UNIVERSITY OF CALIFORNIA, SAN DIEGO

Social Behavior from a Comparative Neuroanatomical Perspective: The Amygdala in Human Evolution

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Anthropology

by

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The Dissertation of Nicole L. Barger is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

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2012

DEDICATION

To my parents and grandparents who, despite not knowing much about the inner workings of the brain, have always excelled at instructing me in its use.

EPIGRAPH

Would I rather have a “miserable” ape for a grandfather or a man highly endowed by nature and possessed of great means and influence and yet who employs these faculties and that influence for the mere purpose of introducing ridicule into a grave scientific discussion?

–I unhesitatingly affirm my preference for the ape.

Thomas Henry Huxley

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF THE DISSERTATION

Social Behavior from a Comparative Neuroanatomical Perspective:

The Amygdala in Human Evolution

by

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Anatomically, the amygdala and its 13 component nuclei integrate higher order perceptual information from the neocortex with subcortical neuroendocrine and autonomic centers. Functionally, it is a central constituent in the neural circuits subserving social and emotional behavior. An increasingly influential hypothesis posits that primate intelligence has arisen in response to challenges in the social *milieu*. However, structures associated with emotion processing, i.e., "limbic" structures, have been historically construed as primitive. Given these competing claims, this dissertation seeks to assess the amygdala's role in human brain evolution by quantifying and

comparing volumes and neuron counts for the amygdala and four of its major nuclei (the lateral, basal, accessory basal, and central nuclei) in a large sample comprising humans and all ape genera.

Analyses revealed three core findings. First, human amygdala volume has tripled in size, but neuron numbers have been maintained in great ape ranges, suggesting that changes in neuron number and volume may be decoupled in human amygdala evolution. Second, the intrinsic organization of the human amygdala is unique compared with ape amygdala. Specifically, the human lateral nucleus is 37% larger and contains 59% more neurons than expected, departing from the organization of ape amygdala which emphasize the basal nucleus. Conversely, the central nucleus was three times smaller in the human amygdala than expected. As the lateral nucleus is the major recipient of neocortical, especially temporal lobe, input and the central nucleus is the major source of autonomic output, human amygdala reorganization may reflect the expansion and conservation of these interconnected anatomical regions, respectively. Third, the human lateral nucleus is one of the most expansive structures in the human limbic system, second only to the hippocampus. Volumetric increases in the lateral nucleus and amygdala also outpace expansion in the dorsal frontal cortex, a region associated both with executive and motor, but not emotional, function, indicating that emotion processing is not uniquely or especially de-emphasized in human brain evolution. Overall, it may be speculated that increases in the human lateral nucleus arose in response to a heightened need to process the emotional salience of stimuli in the complex human social environment.

Chapter 1

Introduction

Humans participate in extensive social networks, employ elaborate verbal and nonverbal communicative repertoires, and form social groups that are disproportionately composed of non-kin, all features which distinguish human social behavior from even that of our closest relatives, the great apes (Dunbar, 1993; Hill et al., 2011). It is perhaps surprising, then, that modern comparative work has revealed only a few distinctions in the gross organization and patterning of the human brain when compared with great apes (Sherwood et al., 2008; Semendeferi et al., 2011). While these findings point to evolutionary continuity in the structure of human and ape brains, very few studies have targeted regions that explicitly subserve social affiliation despite the central role social behaviors play in key human adaptations. As the complexity of the social environment increases, it has been hypothesized that cognitive systems dedicated to interpreting the identities, communicative signals, intentions, and minds of social partners may become increasingly taxed, theoretically spurring the evolution of advanced cognition in primates (Dunbar, 2009; Humphrey, 1976; Jolly, 1966; Byrne, 1996; Byrne and Bates, 2007). The amygdala, a subcortical structure located on the medial surface of the temporal lobe, has long been associated with social behavior in human and non-human primates (Kling, 1986; Brothers, 1990 a). This dissertation investigates the proposition that the human amygdala may have become specialized in human evolution, perhaps as a response to the qualitative and quantitative distinctions in social behaviors that characterize the human species.

SOCIALITY, EMOTION, AND PRIMATE COGNITIVE EVOLUTION

What is "special" about primate social cognition?

This question must first be met with a caveat, as there is some disagreement as to whether the behavior of other large-brained social mammals, e.g., elephants, is analogous to primate social behavior given the paucity of behavioral data available for many species (Shultz and Dunbar, 2007; Byrne and Bates, 2010). Nonetheless, several decades of research converge on the idea that the primate social environment presents unique challenges not experienced by most mammalian taxa. Primates are engaged in navigating complex, rapidly changing social environments, which, it is argued, require an understanding of and adherence to somewhat arbitrary rules, constraints, and conventions and an ability to monitor frequent fluctuations in social contingencies which may tax cognitive systems dedicated to interpreting the identities, communicative signals, intentions, and minds of other social actors (Humphrey, 1976; Jolly, 1966; Byrne and Bates, 2010). Theories of social ("Machiavellian") intelligence emphasize the importance of political behaviors, e. g., alliance formation, coalition building, and reconciliation, presumed to be premised on the social actor's awareness of and capability to monitor the relationships of all relevant conspecifics (Byrne & Whiten, 1997). Essentially, this requires knowledge of other social actors' identities, minds, and probable responses to changing social contingencies (Byrne, 2008) and may be particularly important to nonhuman primates, who, for example, utilize alliance formation more than any other mammal (Byrne & Whiten, 1997). An enhanced ability to solve problem in the social, as opposed to physical, environment may have arisen early in primate evolution in our

shared ancestors with social lemurs (Jolly, 1966) and is clearly still a feature of modern human cognition (Mesoudi et al., 2006).

Early theories of social intelligence were largely based on observations that conspecifics acted as important cognitive stimuli to group-living primates. Jolly (1966) remarked that, in primates, social partners are often more salient, i.e., valuable, stimuli than novel objects. When "intelligence is measured in relation to gadgetry", she suggested, primate cognitive abilities, construed as technical abilities, may be masked by the presence of social partners. Nicholas Humphrey (1977) was also struck by the motivational appeal of primate social partners. Regardless of the complexity or amount of enrichment devices provided for individually housed macaques in research facilities, he was shocked to find that group housed macaques were infinitely more stimulated simply by the presence of other macaques.

In Humphrey's and Jolly's estimation, the social environment provided a distinctly different and more challenging problem than the ecological environment which would influence the evolution of intelligence. Fundamentally, Humphrey (1976) pointed to the intransigent nature of social interactions, which, while intrinsically unstable, still follow a given logic, based on the context and the relationships shared by the participants in an interaction. As an adaptive environment, he posits, the social environment is somewhat unique and highly "problematical". Individual fitness concerns may drive competition for resources, but successful group living requires that individuals adhere to social "contracts". Thus, he theorized that the chief force driving the evolution of intellect was the difficult task of "hold[ing] society together". In complex groups, associations are often highly variable and subject to social rules, conditions, and constraints that

individuals must learn and employ. Successful navigation of these complex contingencies, he posited, can have a "ratchet" effect on intelligence over time. Of course, in primates, social rules are not genetically programmed, but are learned. Jolly (1966) suggested that one other characteristic feature of primates, a particularly protracted developmental period, is best explained by the need to "learn to be social", and it is this social learning requirement that is most likely to have driven intelligence.

Although it can be argued that sociality does not necessitate intelligence (e.g., social insects engage in social interactions with limited neural resources), primate social interactions have been argued to be of a qualitatively different nature. Primates engage in complex "tripartite social relations" in which three animals may undertake different roles in one interaction, directing their behavior towards as well as monitoring the responses of the two other parties involved (Kummer, 1967). To provide an example, female Hamadryas baboons utilize "protected threats", a behavior in which subordinate animal A positions itself behind dominant animal X in order to threaten subordinate animal B, who happens to be facing the dominant animal. The resultant effect is that A may threaten B, but that B may not return the threat without the simultaneous and dangerous act of also perturbing dominant animal X (Kummer, 1967). Cognitive complexity is proposed to underlie this interaction, as one individual (A) manipulates a third party (X) to accomplish the individual's intended goal, e.g., safely threatening B or eliciting protection from X. The primates involved must, at minimum, know their place in the dominance hierarchy, the rules associated with that position (don't threaten dominant individuals), and the relationships between the three animals involved in the interaction. An additional distinction of primate groups is the long duration of group tenure, in

contrast to the more loosely organized "social" groups of herd-dwelling ungulates (Byrne and Bates, 2010; Dunbar and Shultz, 2007). Not only do primates interact with group members differently than ungulates, but they also, ostensibly, need to keep track of a long history of such interactions (Dunbar and Shultz, 2007; Aureli and Schaffner, 2002). Thus, both the complexity and duration of relationships may be increased in primate groups and this may have consequences for intelligence.

Natural selection acts on behaviors, and these conceptualizations of complexity clearly purport ultimate causation linked to the behavioral challenges experienced by group living primates. But what may be occurring at more proximate levels? Several cognitive mechanisms have been outlined (Byrne and Bates, 2007, 2010). At the most basic level of cognitive processing, managing social complexity has been suggested to require a highly attuned perceptual system. This would involve the ability to effectively interpret communicative facial expressions, body postures, vocalizations, and a host of other social signals related to conspecifics' mood and disposition, in addition to enhanced abilities to selectively attend to and sustain focus on key features in the social environment (Byrne and Bates, 2007). Additionally, in primates, the ability to follow the gaze of conspecifics may be essential to assessing salient features of the environment. Built upon the perceptual system, an ability to predict and understand others' perceived actions may be essential to managing complexity and unpredictable social relationships (Byrne and Bates, 2010). Navigating social hierarchies and alliances would require considerable social knowledge of other individuals (Byrne and Bates, 2010) and their relationships with one another (Byrne and Bates, 2007), as well as an understanding of the social rules that govern these relationships (Humphrey, 1976). This would be

facilitated by rapid social learning (Byrne and Bates, 2007). Given that relationships between all individuals in a group need to be kept in mind, increased social group size and duration of tenure in a group would effectively result in exponential increases in memory load, requiring increasingly efficient long-term memory stores (Byrne and Bates, 2010).

Although higher-order cognitive process like executive function could clearly play a role in social intelligence, it is likely that emotional processes mediate every day social decision-making (Casebeer and Churchland, 2003; Aureli and Schaffner, 2002). Social relationships between individuals form the fundamental unit of complex social systems and each social relationship has its own history. However, social relationships may also provide primates with an ability to predict the future actions of social partners, given future interactions will be contextualized within their established relationship (Aureli and Schaffner, 2002). How do animals assess the strength and nature of these relationships? Aureli and Schaffner (2002) have suggested that, in complex social systems, social emotional processing can accomplish a kind of social bookkeeping, in that the emotional system provides a means of summarizing the frequency and quality of past interactions within particular contexts. That is, emotions provide a rapid means of assessing relationships with both related and unrelated conspecifics. Emotion can function as an "intervening" variable, coordinating behavioral responses by reducing the potential range of behavioral options to a manageable few. For example, anxiety can mark the value of a social relationship. When conflict arises between "valuable" social partners, macaques exhibit increased rates of anxiety concomitant with increased tendencies to reconcile post-conflict (Aureli and Schaffner, 2002). The authors suggest

that anxiety reflects relationship assessment and guide behavioral choices, i.e., the choice to resolve the conflict. Anxiety may even function to pre-empt conflict with more valuable partners. This points to the interdependent nature of emotional and social processing in fundamental aspects of primate social cognition.

