that have found higher levels of DAergic $D_{2/3}$ receptor binding in the human ventral striatum that were associated with increased socialization and decreased indirect aggression (Caravaggio et al. 2016). Moreover, individuals self-reported to be socially detached had less endogenous DA D2/3 binding than more socially attached individuals (Caravaggio et al. 2017). Additionally, we expect neuron density to decrease in humans as a reflection of increased brain size (Haug 1987). we also expect glia density and the glia/neuron ratio, an indirect measure of metabolic support, to increase as a measure of metabolic expenditure of the human brain. This hypothesis is supported by a previous study comparing human frontal cortex glia-neuron ratios to those in other nonhuman primates (Sherwood et. al 2006).

While the ultimate goal of this study is to determine uniquely human characteristics of the ventral striatum, we also expected differences among nonhuman primate species. I hypothesize tamarins will also show increased dopaminergic innervation in the ventral striatum as a result of their monogamous mating system (Donaldson and Young 2008, Pitkow et al. 2001, Curtis et al. 2006). Lastly, we expect increased dopaminergic innervation in bonobos as a result of their differential social structure that includes highly affiliative behavior compared to that of their chimpanzee relatives, a hypothesis supported by previous studies that categorized several neurobiological differences (Gruber and Clay 2016; Hopkins et al. 2009).

CHAPTER 2: METHODS

Specimens

Our sample consists of postmortem brains from forty individuals representing seven primate species; six cotton-top tamarins (*Saguinus oedipus*), six tufted capuchins (*Cebus apella*), six pig-tailed macaques (*Macaca nemestrina*), six rhesus macaques (*Macaca mulatta*), five chimpanzees (*Pan troglodytes*), five bonobos (*Pan paniscus*), and six humans (*Homo sapiens*). Sexes were balanced for all species except for *P. troglodytes and P. paniscus*, where 2 of the 5 individuals were male. All nonhuman primates were housed in accordance with their home institution's animal care and use standards. Whole brains or sections were obtained from the Oregon National Primate Research Center, Seattle National Primate Research Center, New England Regional Primate Research Center (Harvard), Alpha Genesis, and Dr. Chet Sherwood's Laboratory for Evolutionary Neuroscience at The George Washington University, Washington D.C. The brains were fixed by immersion in 10% buffered formalin for 7-10 days, then transferred to a preservative solution of 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 0.1% sodium azide and stored at 4º Celsius until further processing. All individuals were adult and free of gross neuropathology. A specimen list can be found in Table 1.

Table 1: Specimen List

Species	Common Name	Sex	Age (yr)
Saguinus oedipus	Cotton-top tamarin	M	10.9
Saguinus oedipus	Cotton-top tamarin	M	8.4
Saguinus oedipus	Cotton-top tamarin	M	9.5
Saguinus oedipus	Cotton-top tamarin	F	10
Saguinus oedipus	Cotton-top tamarin	F	5
Saguinus oedipus	Cotton-top tamarin	F	8
Cebus apella	Tufted capuchin	M	2.9
Cebus apella	Tufted capuchin	M	16.6
Cebus apella	Tufted capuchin	M	15.9
Cebus apella	Tufted capuchin	F	12.6
Cebus apella	Tufted capuchin	F	17.5
Cebus apella	Tufted capuchin	F	18.3
Macaca nemestrina	Pig-tailed macaque	M	15.73
Macaca nemestrina	Pig-tailed macaque	M	4.28
Macaca nemestrina	Pig-tailed macaque	M	2.5
Macaca nemestrina	Pig-tailed macaque	F	15.1
Macaca nemestrina	Pig-tailed macaque	F	14
Macaca nemestrina	Pig-tailed macaque	F	5.95
Macaca mulatta	Rhesus macaque	M	8
Macaca mulatta	Rhesus macaque	M	13
Macaca mulatta	Rhesus macaque	M	13
Macaca mulatta	Rhesus macaque	F	14
Macaca mulatta	Rhesus macaque	F	11
Macaca mulatta	Rhesus macaque	F	12.5
Pan troglodytes	Chimpanzee	F	30.8
Pan troglodytes	Chimpanzee	M	19.8
Pan troglodytes	Chimpanzee	F	18.5
Pan troglodytes	Chimpanzee	M	19.5
*Pan troglodytes**	Chimpanzee	F	17.8
Pan paniscus	Bonobo	M	34
Pan paniscus	Bonobo	M	5
Pan paniscus	Bonobo	M	25
Pan paniscus	Bonobo	F	52
*Pan paniscus**	Bonobo	F	25
Homo sapiens	Human	M	44
Homo sapiens	Human	M	56
Homo sapiens	Human	M	44
Homo sapiens	Human	F	39
Homo sapiens	Human	F	25
Homo sapiens	Human	F	53

* , only included in among-species analyses for nucleus accumbens TH-ir Nv/total Nv

Sample Preparation

All samples were cryoprotected in a graded series of sucrose solutions (10%, 20%, 30%) prior to sectioning. The brain samples were rapidly frozen with dry ice and the sectioned at 40 µm using a freezing sliding microtome (SM2000R, Leica, Chicago, IL). After sectioning, each section was placed into an individual centrifuge tube containing freezer storage solution (30% each distilled water, ethylene glycol, and glycerol and 10% 0.244 M PBS), numbered sequentially, and then stored at -20° C until histological or immunohistochemical processing. Nissl staining was done on every tenth section for each sample. Samples were mounted on gelatin coated slides, dried, and placed in a 1:1 alcohol/chloroform mixture for 30 minutes. Then the sections were rehydrated through an alcohol series of 100%, 90%, 70%, 50%, 30%, dH_20, for 2 minutes each. Samples were stained with cresyl violet for 8 minutes, and then dehydrated in from distilled water to 100% ethanol for 1 minute each. Finally, the samples were placed in citrasolv for 30 minutes and then cover-slipped. These sections were used to visualize the regions of interest for immunohistochemical staining and stereological quantification of neural cell density.

Regions of interest

Figure 1 shows the two areas of interest for the present study are the nucleus accumbens (NAcc), which comprises most of the ventral striatum (VS), and ventral pallidum (VP). The VS, along with the dorsal striatum, comprise the primary input structure of the basal ganglia. The NAcc is heavily involved in the mesolimbic dopaminergic reward pathway, and mediates reward salience and social behavior (Middleton 2000; Middleton & Strick 2000; Haber 2003; Seger 2006; Packard and Knowlton 2002). It sends the majority of its projections to the ventral pallidum. The VP mediates motor function in response to rewarding stimuli and is necessary for assigning the proper valence to reward (Haber 2011; Smith et al. 2009; Ottenheimer 2018).

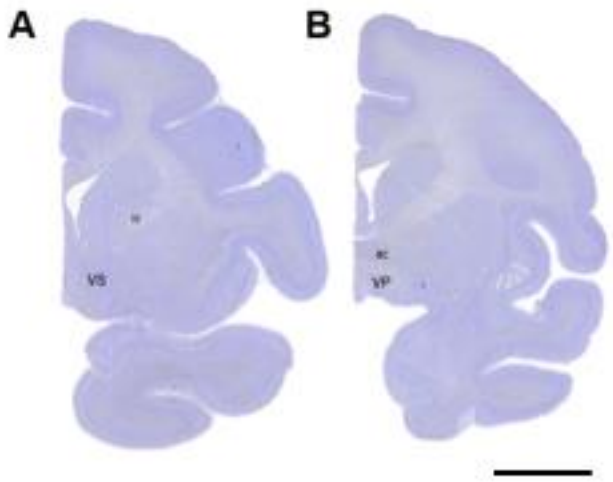

Figure 1: A) Nissl stained coronal section of left hemisphere of *M. mulatta* striatum showing the ventral striatum (**VS**) and internal capsule (**ic**). B) Nissl stained coronal section of left hemisphere of *M. mulatta* showing the ventral pallidum (**VP)** below the anterior commissure (**ac**). Scale bar at .5 inches

Immunohistochemistry

A minimum of three sections containing the NAcc and VP per individual were used for immunohistochemical studies. The sections were stained for tyrosine hydroxylase (TH) to allow for quantification of dopaminergic axon density. TH is the rate limiting enzyme for catecholamine synthesis (Cooper et al., 2002). Floating tissue sections were stained using the avidin-biotin-peroxidase method. Sections were removed from the freezer and rinsed 10 x 5

minutes in phosphate buffered solution (PBS). Sections were pretreated for antigen retrieval by incubating in 0.05% citraconic acid (pH 7.4) at 86°C in a water bath for 30 minutes. Sections were then rinsed, and endogenous peroxidase was quenched using a solution of 75% methanol, 2.5% hydrogen peroxide (30%), and 22.5% distilled water for 20 minutes at room temperature. Sections were preblocked in a solution of 4% normal goat serum, 0.6% Triton X-100 detergent, 90.7% PBS, and 5% bovine serum albumin. Following this, sections were incubated in primary antibody diluted to 1:1,000 in PBS for 24 hours at room temperature and then 24 hours at 4° C. After incubation in primary antibody, the tissue was incubated in in biotinylated secondary antibody (1:200) in a solution of PBS and 2% normal goat serum for 1 hour at room temperature. Sections were then incubated in avidin-peroxidase complex (PK-6100, Vector Laboratories, Burlingame, CA) for 1 hour at room temperature. A 3,3' -diaminobenzidine-peroxidase substrate with nickel solution enhancement was used as the chromogen (SK-4100, Vector Laboratories).

Data Collection

Axon length densities (ALv) were obtained using StereoInvestigator software (MBF Bioscience, Williston, VT, USA, version 9) on an Olympus BX-51 photomicroscope equipped with a Ludl X, Y motorized stage, Heidenhain z-axis encoder, and digital camera that projects images onto a 24-inch LCD flat panel monitor. Three sections, at an interval of every 10 sections, were quantified for each individual. The SpaceBalls program was used at 100x magnification for quantification of TH axon length density. The hemisphere setting was a radius of 7µm and the probe height was 7µm. Section thickness was measured every fifth sampling site. Summary statistics for counting parameters are listed in **Tables 2 and 3.**

Adjacent Nissl-stained sections were used to obtain neuron and glia densities. The NAcc and VP were outlined at 4x magnification and the optical fractionator program was used to manually count neurons and glia at 40x magnification with a counting frame of 2500 x 2500 µm. The optical dissector height was 7 µm and section thickness was measured every 5th sampling site. Neurons were identified by the presence of a large, lightly stained nucleus and a distinct nucleolus, ad lightly stained dendritic processes. Glia cells do not possess a visible nucleolus or dendritic processes.

The major variable for detecting among-species differences was ALv/Nv, as this measure takes into consideration brain size and neuron density. Brain size varies significantly within this sample (9.54-1200cc^3), and as a result, ALv was normalized by dividing by neuron density (Nv). TH-ir axons innervate neurons, therefore ALv/Nv may be loosely interpreted as innervation per neuron. We also evaluated glia densities (Gv) and glia to neuron ratios because they provide a measure of metabolism for different brain regions and were able to be quantified while obtaining neuron densities.

Species	Neuron/ Glia/ Axon	# of markers	# of sites	Section Thickness	Estimated Pop./Axon Length	Sampling Grid Area (XY) (µm2)	Grid (X) (µm)	Grid (Y) (µm)	Planimetric Volume	Guard Zone
S. oedipus	Neuron	150.72	31	12.11	3578.68	34760.28	169.40	203.99	37611444.44	0.1
	Glia	138.44	31	12.11	3434.75	34760.28	169.40	204.01	37611444.44	0.1
	Axon	124.33	29	15.37	759079.19	31546.08	178.88	176.66	37295538.89	0.1
C. apella	Neuron	88.06	31	11.95	6273.86	117968.82	331.45	334.81	134629905.55	0.1
	Glia	200.89	31	12.51	15778.39	100483.66	312.79	284.80	115195644.44	0.1
	Axon	114.56	29	23.98	3571358.85	111529.18	314.93	349.94	131004861.11	0.2
M. nemestrina	Neuron	109.22	31	9.82	13236.69	211174.39	482.88	438.31	244945555.56	0.1
	Glia	172.06	31	9.82	20492.51	211174.39	482.88	438.31	244945555.56	0.1
	Axon	87.17	30	18.47	2908394.42	158820.27	347.86	438.43	187061238.89	0.1
M. mulatta	Neuron	121.72	31	11.67	15767.25	205143.84	444.29	462.95	239566833.33	0.1
	Glia	186.78	31	11.67	26281.35	205143.84	444.29	462.95	239566833.33	0.1
	Axon	80.22	30	27.71	4296389.70	153386.12	434.63	357.47	181666322.22	0.2
P. troglodytes	Neuron	125.60	31	13.65	36962.74	421583.20	729.01	590.53	498967800.00	0.1
	Glia	149.07	31	13.54	41131.47	382428.40	697.27	559.78	454258866.67	0.1
	Axon	90.07	30	26.15	5632852.58	158019.00	499.90	388.60	235849133.33	0.1
P. paniscus	Neuron	121.47	32	16.35	49764.09	469887.73	811.62	569.26	557704866.67	0.1
	Glia	156.33	32	16.35	61802.90	469887.73	811.62	569.26	557704866.67	0.1
	Axon	166.20	30	27.68	10023241.33	175554.00	460.63	385.20	208698800.00	0.2
H. sapiens	Neuron	57.78	30	12.87	38755.27	857711.61	794.86	1093.94	992083277.78	0.1
	Glia	198.67	30	12.87	124145.49	857711.61	794.86	1093.94	992083277.78	0.1
	Axon	185.61	30	20.92	29458285.17	660929.33	907.97	737.33	790594444.44	0.2

Table 2: Summary counting parameter averages by species within the NAcc.