What is "special" about human social cognition?

Given we share a deep ancestry with other diurnal anthropoid primates, many facets of human social behavior are likely have arisen as an extension of the cognitive abilities of nonhuman primates. Nonetheless, human social organization appears to be of a qualitatively and quantitatively different nature (Hill et al., 2011; Hill and Dunbar, 2003). Several propositions have been raised regarding "unique" features of human social cognition.

Herrmann and colleagues (2007) have proposed the "cultural intelligence hypothesis" which posits that, while primates are social, humans are "ultra-social". Humans form distinct cultural groups with their own abstract symbols, artifacts, social practices, and institutions. In addition to advanced communicative capabilities and theory of mind, the authors propose that heightened social learning in childhood facilitates the acquisition of cultural behaviors and knowledge. In an extensive study comparing over 100 great ape individuals with over 100 human children, this group found human children routinely outperformed great apes in tests of social learning (solving a problem after viewing a demonstrated solution), communication (following communicative cues and gestures to obtain reward with or without considering the recipient's attentional state), and theory of mind (following gaze and understanding the intended outcome of an

action). Although one may question the construct validity of using human demonstrators to engage non-humans in "social" cognitive tasks, the authors indicate that choice to use humans or conspecifics interactants in previous analyses did not influence their findings.

Theory of mind has been regarded as the most likely qualitative cognitive specialization distinguishing human and non-human primate cognition (Herrmann et al., 2007; Byrne and Bates, 2007; Hare, 2011; Tomasello and Call, 1997). Higher order theory of mind is one domain in which humans are routinely regarded as outperforming nonhuman primates. Humans are capable of seeing both their behavior and the behavior of others as intentional and come to identify self with others (Tomasello and Call, 1997). Neither chimpanzees nor monkeys have been shown to pass "false belief" tasks, i.e., anticipating what another individual would expect to see when experimental contingencies are changed in that individual's absence (Byrne and Bates, 2010). In contrast, chimpanzees have been shown to be able to both monitor relevant gaze cues from other individuals and deceive competitors in natural and experimental contexts (Byrne and Corp, 2004; Hare et al., 2006), evidencing at least low level theory of mind, but primarily in competitive contexts. These findings have led to an additional hypothesis about human cognitive specializations, the "emotional reactivity hypothesis".

A new role for emotion in theories of human social cognitive evolution

Differences in emotional style limit social problem solving in chimpanzees, and it has been hypothesized that human emotional behavior has changed over the course of evolution to emphasize increased perceptual and emotional sensitivity to conspecifics which allowed for human-specific forms of social cooperation and problem solving (Hare

and Tomasello, 2005 a). Based on a number of experiments performed with humans, apes, and domestic dogs, Hare and colleagues (Hare, 2007; Hare and Tomasello, 2005 b; a) have proposed that major human adaptations occurred in the realm of social and emotional cognition, which released constraints on cooperative problem solving. This would involve heightened tolerance towards, cooperation with, and knowledge sharing with conspecifics (Hare, 2007; Herrmann et al., 2007, 2011). Concordant with previous arguments regarding the importance of joint attention and social motivation (Tomasello and Call, 1997), Hare posits that such flexibility using other's social cues evolved only in the human lineage after species-specific changes in social emotions increased the motivation to attend to the behavior of conspecifics. Some evidence links temperament, particularly anxiety, to human social adaptations. Heightened anxiety in novel contexts distinguishes the temperament of human infants from that of adult great apes (Herrmann et al., 2011) (although no ape infant apes were tested in this study). Several recent studies link emotion to social expertise. Theory of mind capability later in development is heightened in children who exhibit shy, withdrawn temperaments and perceptual sensitivity to social cues, but is inversely related to aggression (Wellman et al., 2011), while poor fear recognition is linked to poor theory of mind (Corden et al., 2006).

Hare's perspective is not singular. Many authors have emphasized the important role that socioemotional behavior may play in human cognitive and behavioral evolution, uniquely or as an extension of our primate ancestry. Attachment and bondedness to both related and unrelated conspecifics is suggested to influence fitness and neocortex volume across primate groups (Dunbar and Shultz, 2010; Silk, 2007; Dunbar, 2009). As the only primate to form cooperative affiliative groups with predominantly non-kin, humans may

exemplify an extreme manifestation of this trend (Hill et al., 2011). The contribution emotion makes to social-decision making and symbolizing is used to argue for the centrality of emotions to complex human social and cultural behaviors (Allman et al., 2001; Barnard et al., 2007; Damasio, 1998). Additionally, emotion's role in social perception, mentalizing, and empathy has been integrated into theories emphasizing the importance of these capacities in human and non-human primate evolution (Aureli and Schaffner, 2002; Byrne and Bates, 2010; Parr et al., 2005; de Waal, 2008). Casebeer and Churchland (2003) have proposed that human moral circuitry relies on emotional, i.e., "limbic", circuitry, as well.

SOCIAL BEHAVIOR AND BRAIN EVOLUTION: THE "SOCIAL BRAIN" HYPOTHESIS

As Humphrey proposed social complexity may "ratchet" up intelligence, it is additionally conceivable that social complexity will leave its mark on neural structure. This supposition is at the core of Dunbar's "Social Brain Hypothesis" (Dunbar, 2009), which purports that social factors led to the considerable expansion of the primate brain. Primates are known to have particularly expansive neocortices (Stephan and Andy, 1964), and a number of studies indicate that neocortical volume is linked to the complexity of social environments, indexed by factors such as social group (Dunbar, 1995) and network size (Kudo and Dunbar, 2001) and rates of tactical deception (Byrne and Corp, 2004).

From a neuroscientific perspective, this experimental paradigm may appear somewhat indiscriminate. The neocortex is not homogenous nor are its components

unified in their evolutionary development, e.g., primary visual cortex is reduced in humans while the temporal lobe is expanded. Ironically, the term "social brain" has also cropped up in the neuroscientific literature. In this case, the "social brain" refers not to the neocortex in its entirety, but rather to a dedicated set of cortical and subcortical structures known to process social stimuli (Adolphs, 2003; Brothers, 1990 a; Kling, 1986). Also, Dunbar's heightened emphasis on the neocortex detracts from the role that subcortical structures may play in the production of complex behavior. In the words of Robert Barton (Barton, 2006), "the neocortex is necessary for many cognitive functions but sufficient for none. It is therefore misleading to view the neocortex as the 'cognitive' part of the brain". Thus, it is conceivable that social complexity may drive enhancements in discrete neocortical and non-neocortical structures associated with social behavioral processing.

A reasonable corollary to the evolutionary "social brain" hypothesis is the proposition that structures which explicitly subserve social behaviors (the neuroscientific "social brain") may be targets of adaptive change resulting in neural specializations in human and nonhuman primate evolution. In primates, the network of cortical structures implicated in the social brain include the orbital frontal, temporal polar, cingulate, insular, and somatosensory cortices (Adolphs, 2003; Brothers, 1990 a; Kling, 1986). Only one subcortical structure, the amygdala, has routinely been included in this group (Adolphs, 2003; Brothers, 1990 a; Kling, 1986). Like the neocortex, increased amygdala volume has been linked to increased social network size in humans and macaques (Bickart et al., 2010; Kanai and Bahrami, 2012; Sallet et al., 2011). Across primates, particular amygdaloid subdivisions also show a correlation between volume and social

network size (Barton and Aggleton, 2000). Proponents of the "cultural intelligence" and "emotional reactivity" hypotheses suggest that social intelligence is driving human cognitive evolution; the significant relationship between amygdala volume and measures of social complexity point to the amygdala as potential target of social cognitive evolution. Moreover, the amygdala's incorporation into the emotional, or "limbic", system provides an additional link to the "emotional reactivity" hypothesis (Hare, 2007).

AMYGDALA FUNCTION IN SOCIAL COGNITION

The amygdala's primary function is to modulate emotional responses to external stimuli, especially fear producing stimuli (LeDoux, 2007; MacLean, 1949), bridging neocortical information about the sensory characteristics of a stimulus with brainstem and subcortical structures that facilitate the production of rapid physiological and motor responses to the stimulus (Price et al., 1987; Freese and Amaral, 2009; Heimer et al., 1999). As such, the amygdala has been characterized as a salience, ambiguity, value, and threat detector (Adolphs, 2010; Morrison and Salzman, 2010; Amaral et al., 2003; Bechara et al., 2003). In gregarious, social mammals, like primates, the amygdala may be particularly involved in processing the emotional salience of stimuli that mark the social status, relationships, and communicative intent of conspecifics as it is routinely engaged in processing emotional social signals (Yang et al., 2002; Sugiura et al., 2001; Adolphs, 2010; Sander et al., 2005; Brothers, 1990 a).

The amygdala processes many rudimentary components of social behavior that may form a foundation for the more complex social behaviors that underpin social intelligence. Generally, the amygdaloid complex subserves functions like implicit

learning, attending to salient stimuli, and memory modulation and consolidation (Phelps, 2006), and each of these functions has been proposed to underlie social intelligence (Byrne and Bates, 2007). One major function of the amygdala is the processing of simple socioemotional signals from multiple modalities. In humans, amygdala activation occurs to a range of social signals, like visual stimuli such as facial expression, familiar faces, and emotionally valenced words (Kesler-West et al., 2001; Yang et al., 2002; Tabert et al., 2001; Sugiura et al., 2001) as well as communicative auditory stimuli like prosody (Sander et al., 2005). Accordingly, in macaques, amygdala activation occurs in response to emotional facial expression, determining the identity of conspecifics, and scenes of other monkeys interacting (Gothard et al., 2007; Brothers et al., 1990) as well as species-specific vocalizations (Gil-da-Costa et al., 2004). Yet, the amygdala also subserves behaviors often lauded by humanists as essential components of the human character, like moral judgment, empathy, resistance to social conformity, and theory of mind (Singer et al., 2004; Berns et al., 2005; Vollm et al., 2006).

Lesions of the amygdala do not extinguish social behavior but, rather, affect the nature of social interactions and may impact important measures of evolutionary "fitness" in primates. Strikingly, amygdala lesioned adult macaques appear more, rather than less, gregarious, although they consistently lose their pre-operative rank and status within their social group and may have more difficulty interacting in larger groups (Machado and Bachevalier, 2006; Machado et al., 2008; Bauman et al., 2006; Kling, 1986). A similar phenomenon may result from human amygdala lesions. Lesion patients experience little discomfort in personal space violations, are less likely to view another individual as

untrustworthy or unapproachable, and perform poorly on theory of mind tasks that require an understanding of another's emotional state (Kennedy et al., 2009; Adolphs et al., 1998; Stone et al., 2003). Although no direct correlation can be made with social rank in primates, patients' social judgments and, consequently, evaluations of social interactions are clearly impacted, as in one case when, after having been mugged, a patient remarked that her assailants were "just larking around" (Broks et al., 1998).