Species	Neuron/ Glia/ Axon	# of markers	# of sites	Section Thickness	Estimated Pop./Axon Length	Sampling Grid Area (XY) (µm2)	Grid (X) (µm)	Grid (Y) (µm)	Planimetric Volume	Guard Zone
S.oedipus	Neuron	72.67	31	13.89	5321.31	95019.27	398.30	242.84	105999755.56	0.1
	Glia	228.61	31	13.89	16234.45	95019.27	398.30	242.84	105999755.56	0.1
	Axon	79.28	29	23.31	1840098.54	357.24	357.24	208.39	88924683.33	0.2
C. apella	Neuron	50.50	30	13.69	9317.77	255692.67	550.98	476.36	295052055.56	0.1
	Glia	211.67	30	13.69	41086.65	255692.67	550.98	476.36	295052055.56	0.1
	Axon	65.39	30	29.37	5546094.21	588.52	588.52	436.57	300893777.78	0.2
M. nemestrina	Neuron	27.06	31	11.76	5049.82	281152.72	908.08	310.97	325041000.00	0.1
	Glia	172.39	31	11.76	34232.89	281152.72	908.08	310.97	325041000.00	0.1
	Axon	56.61	29	19.81	3629205.31	575.47	575.47	478.30	315467111.11	0.1
M. mulatta	Neuron	42.28	31	15.78	9591.85	252562.00	715.60	357.05	293677222.22	0.1
	Glia	217.28	31	15.78	50376.73	252562.00	715.60	357.05	293677222.22	0.1
	Axon	47.89	30	31.46	4118605.63	623.96	623.96	357.44	261969944.44	0.2
P. troglodytes	Neuron	42.92	31	14.19	5641.99	191261.30	318.57	564.68	219229200.00	0.1
	Glia	160.25	31	14.19	22665.64	191261.30	318.57	564.68	219229200.00	0.1
	Axon	46.83	30	26.85	3571410.20	588.35	588.35	387.08	266693083.33	0.1
P. paniscus	Neuron	21.75	31	16.93	3662.37	152620.07	423.71	424.16	174136025.00	0.0
	Glia	168.75	31	16.93	25612.76	152620.07	423.71	424.16	174136025.00	0.0
	Axon	48.25	30	23.03	2122888.61	419.15	419.15	309.97	154750683.33	0.1
H. sapiens	Neuron	28.72	30	14.12	15361.97	546378.89	986.52	564.62	625810944.44	0.4
	Glia	198.72	30	14.12	98428.04	546378.89	986.52	564.62	625810944.44	0.4
	Axon	113.11	30	24.00	14033507.31	928.99	928.99	566.69	581786000.00	0.2

Table 3: Summary counting parameter averages by species within the VP.

Statistical Analysis

IBM SPSS software (MBF Bioscience, Williston, VT, USA, version 11) was used to analyze the data. Among-species differences were evaluated using Analysis of Variance (ANOVA) for each of the variables in the NAcc and VP separately. The dependent variables were TH ALv TH ALv/Nv, Nv, Gv, and glia-to-neuron ratio (G/N). A Brown-Forsythe correction was applied when Levene's test for homogeneity of variance was significant. Finally, significant results were evaluated using Fisher's Least Significant Difference (LSD) post hoc tests. Separate independent sample T-tests were run for each species to test for differences between sexes for each dependent variable. The results from these analyses were non-significant.

Chapter 3: RESULTS

Figure 3 provides examples of TH immunohistochemical staining of the NAcc and VP from a representative individual from each species. Summary data are listed in **Tables 4 and 5.** The mean number of sampling sites for total nucleus accumbens nuclei/individual was 92.7 for Nv and Gv (total sampling sites = 3,708; total neurons = 13,201; total glia counted=20,609) and 89.4 for ALv (total sampling sites = 3,576). The mean number of sampling sites for total ventral pallidum nuclei/individual was 92.32 for Nv and Gv (total sampling sites = 3,508, total neurons counted = 4,758; total glia counted= 22,464) and 89.37 for ALv (total sampling site = 3,396).

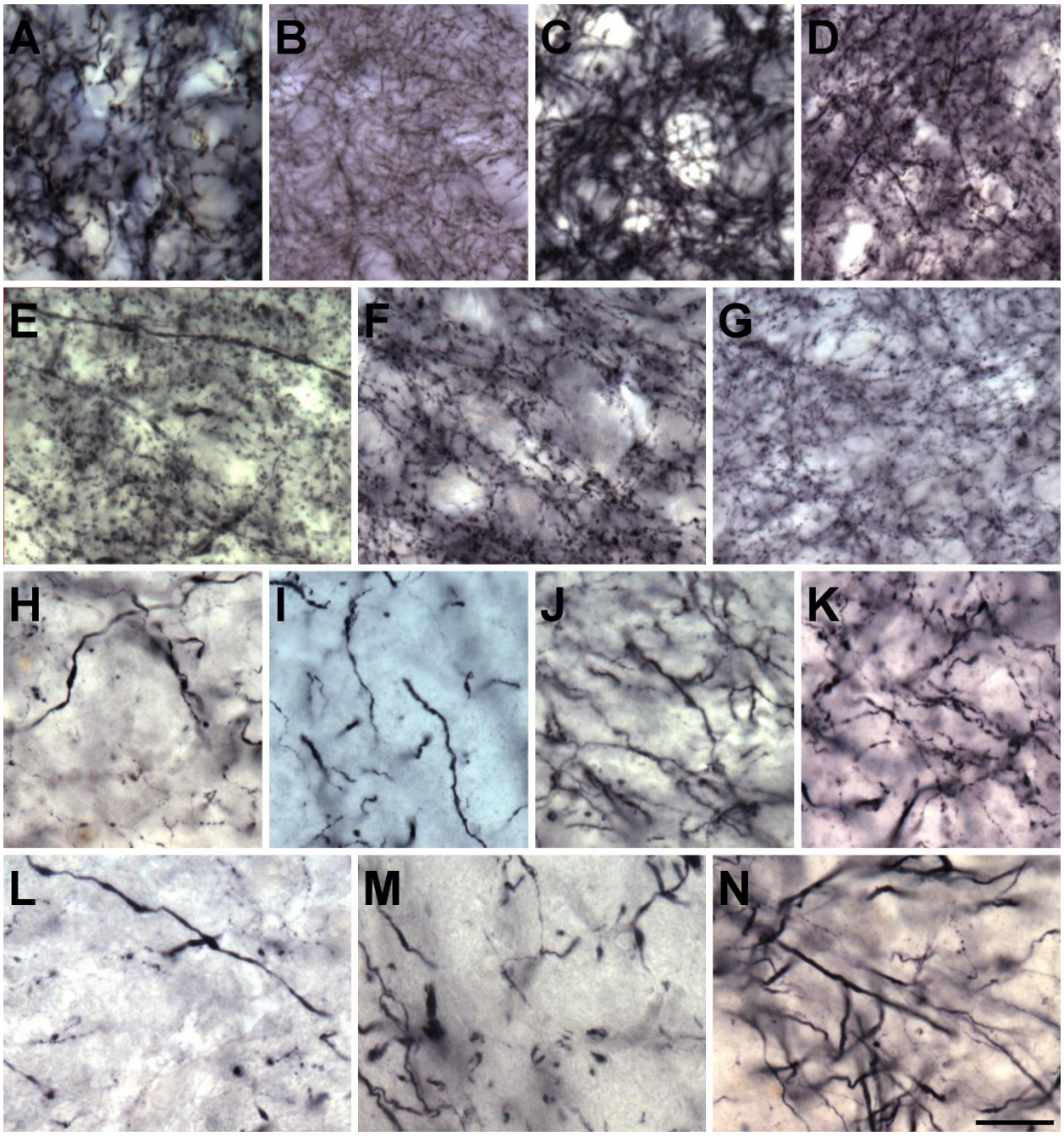

Figure 3: TH-ir axons in the NAcc (A-G) and VP (H-N) in corresponding order: *Saguinus oedipus* (A,H), *Cebus apella* (B,I), *Macaca nemestrina* (C,J) ,*Macaca mulatta* (D,K), *Pan troglodytes* (E,L), *Pan paniscus* (F,M), *Homo sapiens* (G,N). Scale Bar at 25 μm.

	Species	N	Mean	SD	SE
TH ALv/Nv	*S. oedipus*	6	229.338	99.547	40.640
	C. apella	6	467.390	167.437	68.356
	M. nemestrina	6	363.780	172.945	70.604
	M. mulatta	6	256.578	84.694	34.576
	P. troglodytes	5	352.699	47.346	21.174
	P. paniscus	5	526.668	135.067	60.404
	H. sapiens	6	1242.178	801.756	327.315
TH ALv	*S. oedipus*	6	20456808.23	6463875.14	2638865.975
	C. apella	6	29610015.16	15075605.46	6154590.158
	M. nemestrina	6	17355095.46	7203398.79	2940775.242
	M. mulatta	6	17766445.94	7115746.85	2904991.487
	P. troglodytes	5	26979932.71	2958395.40	1323034.645
	P. paniscus	5	47311086.49	13961461.65	6243755.462
	H. sapiens	6	38852153.02	10445364.77	4264302.311
Nv	*S. oedipus*	6	94391.322	25711.777	10496.789
	C. apella	6	61559.232	44520.925	18175.592
	M. nemestrina	6	51287.665	20536.651	8384.053
	M. mulatta	6	67524.679	22498.335	9184.907
	P. troglodytes	5	77714.670	14057.822	6286.849
	P. paniscus	5	94127.918	30620.532	13693.918
	H. sapiens	6	38804.366	17699.873	7225.943
Gv	*S. oedipus*	6	88419.631	20584.537	8403.602
	C. apella	6	137895.891	41112.575	16784.138
	M. nemestrina	6	82575.946	15091.835	6161.216
	M. mulatta	6	110359.182	35704.320	14576.228
	P. troglodytes	5	96138.676	52820.491	23622.042
	P. paniscus	5	120157.353	55220.172	24695.212
	H. sapiens	6	133250.742	47120.744	19236.963
G/N Ratio	*S. oedipus*	6	1.02	0.425	0.173
	C. apella	6	2.998	1.763	0.720
	M. nemestrina	6	1.745	0.508	0.207
	M. mulatta	6	2.014	1.442	0.589
	P. troglodytes	5	1.238	0.624	0.279
	P. paniscus	5	1.298	0.399	0.178
	H. sapiens	6	4.428	3.731	1.523

Table 3: Descriptive Statistics for each variable/species within the NAcc

	Species	N	Mean	SD	SE
TH ALv/Nv	*S. oedipus*	6	391.604	112.072	45.753
	C. apella	6	598.778	169.299	69.116
	M. nemestrina	6	783.925	215.621	88.027
	M. mulatta	6	468.964	103.664	42.321
	P. troglodytes	4	515.247	252.507	126.254
	P. paniscus	4	615.705	171.883	85.941
	H. sapiens	6	1579.374	817.598	333.783
TH ALv	*S. oedipus*	6	19557066.79	4767129.31	1946172.39
	C. apella	6	19984428.32	8288468.73	3383753.19
	M. nemestrina	6	11345480.36	2104688.17	859235.35
	M. mulatta	6	14832980.66	4566302.26	1864185.09
	P. troglodytes	4	12877586.34	1564131.28	782065.64
	P. paniscus	4	11379280.85	3935412.68	1967706.34
	H. sapiens	6	24707901.07	10494620.21	4284410.76
Nv	*S. oedipus*	6	51120.274	8495.719	3468.363
	C. apella	6	33029.964	9107.848	3718.264
	M. nemestrina	6	15333.174	4712.827	1924.003
	M. mulatta	6	31472.746	4827.365	1970.764
	P. troglodytes	4	32984.229	22449.201	11224.601
	P. paniscus	4	19540.773	7938.179	3969.089
	H. sapiens	6	20522.579	12709.677	5188.704
Gv	*S. oedipus*	6	162862.424	41059.708	16762.556
	C. apella	6	141935.992	35705.324	14576.637
	M. nemestrina	6	101138.521	20833.839	8505.379
	M. mulatta	6	167385.048	47208.166	19272.653
	P. troglodytes	4	118253.754	34197.816	17098.908
	P. paniscus	4	139552.321	44864.172	22432.086
	H. sapiens	6	145732.409	62153.404	25374.021
G/N Ratio	*S. oedipus*	6	3.236	0.835	0.341
	C. apella	6	4.391	0.840	0.343
	M. nemestrina	6	6.866	1.517	0.619
	M. mulatta	6	5.249	0.828	0.338
	P. troglodytes	4	5.214	4.199	2.100
	P. paniscus	4	7.842	2.662	1.331
	H. sapiens	6	9.443	6.052	2.471