Generally, the amygdala can be seen to facilitate the production of emotional, neural, and bodily responses to various forms of external social stimuli, which serve to direct attention based on the emotional significance of the stimulus and may be important for the rapid assessment of conspecifics in the social environment (Adolphs, 2010). Such rapid environmental assessment may be a key factor determining an individual's ability to respond to the complexity inherent in the primate social environment, which is suggested to have fostered the evolution of complex cognition (Humphrey, 1976; Byrne and Bates, 2007; Dunbar, 2009). Moreover, neuroscientists have stressed the functional links between subcortical limbic structures and neocortex in this network, underscoring the interdependence of emotion and higher order cognition in the production of social behavior (e.g., Damasio, 1998). Because it participates in behaviors theorized to underlie social complexity, the amygdala may be a good candidate for comparative analysis at the neuroanatomical level.

EVOLUTIONARY ANATOMY OF THE PRIMATE AMYGDALA: WHAT WE KNOW

Anatomically, the amygdala is situated at the confluence of several functional systems. It comprises 13 distinct, highly interconnected nuclei that may be divided into several functional groups in human and nonhuman primates (Pitkänen and Amaral, 1998; Price et al., 1987; Solano-Castiella et al., 2010; Saygin et al., 2011; Bach et al., 2011). Four nuclei are frequently implicated as major players in amygdala function: the lateral, basal, and accessory basal nuclei, collectively called the basolateral nuclei, and the central nucleus. The basolateral complex shares a strong connective relationship with neocortical structures subserving all sensory modalities. The superficial nuclear group on the medial surface of the amygdala is more integrated into the olfactory system, while the remaining nuclei, particularly the central nucleus, communicate with brainstem and hypothalamic regions associated with producing physiological responses to external stimuli (Price et al., 1987). The connectivity and chemoarchitecture of the amygdala is complex and beyond the scope of this review. It has been addressed in detail in several publications (Heimer et al., 1999; Price et al., 1987; Freese and Amaral, 2009).

Limbic structures, like the amygdala, have traditionally been conceived of as evolutionarily conserved (MacLean, 1990), but considerable evidence points to the fact that limbic structures are more evolutionarily dynamic than previously understood. For example, subcomponents of thalamus (Armstrong, 1986) show differential volumetric increase across primate species indicating that they are reorganized, an important mechanism of evolutionary change (Holloway, 1968). Early comparative analyses of the amygdala indicated that this structure may be emphasized in human evolution, as well (Stephan and Andy, 1977; Stephan et al., 1987). Due to difficulties in identifying individual nuclei across the broad array of primates and insectivores they analyzed,

Stephan and colleagues (1977; 1987) subdivided the 13 nuclei into only 2 divisions, the corticobasolateral and centromedial divisions. The corticobasolateral division appeared expanded, while the centromedial division appeared more conserved, indicating that the amygdala may also be a target of evolutionary reorganization. The authors suggested that the strong connections between the basolateral division and the neocortex led to the expansion of the corticobasolateral division, and this interpretation was statistically corroborated by later researchers (Barton and Aggleton, 2000). Unfortunately, this group used an eccentric means of analyzing their data, which renders these findings obsolete in modern evolutionary discourse (Reviewed in Ch. 4). Moreover, as the neocortex is not functionally homogeneous, neither is the basolateral division, as evidenced by the differential connectivity (Stefanacci and Amaral, 2002) and potential functional importance of individual basolateral nuclei (Freese and Amaral, 2009). This, of course, complicates any claim made about the evolution of the human basolateral division based on the Stephan study.

OUTLINE OF THE ANLYSIS

In this analysis, we attempt to ascertain whether aspects of amygdala organization may be derived, uniquely human features as opposed to an ancestral features shared with other hominoids. This dissertation targeted the individual basolateral nuclei, the lateral, basal, and accessory basal nuclei to further test and also extend the findings of the Stephan group. In addition, data for the central nucleus, a constituent of the centromedial division, was collected for contrast. Because we are predominantly interested in human evolution, we sought to augment previous findings by focusing heavily on the

evolutionary relationship between humans and great apes. Using design-based stereological methods, I estimated volumes (Ch. 2, 4) and neuron numbers (Ch. 3) in the amygdala and the lateral, basal, accessory basal, and central nuclei, in a comparative sample of humans and apes (and macaques in Ch . 3). Chapter 4 contains a meta-analysis contextualizing the evolution of the human amygdala within the evolution of the limbic system, as a whole, to address the potential importance of emotion in human brain evolution. Given its participation in cognitive processes posited to underlie complex social behavior, the amygdala, in particular, may be a target of evolutionary change in human and non-human primates. Across primates, it is hypothesized that the amygdala will exhibit differential expansion in and distribution of neurons to the lateral, basal, accessory basal, and central nuclei. In humans, we expected to see expansion in the basolateral nuclei due to their strong connective relationship with the expansive neocortex, while we anticipated that the central nucleus will be more conserved due to its connectivity with more conserved neural areas.

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Chapter 2

A comparative volumetric analysis of the hominoid amygdala and basolateral division[1]

ABSTRACT

The amygdaloid complex functions to facilitate effective appraisal of the social environment and is an essential component of the neural systems subserving social behavior. Despite its critical role in mediating social interaction, the amygdaloid complex has not attracted the same attention as the isocortex in most evolutionary analyses. We performed a comparative analysis of the amygdaloid complex in the hominoids to address the lack of comparative information available for this structure in the hominoid brain. We demarcated the amygdaloid complex and the three nuclei constituting its basolateral division, the lateral, basal, and accessory basal nuclei, in 12 histological series representing all six hominoid species. The volumes obtained for these areas were subjected to allometric analyses to determine whether any species deviated from expected values based on the other hominoids. Differences between groups were addressed using nonparametric comparisons of means.

The human lateral nucleus was larger than predicted for an ape of human brain size and occupied the majority of the basolateral division, whereas the basal nucleus was the largest of the basolateral nuclei in all ape species. In orangutans the amygdala and

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basolateral division were smaller than in the African apes. While the gorilla had a smaller than predicted lateral nucleus, its basal and accessory basal nuclei were larger than predicted. These differences may reflect volumetric changes occurring in interconnected cortical areas, specifically the temporal lobe and orbitofrontal cortex, which also subserve social behavior and cognition, suggesting that this system may be acted upon in hominoid and hominid evolution.

INTRODUCTION

The evolution of human cognition and its material substrate, the brain, has been a central concern in the study of human evolution. Despite this fact, very few comparative neuroanatomical analyses have focused on hominoid evolution. This study provides volumetric information about an important subcortical structure, the amygdaloid complex (AC) and the nuclei of its basolateral division (BLD), the lateral (L), basal (B), and accessory basal (AB) nuclei, for the often omitted or undersampled hominoids. Because many reports indicate that the AC is integral to social behavior (Adolphs, 2003; Brothers and Ring, 1992; Kling and Brothers, 1992) and that social pressures may have influenced the evolution of the human and primate brain (Dunbar, 2003), this analysis focuses on potential changes in the size and organization of the AC.

Many researchers consider the AC to be a central component of the neural system underlying social cognition and affiliation (Adolphs, 1999; Brothers, 1990; Kling, 1986). Generally, the AC subserves several functions relevant to social behavior like implicit learning, attending to salient stimuli, and memory modulation and consolidation (Phelps, 2005). In humans, AC activation occurs in response to a

number of behaviors associated with appraising the social environment, including gaze monitoring (Emery, 2000), processing emotional vocal, facial, and full body expressions (Yang et al., 2002, Glascher et al., 2004; Hadjikhani and de Gelder, 2005; Sander et al., 2005), evaluating another's trustworthiness (Singer et al., 2004; Grezes et al., 2004), and deciding whether to conform to peers' suggestions (Berns et al., 2005). Neurons in the macaque AC respond selectively to dynamic aspects of social behaviors, e.g., social interactions (Brothers and Ring, 1992; Brothers and Ring, 1993) and the social approach of other monkeys (Kling et al., 1979), as well as static representation, e.g., images of faces (Brothers, 1990). Generally, the AC can be seen to facilitate the production of emotional, neural, and bodily responses to various forms of external social stimuli, which serve to direct attention based on the emotional significance of the stimulus and may be important for the rapid assessment of conspecifics in the social environment (Adolphs, 1999*).*

Such rapid environmental assessment may be a key factor determining an individual's ability to respond to the complexity inherent in the primate social environment, which is suggested to have fostered the evolution of complex cognition (Humphrey, 1988; Whiten and Byrne, 1988; Dunbar, 2003). Many studies have statistically assessed the relationship between the volume of brain components and measures of sociality (Dunbar, 1998), but most of these evolutionary analyses have relied on gross measures like isocortex (neocortex) volume (e.g., Reader, 2003; Kudo and Dunbar, 2001; Byrne and Corp, 2004) despite the fact that not all isocortical components, e.g., the dorsolateral frontal cortex (Raleigh and Steklis, 1981), directly

participate in the specific network of neural structures thought to subserve social behavior (Brothers, 1990; Adolphs, 1999). Moreover, neuroscientists have stressed the functional links between subcortical limbic structures and isocortex in this network, underscoring the interdependence of emotion and higher order cognition in the production of social behavior (e.g., Damasio, 1994). Thus, to assess this theory from a neuroscientific perspective, more information is needed about both subcortical and cortical structures functionally associated with social behavior.

Limbic structures have traditionally been conceived of as evolutionarily conserved (MacLean, 1990), but considerable evidence points to the fact that limbic structures are more evolutionarily dynamic than previously understood. For example, subcomponents of the orbitofrontal cortex (Semendeferi et al., 1998; Semendeferi et al., 2001) and thalamus (Armstrong, 1986), two limbic structures strongly connected with the AC, show differential volumetric increase across primate species indicating that they are reorganized, an important mechanism of evolutionary change (Holloway, 1973). Stephan and Andy (1977) found that the primate AC appeared to be undergoing evolutionary expansion driven in large part by the "marked development" of, what they termed, the corticobasolateral amygdala (a division of the AC predominantly composed of the BL). In contrast, the remaining portion of the AC (including predominantly the medial and central nuclei) appeared more conserved. This differential expansion indicates that the AC also may be reorganized. Further, although Joffe and Dunbar (1997) found no relationship between measures of sociality and the limited number of anthropoid volumes reported in Stephan and colleagues' (1981) early AC data set (n=8), Barton and Aggleton

(2000) determined that corticobasolateral volume was significantly related to primate social group size using Stephan and colleagues' (1987) later, more comprehensive anthropoid AC data set (n=27) that included a larger number of hominoids and a more diverse array of primate species overall. Taken together, these studies suggest that the evolutionary reorganization of the primate AC may have been influenced by social factors.

Previous evolutionary analyses of the AC have highlighted the potential importance of functionally related brain regions on the elaboration of amygdaloid subcomponents in primates. The corticobasolateral division's strong interconnections with the expansive isocortex are thought to influence its proliferation, especially given that the constituent nuclei of the more conserved portion of the AC generally target brainstem and olfactory centers (Stephan and Andy, 1977; Barton et al., 2003). Thus, because they communicate with isocortex the BLD nuclei are most likely to show evolutionary change. Yet, the individual BLD nuclei also show different patterns of isocortical connectivity that may influence their development. The L exhibits the most specialized connectivity, predominantly receiving information from the temporal lobe (Stefanacci and Amaral, 2000). The B and AB are more connected to the orbitofrontal cortex, e.g., Brodmann's areas (BA) 12 and 13 (Carmichael and Price, 1995). The AB also receives strong projections from the frontal pole (BA 10) and from the superior temporal sulcus (Stefanacci and Amaral, 2002). Although the role that each nucleus plays in stimulus processing is not certain, it has been hypothesized (Emery and Amaral, 2000; Stefanacci and Amaral, 2002) that the L receives and categorizes

sensory information from the temporal cortex, which is then distributed to the B. In the B the processed information is paired with information from the orbitofrontal cortex concerning the social context in which the signal is occurring. After this evaluation, a context appropriate response may be directed through the B's connections with the striatum and the central nucleus (which subsequently targets hypothalamic and brainstem nuclei). Thus, the BLD is in a position to influence a number of functions important for social behavior, e.g., pairing affective values with incoming stimuli, associative learning (Sah, et al., 2003), and modulating memory consolidation (McIntyre, et al., 2003), which are made possible through the extensive interconnections its constituent nuclei share with specific isocortical areas.