Table 4: Descriptive Statistics for each variable/species in the VP

Among Species Analysis

Among-species differences were evaluated using Analysis of Variance (ANOVA) for TH ALv/Nv, TH ALv, Nv, Gv, and G/N ratio in the NAcc and VP separately. **Table 5** offers a summary of among species post hoc analyses for both regions**.**

NAcc

NAcc TH ALv/Nv results show a significant species difference ($F_{6, 33}$= 6.891, $p < 0.025$; **Figure 4**). Post hoc analyses reveal *Homo sapiens* possessed significantly higher TH ALv/Nv in the NAcc compared to nonhuman primate species. **NAcc TH ALv** was also different among species ($F_{6,33}$= 7.228, $p < 0.025$; **Figure 5**). Post hoc analyses revealed that *Pan paniscus* possessed significantly higher absolute TH ALv than every other species. *H. sapiens* possessed significantly higher TH ALv than *Saguinus oedipus*, *Macaca nemestrina*, and *Macaca mulatta*. Additionally, *Cebus apella* possessed significantly higher TH ALv compared to both species of macaque. **NAcc Nv** results reveal a significant difference among species ($F_{6,33}$= 3.464, $p < 0.025$; **Figure 6**). Post hoc analyses revealed that *S. oedipus* possessed significantly higher neuron density than *C. apella*, *M. nemestrina*, and *H. sapiens. M. nemestrina* possessed significantly higher neuron density than *P. paniscus*. *P. paniscus*, *Pan troglodytes*, and *H. sapiens* all differed significantly from each other, with neuron density lower in *H. sapiens* and higher in *P. paniscus*. **NAcc Gv** ($F_{6,33}$= 1.658, $p > 0.025$; **Figure 7**) as well as **NAcc G/N** ratio analyses ($F_{6,33}$= .060 $p > 0.025$; **Figure 8**) did not exhibit among-species differences.

VP

VP TH ALv/Nv was significantly different among species ($F_{6,31}$= 7.925, p < 0.025; **Figure 4**). Post hoc analyses revealed that *H. sapiens* possessed significantly higher ALv/Nv than nonhuman primate species. **VP TH ALv** analyses revealed a significant difference as well ($F_{6,31}$= 3.751 p < 0.025; **Figure 5**). Post hoc analyses found that humans possessed higher TH ALv than *M. nemestrina, M. mulatta, P. troglodytes*, and *P. paniscus*. Moreover, *S. oedipus* and *C. apella* possessed significantly higher TH ALv than *M. nemestrina*. A significant effect of species was detected for **VP Nv** ($F_{6, 31}$= 6.199, p< 0.025; **Figure 6**). Post hoc analyses revealed that *S. oedipus* possessed significantly higher neuron density than all other species. *M. nemestrina* possessed significantly lower neuron density than *C. apella*, *M. mulatta*, and *P. troglodytes*. **VP Gv** ($F_{6,31}$= 1.704, p > 0.025; **Figure 7**) and **VP G/N ratio** ($F_{6,31}$= 2.761, p > 0.025; **Figure 8**) analyses were not different among species.

Region	Species	TH Alv/Nv	TH Alv	Nv	Gv	G/N Ratio
NAcc	*S. oedipus*	<HS	<HS,PP	>CA,MN,HS	-	-
	C. apella	<HS	>MN,MM;<PP	<CA	-	-
	M. nemestina	<HS	<CA,HS,PP	>PP	-	-
	M. mulatta	<HS	<CA,HS,PP	-	-	-
	P. troglodytes	<HS	<PP	<PP	-	-
	P. paniscus	<HS	>all	>PP,PT;<MN	-	-
	H. sapiens	>all	>SO, MN,MM;<PP	<PP,PT	-	-
VP	*S. oedipus*	<HS	>MN	>all	-	-
	C. apella	<HS	>MN	<SO;>MN	-	-
	M. nemestina	<HS	<SO,CA,HS	<CA,MM,PT,SO	-	-
	M. mulatta	<HS	<HS	>MN;SO	-	-
	P. troglodytes	<HS	<HS	<SO;>MN	-	-
	P. paniscus	<HS	<HS	<SO	-	-
	H. sapiens	>all	>MN,MM,PT,PP	<SO	-	-

Table 5: Summary of among species analyses. Dashes indicate non-significant results. Abbreviations are as follows: *Saguinus oedipus* (SO), *Cebus apella* (CA), *Macaca nemestrina* (MN), *Macaca mulatta* (MM), *Pan troglodytes* (PT), *Pan paniscus* (PP), *Homo sapiens* (HS).