Here we present volumes for the AC (Fig. 1) and the lateral (L), basal (B), and accessory basal (AB) nuclei (Fig. 2), which form the basolateral division (BLD) of the AC. Our study uses a considerably larger sample of hominoids than previous comparative analyses (Stephan and Andy, 1977; Stephan et al., 1987) and includes humans (*Homo sapiens*), all great ape *(Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla*, and *Pongo pygmaeus*), and some lesser ape species (*Hylobates lar*, *Hylobates concolor*), to investigate the evolution of the AC in this taxon. Because recent analyses indicate that the L is the largest of the human BLD nuclei (Schumann and Amaral, 2005), and this is not true of Old World monkeys (Amaral et al., 1992; Emery et al., 2001), we were particularly interested in determining whether this volumetric increase is a shared hominoid feature or is derived in humans, suggesting reorganization of the AC. Equally, because it subserves social interaction, the proliferation of the AC and its nuclei may be

influenced by a species' socioecology; thus, we wanted to examine the potential influence of sociality on the evolution of the AC by addressing differences between the orangutan AC and the AC of the other, more gregarious, great apes.

MATERIALS

Our sample (Table 1) consisted of 12 complete series of histologically processed brains (24 hemispheres) from all six hominoid species including *Homo sapiens (n=1*), *Pan troglodytes (n=3*), *Pan paniscus (n=2*), *Gorilla gorilla (n=1*), *Pongo pygmaeus (n=3*), *Hylobates concolor (n=1), and Hylobates lar (n=1*). For each series, brains were extracted within 12 hours after death and submerged in a 4% formalin solution. They were then embedded in paraffin and sectioned in the coronal plane at a thickness of 20 μm. Every 10^{th} to 16^{th} section was Nissl stained using a modification of the Gallyas silver stain for neuronal perikarya (Gallyas, 1971; Merker, 1983). On exception, the chimpanzee YN89-278, was cut in the axial plane at 15 μm thick and every 30^{th} section was stained.

The individuals in our sample were donated by zoological institutes and the Yerkes National Primate Research Center following their natural deaths. Only three older ape specimens (two orangutans, Harry and Briggs, and one chimpanzee, Bathsheba) were wild born. Specimens ranged from 2-75 years of age and represented both sexes (Table 1). Despite this large age range, age related differences in AC volume, independent from whole brain size, are not expected to be large enough to influence interspecific comparisons. Adult humans generally show no significant age-related variation in AC volume (Pruessner et al., 2001; Mu et al., 1999). Although one meta-analysis found

that adult human AC volumes uncorrected for overall brain volume show a loose negative correlation with age (approx. r =0.3/ r^2=.09; Brierley et al., 2002), volumetric differences in the AC generally reflect overall patterns of decrease in gross brain size, gray matter, and neighboring cortical structures (Allen et al., 2005; Walhovd et al., 2005; Good et al., 2001). Thus, the use of relative measures in group comparisons should factor out possible minor differences in adult AC size due to age effects. This is the largest sample of hominoids ever used in a histological comparative study of the AC. Nevertheless, because of specimen availability the sample is necessarily limited and our findings will be treated accordingly.

METHODS

In both hemispheres, the boundaries of the AC, L, B, and AB were hand traced in serial sections by NB with the StereoInvestigator software (MicroBrightField,Williston, VT) using the 1x and 2x objectives (N.A. 0.4 and 0.06) of the Nikon Eclipse E400 microscope.

Volumetric data were collected by NB with the Cavalieri method, using a 150 μm point counting grid in the StereoInvestigator program. In each hemisphere, 9 to 12 sections were analyzed at intervals of 30 to 150 μm for each region of interest (ROI), depending on absolute brain size. As required by the Cavalieri method, sampled sections from each specimen were chosen at standard intervals for each ROI and the starting section was picked randomly from the first interval. The coefficient of error (Gundersen, m=1) was less than 0.012 for each specimen; such low values indicate that the precision of the volume estimates was high and that the sampling parameters were sufficient.

We calculated a correction factor (CF) for each specimen to account for much of the shrinkage that occurs during tissue processing. Although CFs cannot account entirely for the possibility that white and gray matter shrink differentially (Kretschmann et al., 1982), procuring a CF for each individual is the best means available to us for nullifying variation due to processing. For each specimen, we divided the pre-processing brain volume by the post-processing volume, and then multiplied ROIs by this factor. Brain volumes before processing were determined by dividing brain weight by the specific gravity of brain tissue (1.036). Fixed brain volumes were estimated with the Cavalieri method using 50 sections in each series. All CFs were obtained by NB, except for one. We used a CF provided by Carol MacLeod (Langara College, Vancouver) for Schimp because she based her measurement on a complementary series in the C and O Vogt Institute's collection in Düsseldorf, Germany, which included the cerebellum, unlike our series.

Anatomical regions of interest

The amygdaloid complex (AC) is a roughly ovoid structure located in the anteromedial temporal lobe (Fig. 1), containing at least 13 distinct nuclei in primates (Amaral et al., 1992). The basolateral (BLD) nuclei (L, B, and AB) are located in the ventrolateral portion of the AC (Fig. 2). The AB is the most medial and dorsal of the BLD nuclei, the L occupies the entire lateral extent of the BLD, and the B lies intermediate between the two (Fig. 2). For this study, definitions of the hominoid AC, L, B, and AB were based on anatomical descriptions of the macaque (Price et al., 1987) and human (Heimer et al., 1999; de Olmos, 1990; Schumann and Amaral, 2005) AC. Because

the AC is structurally similar across the primates (Heimer et al., 1999) the boundaries of the ape AC were consistent with these sources. The anatomical criteria for each region of interest (ROI) delineated in this project were as follows.

Amygdaloid complex. The rostral pole of the AC was marked by the appearance of one of the BLD nuclei. In anterior sections the internal capsule borders the AC laterally (Fig. 1). Medially and rostrally, the striated composition of the adjacent piriform cortex contrasts with the diffuse cellular arrangement of the amygdaloid subnuclei. White matter forms the dorsal and ventral borders at this level. In posterior sections, the putamen forms a portion of the dorsal and lateral border, but it is distinguishable from the AC based on differences in cell structure, density, and organization. The AC is also bounded dorsally by the substantia innominata, marked by the presence of the basal nucleus of Meynert in this analysis (Fig. 1). In caudal sections, the sulcus semiannularis (Fig. 1) separates the entorhinal cortex from the AC medially (Schumann and Amaral, 2005), while the lateral ventricle and hippocampus form the dorsal borders.

Lateral. Because the L is the most lateral of all of the nuclei, its lateral, dorsal, and ventral borders are consistent with those of the AC. Rostrally, the L is in close proximity to the claustrum, but the latter structure is distinguishable by its larger, more darkly staining cells. The medial border is defined by the presence of the lateral medullary lamina (Fig. 2) and the fact that the cells of the L are smaller and more compact than those in the neighboring B. Caudally, the comparatively larger cells of the dorsal part of the L distinguish it from the adjacent putamen.

Basal. The B is separated from other nuclei by the longitudinal association fiber bundle. The lateral medullary lamina divides the lateral aspect of the B from the L, while the intermediate medullary lamina divides the medial aspect of this division from the AB (Fig. 2). The dorsomedial border separating the B and AB is best discerned by changes in cell size.

Accessory basal. The intermediate medullary lamina marks the lateral border of the AB as does the absence of the larger cells that characterize the neighboring B. The medial border is demarcated by the medial medullary lamina, which divides the AB from the superficial cortical nuclei. The ventral border is the same as that for the dorsomedial border of the B.

Data analysis

Absolute volume measures for each ROI discussed in the text include the summed volumes of the structure in the right and left hemisphere. The relative volume of the ROI is this absolute volume divided by the total volume of both hemispheres, in other words, the percent of the hemispheres occupied by the ROI. The cerebral hemispheres ("hemispheres") were defined as the whole brain minus cerebellum, midbrain, and brainstem. To assess the possibility of volumetric lateralization, the relative degree of asymmetry was measured as: |(Left ROI – Right ROI)/[(Left ROI+ Right ROI)/2]|.

Regression analyses were performed using log-transformed data. Equations and 95% confidence intervals from standard least squares regression (SLS) were obtained using JMP IN 4.04 (SAS Institute, 2000) statistical software. In previous studies (Semendeferi and Damasio, 2000) we found minor differences between SLS and major

axis regressions, so here we used only the more common SLS. We also performed independent contrasts (IC) analysis with the PDAP program (Garland et al., 1999; Garland and Ives, 2000), using Purvis's (1995) phylogeny to set branch lengths (which were squared to ensure that standardized contrasts did not correlate with their standard deviation). Regressions drawn from the PDTREE module of PDAP were mapped back into original data space with individual values for each specimen plotted over this regression line. For the independent variable in all regressions, ROI volume was subtracted from hemisphere volume to avoid statistical artifacts related to regressing an ROI against a region of which it is a part (Deacon, 1990). We report results from both regressions focusing on the allometric relationship between ROI volume and hemisphere volume in two conditions: including or excluding the human datum. In all cases, SLS regressions yielded considerably narrower 95% confidence intervals (CI) than IC analysis. Differences between slopes were tested using a modified version of the t-test (Zar, 1996: section 18.1). Because this sample is unlikely to satisfy the requirements of parametric analyses, e.g., normal distribution, the nonparametric Wilcoxon two-sample rank test was used to test for differences between means in JMP IN 4.04.

RESULTS

Absolute volumes

Amygdala (AC) and Basolateral Division (BLD). In absolute volume, the human set the upper bound for the hominoid AC and BLD at 3.805 cm^3 and 2.424 cm^3 respectively, while the gibbons set the lower bound, ranging from 0.407-0.637 cm^3 (AC) and 0.259-0.348 cm^3 (BLD) (Table 1). Great ape AC volumes ranged from 1.048 cm^3 in

an orangutan to 1.418 cm^3 in a chimpanzee. Also, a chimpanzee had the largest (0.909 cm^3), and an orangutan had the smallest (0.616 cm^3) absolute BLD volume. All great ape species overlapped for both ROIs (Table 1).

Basolateral Nuclei (L, B, AB). The human exhibited the greatest absolute volumes for the L (1.146 cm^3), B (0.881 cm^3), and AB (0.3964 cm^3), and the gibbons exhibited the smallest L (0.087-0.114 cm^3), B (0.128-0.172 cm^3), and AB (0.0442-0.0610 cm^3) (Table 1). In the great apes, volumes for the L fell between those set by a bonobo (0.323 cm^3) at the high end and a gorilla (0.200 cm^3) at the low end. The values for the B overlapped with the gorilla (0.485 cm^3) setting the upper limit and an orangutan (0.302 cm^3) setting the lower one. AB volume was distinctly largest in the gorilla (0.1813 cm^3) and smallest in the orangutans (0.1032-0.1111 cm^3), while the bonobo and chimpanzee values (0.1283-0.1571 cm^3) were intermediate and overlapped one another.

As expected based on differences in overall brain size, absolute AC volumes were greatest in the human, smaller and overlapping in the great apes, and smallest in the gibbons. In the great apes, the BLD and its constituent nuclei followed this pattern, with the exception of the AB, which did not show overlap in all great ape species. It was distinctly largest in the gorilla and smallest in the orangutans.

Regression against hemisphere volume

Amygdala. AC volumes were negatively allometric with hemispheres (SLS slope=0.737 SE=0.0485; IC slope=0.755 SE=0.0735; slope difference: *n.s.*, p=0.439). The AC of two of the three orangutans fell below the CI and the third fell on its lower

periphery (Fig. 3a). Excluding the human from the analysis resulted in a further decrease in slopes (SLS slope=0.686 SE=0.0469; IC slope=0.582 SE=0.140; slope difference: *n.s.*, p=0.0928).

Basolateral Division. The volume of the BLD was also negatively allometric with the hemispheres (SLS slope=0.769 SE=0.053; IC slope=0.749 SE=0.093; slope difference: *n.s.*, p=0.642). The BLD of two orangutans fell below the CI, and all three fell below the regression line (Fig. 3b). When the human BLD was excluded, the slope changed slightly in the SLS analysis (slope=0.736 SE=0.0548) and clearly decreased in the IC analysis (slope=0.594 SE=0.258; slope difference: *n.s.*, p=0.229*).* The rest of the AC (AC minus BLD) and the hemispheres were negatively allometric with the human included (SLS slope=0.769 SE=0.0623; IC slope=0.784 SE=.0430; slope difference: *n.s.*, p=0.799) and with the human excluded (SLS slope=0.603 SE=0.0256; IC slope=0.585 SE=0.0352; slope difference, *n.s.*, p=0.904).

Lateral Nucleus. The relationship between the L and the hemispheres (Fig. 4a) was the closest to isometric of all the analyses in which all species were included (SLS slope=0.852 SE=0.090; IC slope=0.991 SE=0.216; slope difference: *n.s.*, p=0.392). When the human value was excluded (Fig. 4b), the relationship between the L and the hemispheres became more negatively allometric (SLS slope=0.674 SE=0.066; IC slope=0.361 SE=0.508; slope difference: *n.s.*, p=0.666). Unlike any other region analyzed, the value for the human L fell above the regression line and the CI in this analysis.

Basal Nucleus. The relationship between the B and the hemispheres was the most negatively allometric of all tested combinations when all species were included (SLS slope=0.689 SE=0.073; IC slope=0.550 SE=0.083; slope difference: *n.s.*, p=0.473). The gorilla's B fell above the confidence interval in this analysis (Fig. 4c). Without the human datum, the slope of the regression line increased (SLS slope=0.770 SE=0.071; IC slope=0.680 SE=0.236; slope difference: n.s., p=0.979), indicating that the human value may be driving the slope downward.

Accessory Basal Nucleus. The relationship between the AB and the hemispheres was negatively allometric with all species included (SLS slope=0.754 SE=0.075; IC slope=0.733 SE=0.085; slope difference: *n.s.*, p=0.958). The AB of both analyzed orangutans fell below the confidence interval and the gorilla fell above it (Fig. 4d). Excluding the human resulted in only a minor change in slope (SLS slope=0.732 SE=0.079; IC slope=0.770 SE=0.254; slope difference: *n.s.*, p=0.973).

The human L was significantly larger than predicted (Fig. 4b). Also, in the analysis of the B, including the human appeared to negatively influence the slope. In all of the analyses, the orangutan data fell below the regression line, and they fell significantly below the confidence interval for the BLD, the L and the AB (Figs. 3, 4). While the gorilla possessed a larger B than predicted, its L fell below the confidence interval (Fig. 4a). Only in these three species did all members fall outside the confidence interval. Several other species did exhibit interesting intraspecific variation with at least one individual falling outside of the range of predicted values in regressions that included all species, e.g., the two bonobos both fell outside the confidence interval for several

analyses, but never jointly on the same measure. Unfortunately, the small sample size limits our ability to draw any strong conclusions about this variation. For example, the bonobo which falls above the line for the lateral nucleus (Fig. 4a) was both young and language trained, which may influence her position on the line, but we do not have a suitable comparative sample of language trained or juvenile primates to test these assumptions. Equally, it must be noted that the sample includes only one gorilla and one human and that a larger sample might yield a greater range of volumes due to individual variation. Our findings for the human are consistent with other volumetric studies (see discussion), but few data are available for the gorilla AC and further investigation is needed in this species.

Relative Volumes

Amygdala relative to hemispheres. The AC occupied the smallest percentage of the hemispheres in the human (0.33%), the largest in the gibbons (0.60%-0.68%), and overlapped in the African apes (0.40%-0.52%) (Fig. 5a). The mean percent occupied by the orangutan AC (0.37%-0.38%) was significantly smaller than that of the average African ape ($Z=-2.09$; $p=0.0364$).

Basolateral division relative to hemispheres. The BLD also occupied the smallest percentage of the hemispheres in the human (0.21%) and the largest in the gibbons (0.37%-0.38%). Similar to the AC, the percentage of the hemispheres occupied by the BLD (Fig. 5b) was smaller in the orangutans (0.22%-0.25%) than in the African great apes (0.26%-0.35%) and the difference between the two group means was significant ($Z=-2.10$; $p=0.0358$). In contrast, in all of the great apes the relative volumes of the rest

of the AC (all non-BLD nuclei) fell within a similar range (Fig. 5c) and the mean value of this region was not significantly smaller in orangutans ($Z=-1.19$; $p=0.233$) when compared with the African apes.

Basolateral division relative to amygdala volume. In all hominoids, the BLD occupied a majority of the AC, from 55-67% for most animals with overlap across all species.

Lateral, basal, and accessory basal nuclei volume relative to basolateral volume. The organization of the human BLD was distinct from that of the apes. The L occupied the largest percentage of the human BLD, 47%, while the B occupied 36%, and the AB occupied 16%. In contrast, in the apes the B occupied the largest percentage of the BLD followed in succession by the L and the AB (Fig. 6). In the gibbons, orangutans, bonobos, and chimpanzees, the B occupied 46-53%, the L occupied 29-38%, and the AB occupied 15-18% of the BLD. The gorilla is the only species in which the L and AB occupy similar percentages within BLD; the percentages occupied by the B and AB (56% and 21%, respectively) fell above the range of values for the other apes and percentage occupied by the L (23%) fell below this range.

The orangutans had significantly smaller relative AC and BLD volumes than did the African apes. In all species, the BLD occupied the majority of the AC. When the volumes of the individual BLD nuclei were compared relative to BLD volume, the human L appeared exceptional in that it forms the largest component of the BLD, while the B was the largest in the apes. Concordant with the regression analysis, the gorilla had the smallest relative L, but a large AB and B.

DISCUSSION

Our values for the amygdaloid complex (AC) in the two human hemispheres fall within the range published in a recent meta-analysis of 39 papers (Brierley et al., 2002) using MR images of living humans (Table 2). Our hominoid data largely overlap with previously reported postmortem human, chimpanzee, orangutan, and gibbon AC volumes procured by Stephan and colleagues (1977; 1987) and Zilles and Rehkamper (1988). The only previously reported gorilla AC volume (Stephan et al., 1987) was more than twice as large as the AC of the gorilla in the present study (Table 2). Although sexual dimorphism may account for the differences between the two studies (the Stephan gorilla was male, while the one in this analysis was female), more specimens are needed to investigate this possible source of difference. The only other postmortem histological analysis of the AC (Schumann and Amaral, 2005) reported volumes uncorrected for shrinkage and thus cannot be directly compared to either our values or the MR studies above (as would be expected, their uncorrected values fall at the lower end of that range; Table 2).

Humans. The L has been suggested to be large in humans (Stephan et al., 1987) and is the largest nucleus in the human basolateral division (BLD) (Schumann and Amaral, 2005). In contrast, the B is the largest nucleus in the macaque BLD (Amaral et al., 1992; control data in Emery et al., 2001). In this analysis, we report that the human AC, specifically the BLD, shows a unique pattern of organization which also deviates from that of our closest phylogenetic relatives, the great apes (Fig. 6). Our data suggest that the organization of the human BLD is distinguished by a volumetric increase in the L and also, potentially, a decrease in the B.

The unique organization of the human BLD, in which the L occupies most of the BLD, may reflect the L's primary connective relationship with the temporal lobe (Stefanacci and Amaral, 2002). In addition to the large L, humans appear to have relatively larger temporal lobes (Semendeferi and Damasio, 2000; Rilling and Seligman, 2002) and more temporal white matter (Semendeferi and Schenker, 2001; Rilling and Seligman, 2002) than other apes. Although increases in temporal lobe white matter most likely reflect increased connectivity intrinsic to this region (Schenker et al., 2005), the temporal lobe is an important component of the system proposed to underlie social cognition (Adolphs, 2003; Brothers, 1990) and overall volumetric increases may have a functional, evolutionary significance. Indeed, Rilling and Seligman (2002) suggest that the elaboration of the human temporal lobe is likely to reflect the importance of language processing in this region. Stephan and Andy (1977) suggested that portions of the AC which include the BLD show greater expansion than other portions of the AC due to the influence of the BLD's strong isocortical connections. Thus, it is likely that the L had expanded, and consequently the BLD is reorganized, in humans at least in part due to evolutionary changes in the temporal lobe and the strong relationship shared between this structure and the L.

The human exhibits the smallest proportion of AC and BLD relative to hemispheres, but shows a positive residual for the latter value. It is interesting that, while the AC residual was not positive, the BLD residual was. This may reflect increased processing demands within the BLD due to a preponderance of information flowing into the human AC, or it may even indicate greater cortical control over the AC. However, the

residual was not significantly positive, so any conclusions on this issue require increased samples.

Orangutans. Our results suggest that orangutans are unique among the apes in having a smaller AC, possibly driven by their smaller BLD. Within the BLD we found that the accessory basal (AB) and basal (B) nuclei were smaller in orangutans than in the African apes. The BLD and especially AB receive considerable projections from orbitofrontal cortices (Stefanacci and Amaral, 2002: Ghashghaei and Barbas, 2002), which are also smaller in orangutans (Semendeferi et al., 1997; Schenker et al., 2005). Further, the organization of BA 13, a major constituent of the orangutan orbitofrontal cortex, appears more "prefrontal" than "limbic" (Semendeferi et al., 1998). Thus, it is likely that specializations are present in the orangutan brain that deemphasize limbic structures.