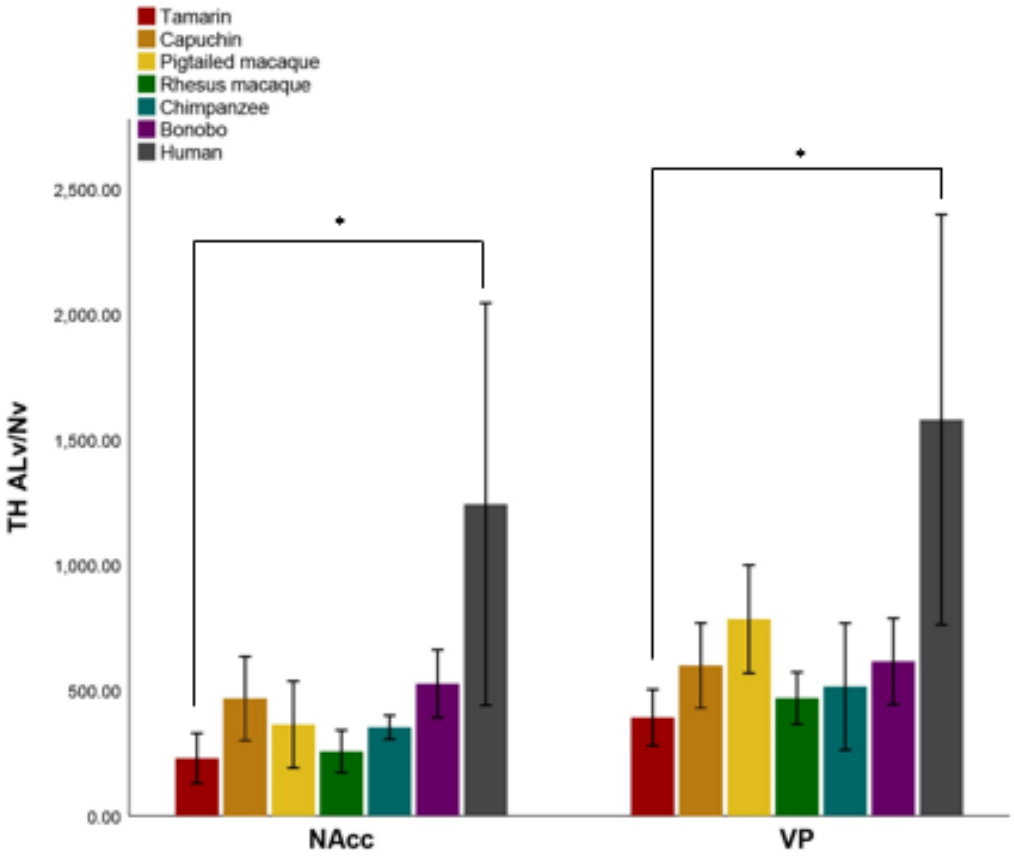

Figure 4: Mean TH-ALv/Nv in the nucleus accumbens (NAcc) and ventral pallidum (VP) by species. Error bars: +/- 1 SD

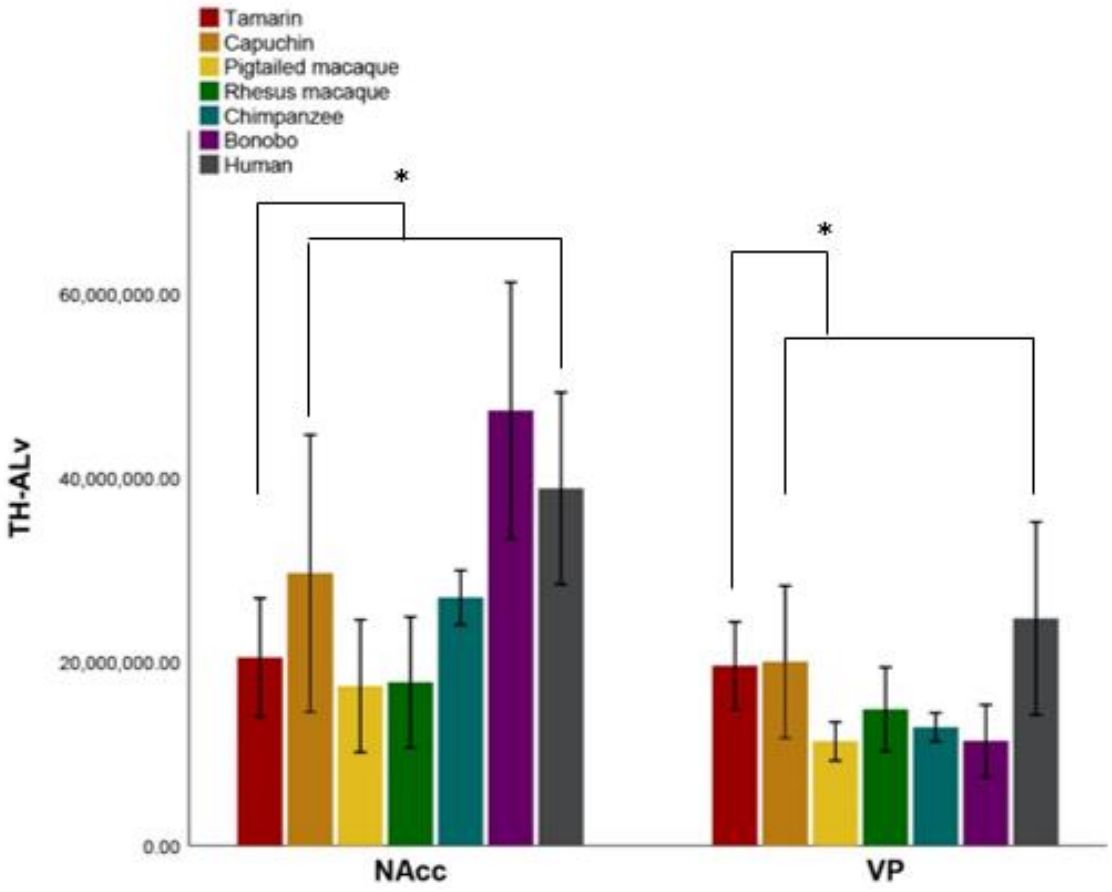

Figure 5: Mean TH Alv in the nucleus accumbens (Nacc) and ventral pallidum (VP) by species. Error bars: +/- 1 SD

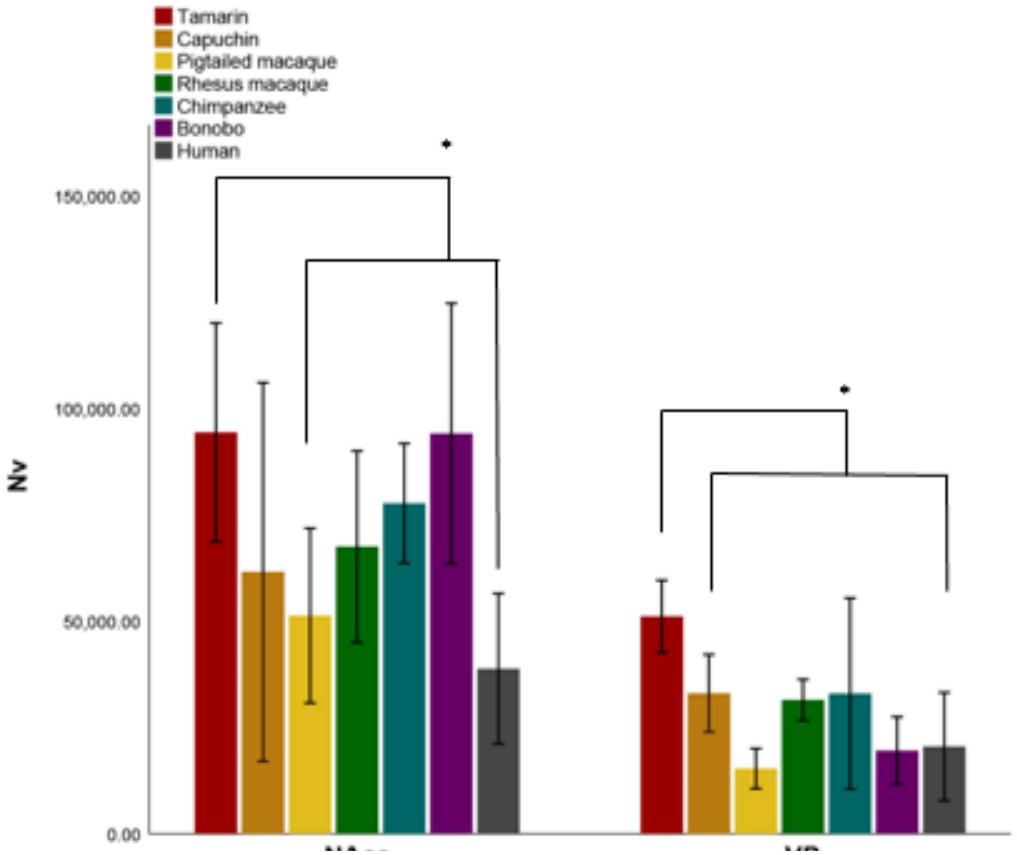

Figure 6: Mean Nv in the nucleus accumbens (NAcc) and ventral pallidum (VP) by species. Error bars: +/- 1 SD

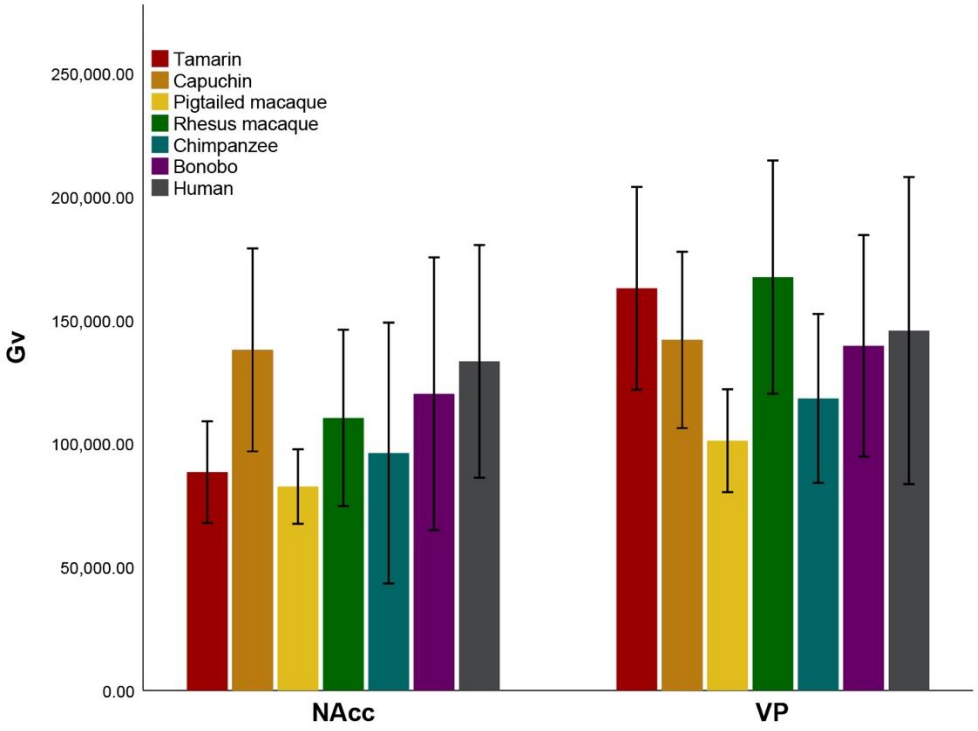

Figure 7: Mean Gv in the nucleus accumbens (NAcc) and ventral pallidum (VP) by species. Error bars: +/- 1 SD

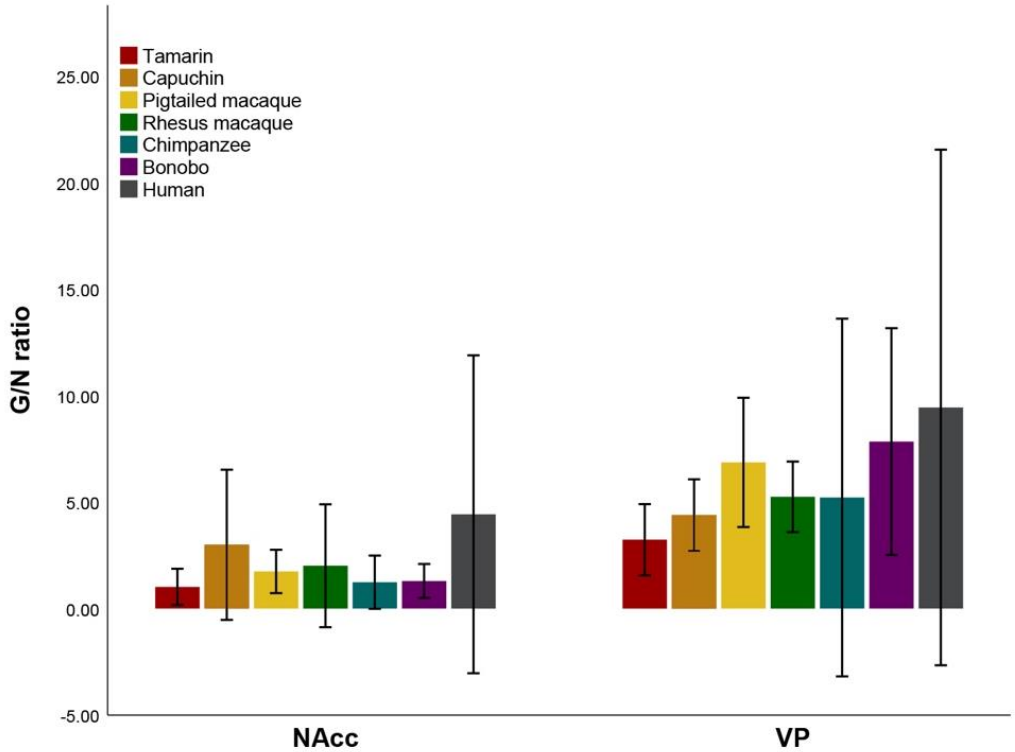

Figure 8: Mean G/N ratio in the nucleus accumbens (NAcc) and ventral pallidum (VP) by species. Error bars: +/- 1SD