Behavioral evidence accords with this possible neural difference in orangutan limbic structures, which generally mediate affect as well as social interactions. While few comparative analyses remark upon affective differences between ape species, Shumaker and colleagues (2001) found that orangutans appeared to engage in less impulsive decision making than chimpanzees when presented with edible stimuli in a numerical ordination task. The authors postulated that this may be evolutionarily related to the reduced amount of direct competition for food in the orangutan social environment. Wild orangutans are unique among the hominoids as they are the only semi-solitary species, occasionally forming bands averaging 2 individuals (Delgado and van Schaik, 2000). In contrast, members of the highly gregarious African genus *Pan* live

in multimale-multifemale groups of up to 150 individuals (bonobos: Kano, 1992; chimpanzees: Mitani and Amsler, 2003), and western lowland gorillas typically form single male groups of up to 20 individuals (Yamagiwa et al., 2003). Given this difference in socioecology, the finding that orangutans show both emotional and neural differences compared with other apes is highly provocative. Unfortunately, our certainty as to whether these results reflect real population level differences that are completely genetic in origin is limited by the small samples available and the diverse rearing conditions experienced by the apes in them. Future studies with larger samples are needed to confirm these ideas.

Gorillas. The gorilla's BLD comprised an exceptionally large B and AB and a diminished L, a more extreme manifestation of the pattern found among the other apes. The fact that the gorilla had a particularly small L corresponds with a report indicating that gorillas have the smallest temporal lobe among the apes (Semendeferi and Damasio, 2000; Rilling and Seligman, 2002), representing a reversal of the pattern found in humans (large L and large temporal lobe). One could argue that this supports the idea that changes in isocortical volume inspire concomitant changes in related AC nuclei volume. Accordingly, one would expect that the large size of the gorilla AB would correspond to an expansion in interconnected isocortical regions. This is not patently the case. While the AB shares strong connections with the superior temporal gyrus and the orbitofrontal cortex (Stefanacci and Amaral, 2002), gorillas do not have larger superior temporal gyri (Rilling and Seligman, 2002) and only a slightly larger percentage of the frontal lobes is devoted to the orbitofrontal cortex in gorillas than in the other apes (Semendeferi et al.,

1997). Still, they do not have appreciably smaller superior temporal gyri than the other apes, despite having relatively smaller temporal lobes (Rilling and Seligman, 2002; Semendeferi and Damasio, 2000). Also, BA 13 of the gorilla's oribitofrontal cortex, while not larger than in the other apes, is more cell dense and contains higher numbers of neurons (Semendeferi et al., 1998). Thus, it is possible that the processing capacity and extrinsic connectivity of portions of the gorilla's orbitofrontal cortex are higher (Semendeferi et al., 1998). As such, it may be that changes in the gorilla orbitofrontal cortex drive changes in the AB, which explains why it would not be affected by the lack of extreme volumetric increase in the superior temporal gyrus.

While theories of connectivity can be invoked to explain volumetric differences in the hominoid AC, the difficulty of this claim, overall, is the fact that the some nuclei share sparser connections with an array of isocortical areas. The influence of these connections must also be considered in our explanatory framework. While the L is more selectively connected to the temporal lobe, the B receives light projections from the temporal lobe (Ghashagaei and Barbas, 2002) in addition to the strong connections it receives from orbitofrontal cortex. It projects extensively to frontal, temporal, and occipital cortices (Amaral et al., 1992; Amaral et al., 2003), making it more difficult to identify any one region that might influence the B's evolution. Although the AB receives strong connections from the orbitofrontal cortex, it also is heavily innervated by some areas within the superior temporal gyrus (Stefanacci and Amaral, 2002). Because our findings are more focused on the AB and L and are consonant with the general

connective relationships of these two nuclei, it is very likely that isocortical regions are influencing hominoid AC development.

Other considerations. Although we have focused on volumetric differences across species, other factors might also influence a structure's size. A short discussion of our findings regarding sex and hemispheric differences is warranted, keeping in mind that none of the results were reliably significant, possibly due, in part, to the size of our sample.

Most species in our sample include members of only one sex (Table 1), a fact which precludes thorough assessment of the effects of sex on AC volume in individual species. Nonetheless, we investigated sex differences across AC volumes in the sample to test the possibility that some of our findings, especially the small orangutan AC, may be the result of variation due to sex and not species. Females in the sample had relatively larger AC than males as a group (Fig. 7). Yet, in the chimpanzees, the only species in which both sexes were sampled, the relative volume of the male's AC fell within the range of the females' volumes, suggesting that this sex difference may be an artifact of sex bias in the sample. This is not surprising, given that there is no concrete evidence for sex differences in the human AC literature. , AC volumes (corrected for brain volume) are reported to be larger in males (Goldstein et al., 2001), in females (Szabo et al., 2003), similar between the sexes (Giedd et al., 2002; Gür et al., 1996), or larger in only one hemisphere in males (Brierley et al., 2002). No clear sex difference in AC volume is apparent in chimpanzee (Freeman et al., 2004) or macaque samples either (Franklin et al., 2000). This casts doubt on the presence of sex differences in the sample and also provides

partial support for our hypothesis that orangutan AC volumes are distinct due to phylogenetic and ecological influences and not the composition of the sample.

No consistent direction of asymmetry was present in the hominoid AC (Fig. 8) or BLD nuclei, nor did these regions exhibit a large degree of asymmetry across the specimens. Only two specimens, an orangutan (YN85-38) and a gibbon (Disco), showed a degree of asymmetry that exceeded 0.26, i.e., the volume in one hemisphere was over 1.5 times the volume of the other, and this was only for one nucleus in each specimen. These results are consistent with MR analyses using larger human (Brierley et al. 2002) and chimpanzee (Freeman et al., 2004) samples which found no asymmetries in the AC.

CONCLUSIONS

Our findings largely support hypotheses of AC evolution that highlight the importance of functional networks within the brain, specifically those related to social behavior. Although this is the most expansive comparative data set for the hominoid AC to date, the sample is small and all conclusions are, thus, somewhat contingent. The results of this analysis suggest several profitable directions for future research. The species differences we found in the hominoid AC may be related to the connections that the AC shares with other portions of the brain. The large size of the human L may reflect the proliferation of the temporal lobe over the course of hominid evolution, while the inverse may be true of the gorilla. The smaller size of the orangutan AC and BLD may be related to the diminished importance of interconnected limbic structures in this species. Further, there is some evidence that the orangutan, which exhibits one of the smallest group sizes on the continuum of primate sociality, may also be distinguished

neuroanatomically from the other great apes, suggesting that social pressures may play a role in the development of the AC in association with other limbic regions. It is beyond the scope of this analysis to test measures of social complexity, because such an investigation would require a much larger sample. Given our preliminary findings, though, each of these hypotheses is viable and certainly not mutually exclusive, providing a solid foundation for future investigations of the hominoid AC.

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Chapter 2: Tables

Table 2.1. Volumetric estimates of the amygdaloid complex and the basolateral division.

	Specimen ID	Age	Sex	Hemi[1,2]	Amygdaloid Complex[1]		Basolateral[1,3]		*Lateral*[1]		*Basal*[1]		*Accessory Basal*[1]	
					L	R	L	R	L	R	L	R	L	R
Human	SN207/84	*75*	*M*	1151	2.032	1.773	1.191	1.233	0.551	0.595	0.452	0.429	0.1879	0.2085
Chimpanzee	Bathsheba	*24*	*F*	286	0.584	0.561	0.379	0.353	0.126	0.105	0.183	0.187	0.0701	0.0615
	Schimp	*22*	*F*	318	0.685	0.733	0.432	0.477	0.138	0.136	0.224	0.255	0.0701	0.0870
	YN89-278[4]	*22*	*M*	326	0.634	0.748	--	--	--	--	--	--	--	--
Bonobo	Zahlia	*11*	*F*	248	0.623	0.660	0.417	0.445	0.124	0.137	0.223	0.236	0.0695	0.0721
	YN86-137	*2*	*F*	304	0.654	0.634	0.425	0.420	0.159	0.164	0.203	0.191	0.0623	0.0660
Gorilla	YN82-140	*20*	*F*	300	0.651	0.655	0.442	0.423	0.100	0.100	0.247	0.238	0.0958	0.0855
Orangutan	Harry[5]	*37*	*M*	351	0.688	0.660	0.447	0.422	0.121	0.127	--	--	--	--
	Briggs[6]	*34*	*M*	279	0.528	0.520	--	0.308	--	0.105	--	0.151	--	0.0516
	YN85-38	*16*	*M*	309	0.637	0.514	0.415	0.307	0.124	0.105	0.228	0.153	0.0622	0.0489
Gibbon	Disco	*22*	*F*	93.2	0.270	0.367	0.169	0.179	0.060	0.054	0.086	0.086	0.0223	0.0387
	YN81-146	*Adult*	*F*	68.3	0.203	0.204	0.130	0.129	0.046	0.041	0.063	0.065	0.0206	0.0236

[1] All volumes are in cm^3.

[2] "Hemi" represents the summed volume of both cerebral hemispheres.

[3] The volume of the basolateral division is equal to the sum of the volumes of the accessory basal, basal, and lateral nuclei.

[4] Specimen is sectioned in the axial plane. Only whole amygdaloid complex volumes for right and left hemispheres were collected to avoid inconsistencies between boundary definitions in the coronal and axial planes for the less distinct nuclei.

[5] The basal and accessory basal nuclei could not be accurately discriminated in this specimen due to localized tissue damage.

[6] Tissue damage in the left temporal lobe prevented collection of lateral, basal, and accessory basal nuclei in the left hemisphere of this specimen. Volumes in the right hemisphere were doubled in this specimen in the analysis.

Table 2.2. Hominoid amygdaloid complex volumes reported in the anatomical literature.

Species	***Range of Amygdaloid Complex Volumes (cm^3)***				
	Present study	*Stephan et al.*[1]	*Zilles & Rehkamper*[2]	*Brierley et al.*[3,5]	*Schumann & Amaral*[4,5,6]
Human	3.80	3.02 & 5.29	--	2.10-7.76	2.40-3.18
Chimpanzee	1.15-1.42	1.42	--	--	--
Bonobo	1.28 & 1.29	--	--	--	--
Gorilla	1.31	2.75	--	--	--
Orangutan	1.0 -1.35	--	1.4	--	--
Gibbon	0.41 & 0.64	0.67	--	--	--

[1] Histological volumes for three apes and one human taken from Stephan, Frahm, and Baron (1987). A different human volume was also presented in Stephan & Andy (1977).

[2] Histological volume from Zilles and Rehkamper (1988).

[3] MR volumes from Brierley, Shaw, & David (2002), a meta-analysis of 39 studies with 51 human datasets.

[4] Histological volumes from Schumann and Amaral (2006). Sample included 10 human hemispheres.

[5] The original volumes reported in these studies were from only one hemisphere. To provide a better comparison with other studies in the table, these reported volumes were doubled before adding them to the table.

6 Information from Schumann & Amaral (2005) is not directly comparable to the rest of the table as the study reports post-processing volume and did not use corrections factors to estimate pre-processing volume.

Chapter 2: Figures

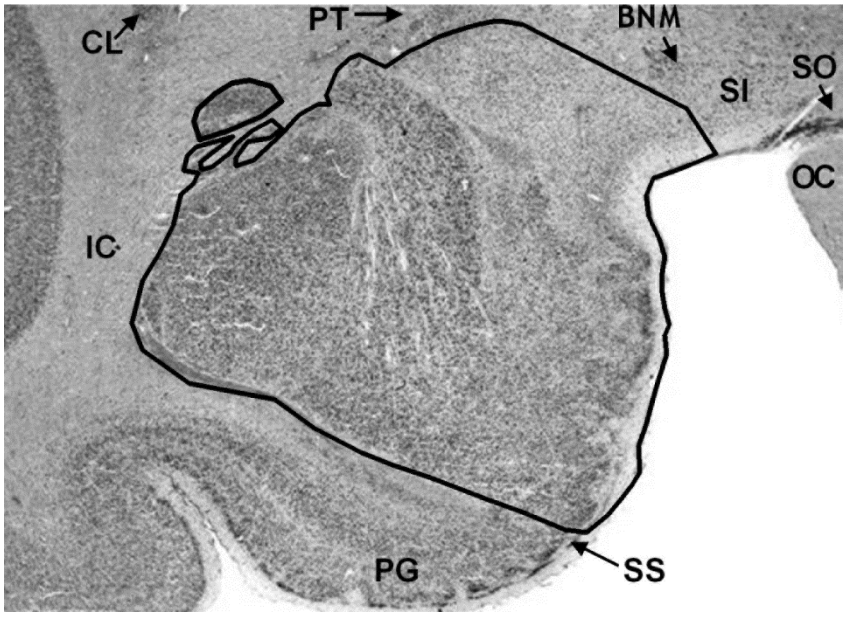

Figure 2.1. Gibbon amygdaloid complex in the left hemisphere at the midrostrocaudal level demarcated on a coronal Nissl section. The superimposed lines indicate the anatomical boundaries of the amygdaloid complex at this level.

Abbreviations:

BNM Basal Nucleus of Meynert
CL Claustrum
IC External Capsule
OC Optic Chiasm
PG Parahippocampal Gyrus
PT Putamen
SI Substantia Innominata
SO Supraoptic Nucleus
SS Sulcus Semiannularis

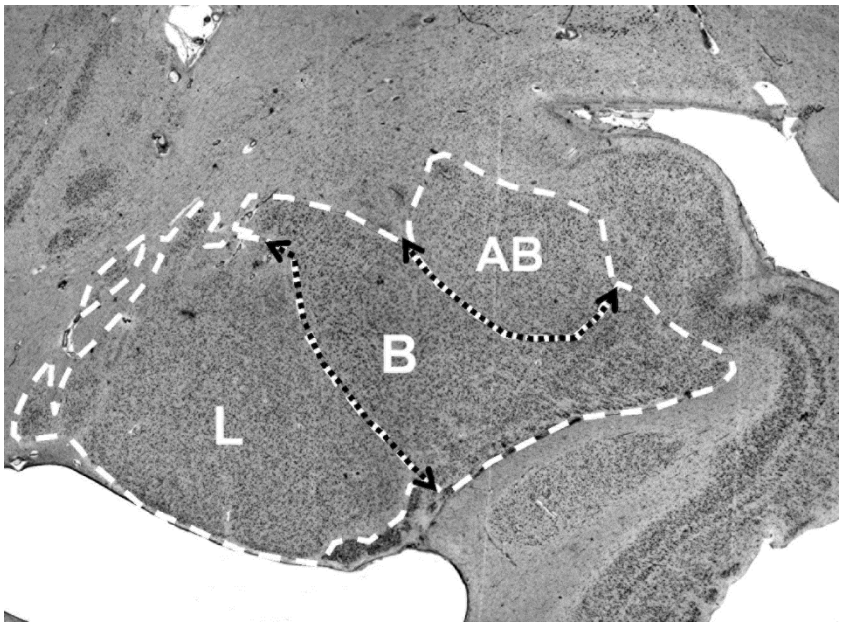

Figure 2.2. Human basolateral division in the left hemisphere at the midrostrocaudal level demarcated on a coronal Nissl section. The superimposed white lines represent the anatomical borders of the lateral (L), basal (B), and accessory basal (AB) nuclei. Black dashed lines show the position of the lamina dividing the basolateral nuclei.

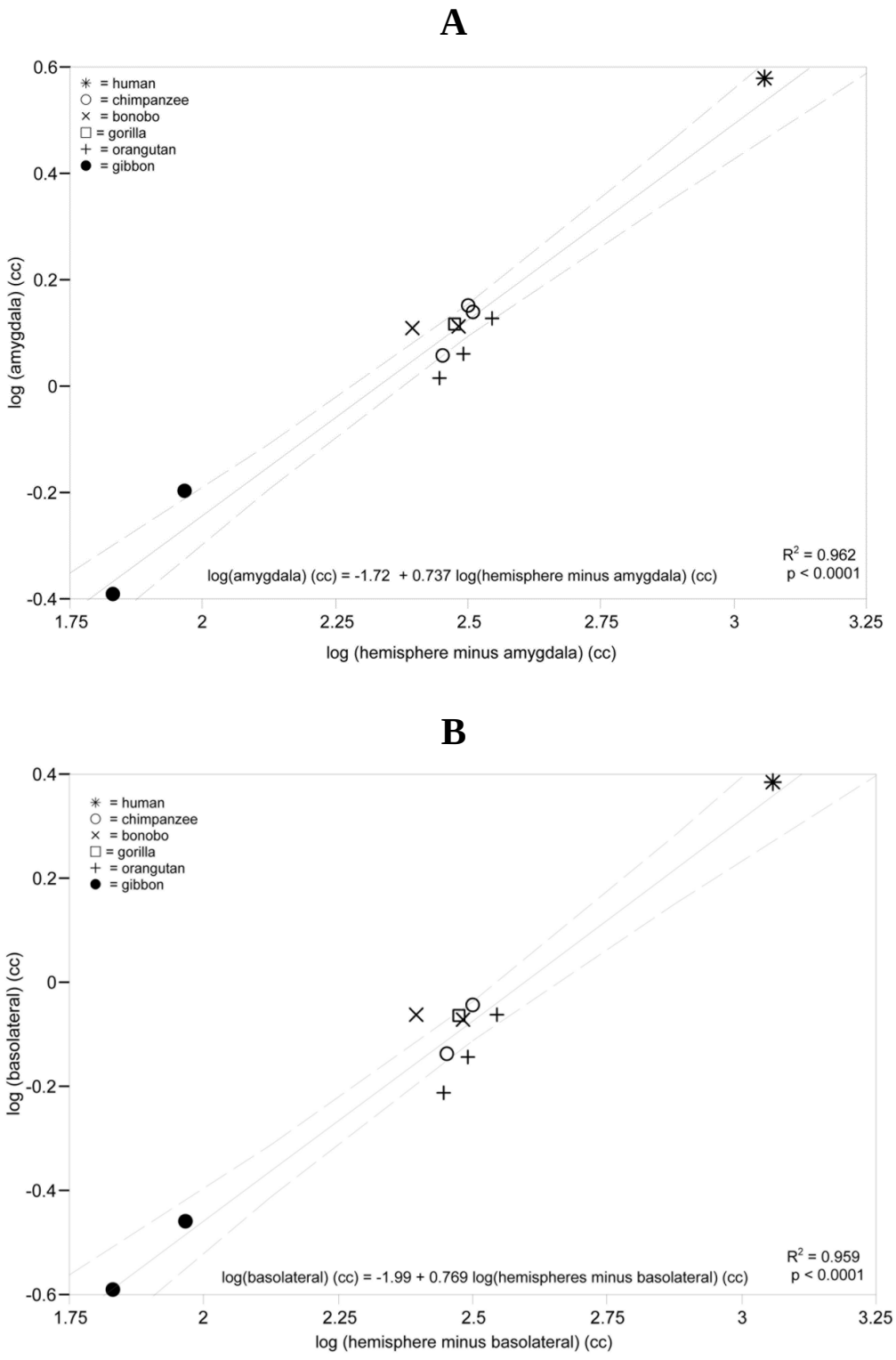

Figure 2.3. Least squares regression plotting the log of hemisphere volumes against the log of the volumes of a) the amygdala (amygdaloid complex) and b) the basolateral division with all species of hominoid included in the regression.

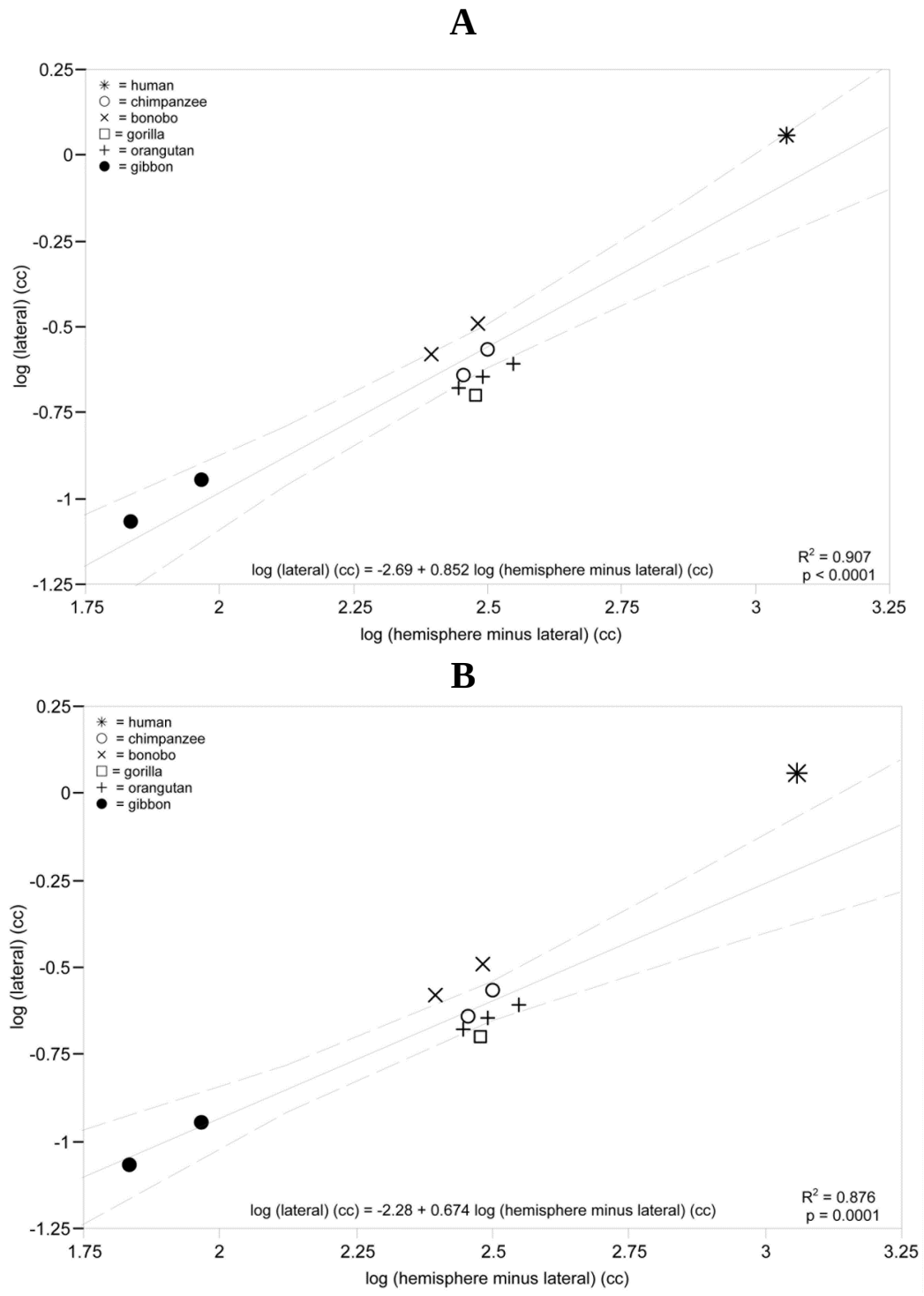

Figure 2.4. Least squares regression plotting the log of hemisphere volumes against the log of the volumes of a) the lateral nucleus with all species included, b) the lateral nucleus with the human excluded from the regression, c) the basal nucleus with all species included, and d) the accessory basal nucleus with all species included. The results of additional regression analyses are discussed in the text.