Chapter 4: DISCUSSION

The present study compared dopaminergic innervation within the NAcc and VP among seven primate species. Neuron density, glia density, and glia-to-neuron ratio were also examined. The major variable for detecting among-species differences was ALv/Nv, as this measure takes into consideration brain size and neuron density. Our results revealed higher dopaminergic innervation in the human ventral striatum and ventral pallidum compared to nonhuman primates. This pattern is in accordance with the unique neurochemical profile in the dorsal striatum that was reported recently (Raghanti et al. 2018). Evidence for higher DA innervation in both the dorsal and ventral striatum in humans compared to nonhuman primates supports the hypothesis that humans possess a dopamine-dominated striatum (DDS) personality style, and initial changes towards this striatal neurochemistry would have been favored in our early ancestors (Raghanti et al. 2018). Additionally, differences across species were found for absolute TH ALv and Nv, with neuron density presenting a different pattern than what is typically expected in cortical areas (Haug 1987). Interestingly, the species that exhibits pair-bonds, *Saguinus oedipus*, did not possess significantly greater TH innervation in the ventral striatum than did the polygynous species. Although investigations of pair-bonded species and their neurochemistry are rare, a position emission tomography (PET) study on titi monkeys found differences in glucose uptake in the NAcc and VP in pair-bonded verses lone males (Bales et al. 2007). Species differences in voles indicate that higher D2 receptor densities in the NAcc play a pivotal role in monogamy (Aragona et al. 2004, Liu 2003). While the NAcc is undoubtedly involved in the formation and maintenance of selective social bonds, more research is needed to

understand the long-term maintenance, as well as any species-specific differences that are involved in this ultimately rare mating strategy. Examination of additional pair-bonding species would be required to clarify the relationship between dopamine, the ventral striatum, and pair-bonding. Moreover, *Pan paniscus* did not possess greater innervation compared to their *Pan troglodytes* relatives. This finding was unexpected as *P. paniscus* exhibits behavioral and physiological characters that were likely pivotal in early hominids more than does *P. troglodytes*. However, these species differences may be mediated by other previously reported neurotransmitter systems and brain regions (Hopkins et al. 2009, Rilling et al. 2012, Stimpson et al. 2015). *P.paniscus* individuals have more gray matter and connectivity in brain regions involved in social behaviors, including the amygdala and anterior insula, a region of the brain involved in social engagement and perception (Rilling et al. 2012). *P. paniscus* also exhibits striatal asymmetry and has less putamen volume than *P. troglodytes*. Moreover, differential serotonergic innervation of the amygdala between the two species has been documented, revealing that *P.paniscus* possessed more than twice the density of serotonergic axons than their *P. troglodytes* relatives (Stimpson et al. 2015).

Support for the Neurochemical Hypothesis of Hominid Origins

The current study supports the hypothesis that selection for a prosocial neurochemistry in the basal ganglia of earliest hominids facilitated the emergence of our species from the last common ancestor (LCA) we shared with the *Pan* clade. Although the fossil record cannot provide access to the neurochemistry of extinct species, *Ardipithecus* informs us that a unique, comprehensive suite of adaptations was necessary to overcome extensive disadvantages such as bipedal locomotion, intensely K-selected demography, and loss of the SCC (Lovejoy 1981,

White et al. 2009, Lovejoy 2009). We propose the presence of a DDS personality style would have modulated affiliative behaviors that encouraged pair-bonding and provisioning, a positive-feedback scenario that increases both individual reproductive success and socialization (Raghanti et al. 2018). Increased awareness of habitat, decreased aggression, and social conformity all associated with the DDS personality style would have led to improve survivorship and reproductive success, and ultimately would have been under strong selection (Raghanti et al. 2018). Moreover, the exaggeration of the DDS style would provide the social intelligence associated with the intense characteristics we see today, such as language, empathy, and altruism (Raghanti et al. 2018).

Future Directions

The present study is limited in its ability to serve as a basis for drawing conclusions about the neural substrates of social reward, pair-bonding, and affiliative behavior mainly because only a single neurotransmitter system in one brain region was examined. Social behaviors are modulated by widespread, complex neural networks, and investigations of additional brain regions and neurotransmitter systems must be conducted to further clarify our hypothesis.

A holistic neurochemical profile has been constructed for the dorsal striatum in numerous primate species and has revealed that humans possess higher levels of serotonin and neuropeptide Y as well as lower acetylcholine than do nonhuman primates (Raghanti et al. 2018). A future study may further characterize the ventral striatum in this fashion, including analyses of these neurotransmitters, each of which is implicated in various aspects of social behavior (van den Bos 2015, Cools et al. 1975, Raleigh et al 1991, Higley et al 1996). For example, striatal serotonin activity levels appear to in part mediate behavioral inhibition and

cognitive control with respect to emotions (van den Bos 2015, Raleigh et al. 1991, Higley et al. 1996). Moreover, serotonin has been found to be co-regulated by dopamine signaling, making serotonin innervation in the ventral striatum a further point of interest (Seo et al. 2008). A complete neurochemical profile of the striatum in several primate species, including humans, can provide additional insights into human evolution and the nature of *Homo* clade's fitness and social behavior.

Moreover, investigating additional brain areas that communicate extensively with the striatum can illuminate its role in social behavior and cognitive processing. The ventral striatum uniquely communicates with the amygdala, the center of emotional processing, and in-depth analyses of this region would provide additional species comparisons (Haber 2011).

Additionally, another future study could include a detailed analysis on subjecting the NAcc and its composite regions, the core and the shell, to further analyses. Since the core and shell are responsible for modulating distinct aspects of reward, the localization would provide further inferences into species-specific differences associated with reward encoding and social behavior (Haber 2011). Histologically, the core and shell are homogenous. However, immunohistochemical labeling with calbindin (CB) reveals core-shell boundaries (McCollum & Roberts 2014). CB staining of adjacent sections to the Nissl and TH-labeled sections in the current study, and consequent stereological quantification with intentional core-shell boundaries, could be future focus and useful addition to the current study.

Exploring neurochemical characteristics of other group-living animals outside the primate order that are known to create long-lasting affiliative and emotional ties is another possible future study. For example, several elephant species have been shown to exhibit cooperative social behavior (Shulte 2000). Similarities in social structure between other animals

and humans can be used to further explore questions about the evolution of affiliative social behavior.

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