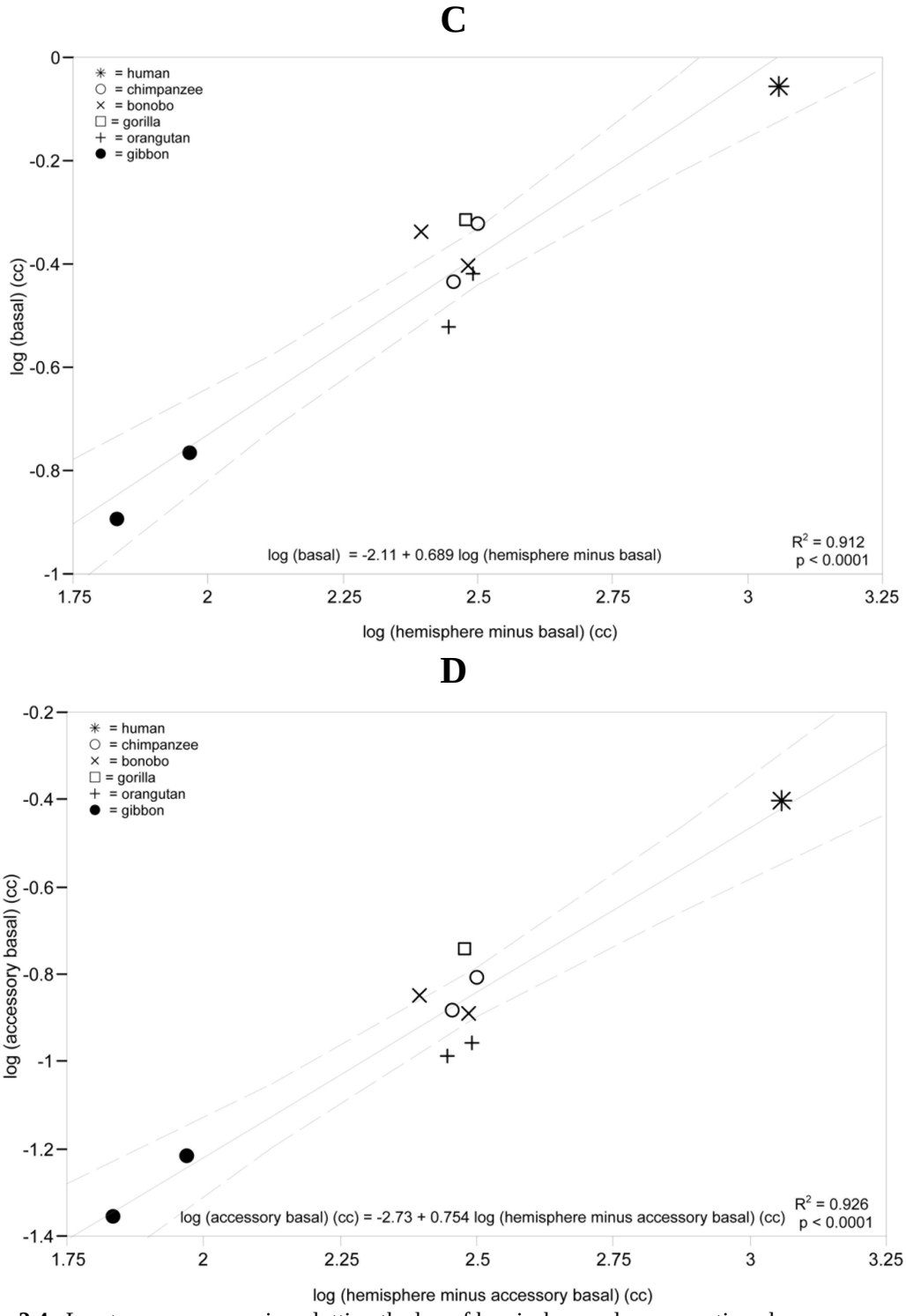

Figure 2.4. Least squares regression plotting the log of hemisphere volumes, continued.

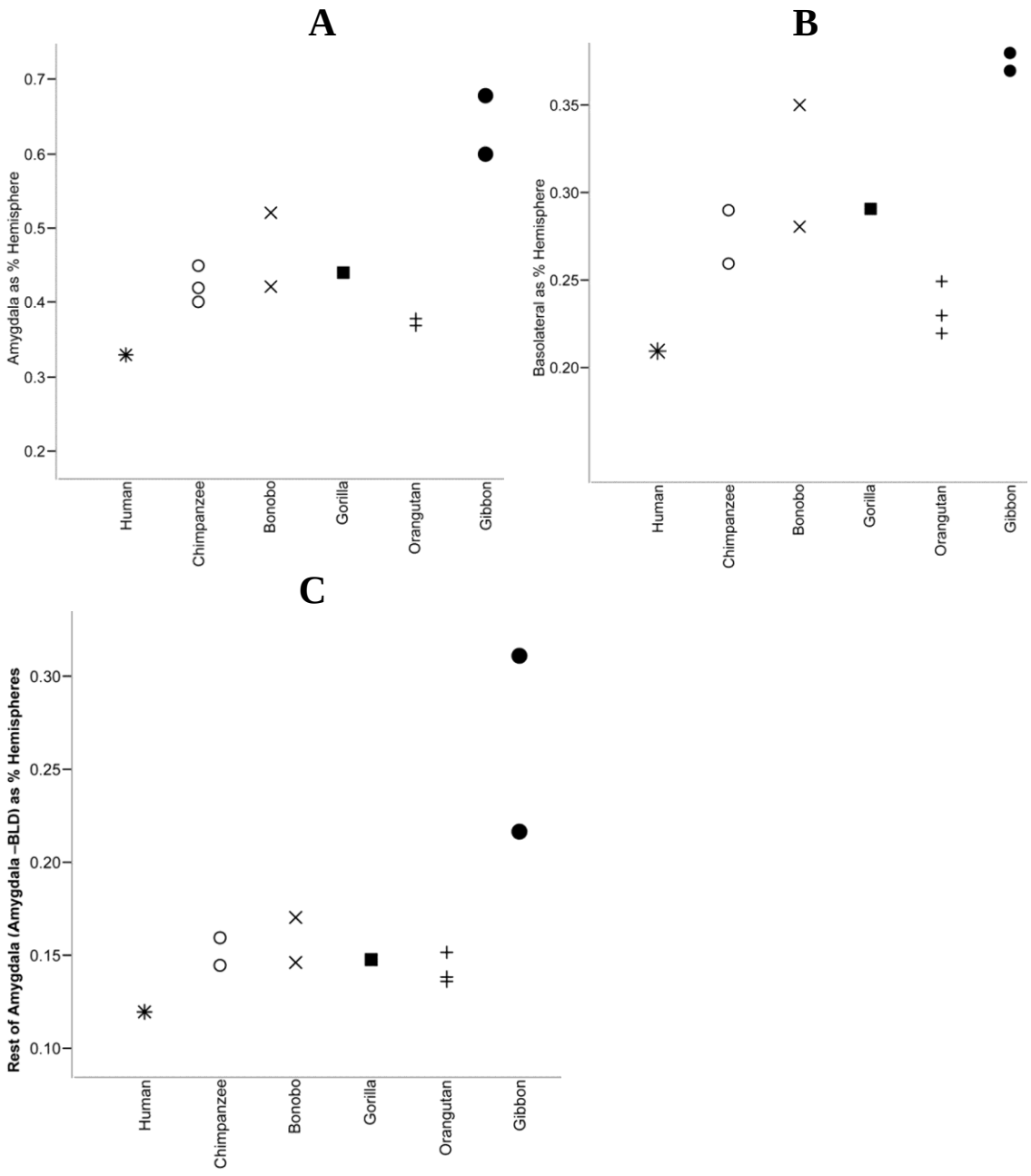

Figure 2.5. Comparison of relative volumes (percentage of hemisphere volumes) of a) the amygdala (amygdaloid complex), b) the basolateral division, and c) the rest of the amygdala (amygdaloid complex minus basolateral division) in the hominoids.

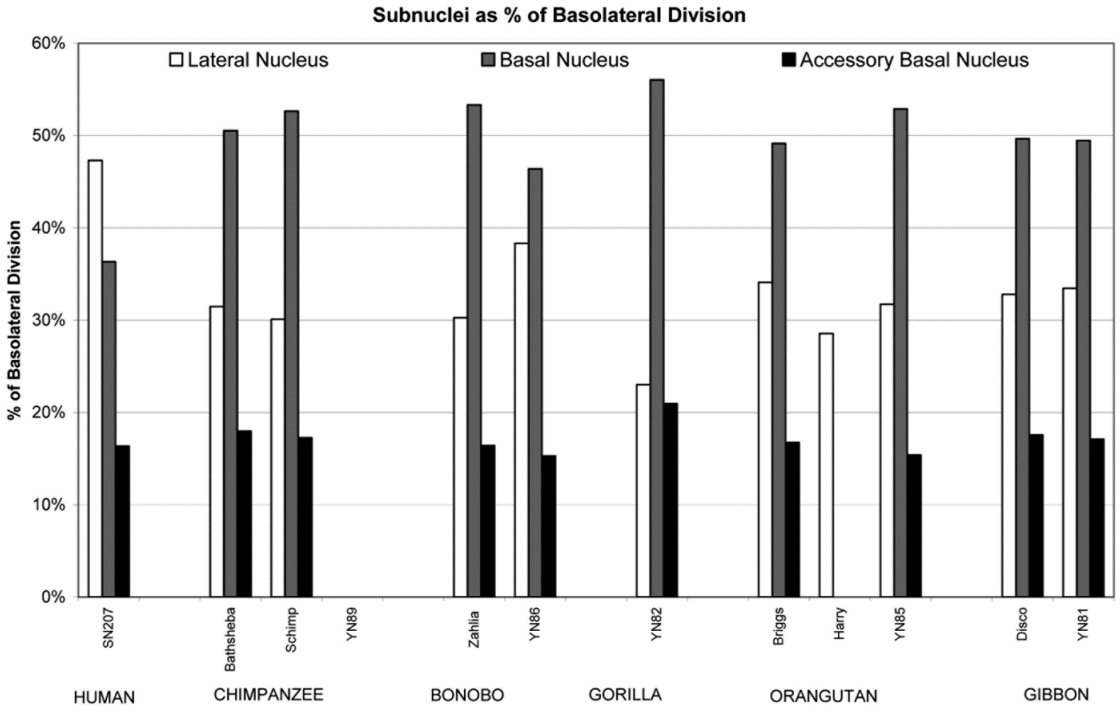

Figure 2.6. Percentage of basolateral division occupied by the lateral, basal, and accessory basal nuclei in each specimen.

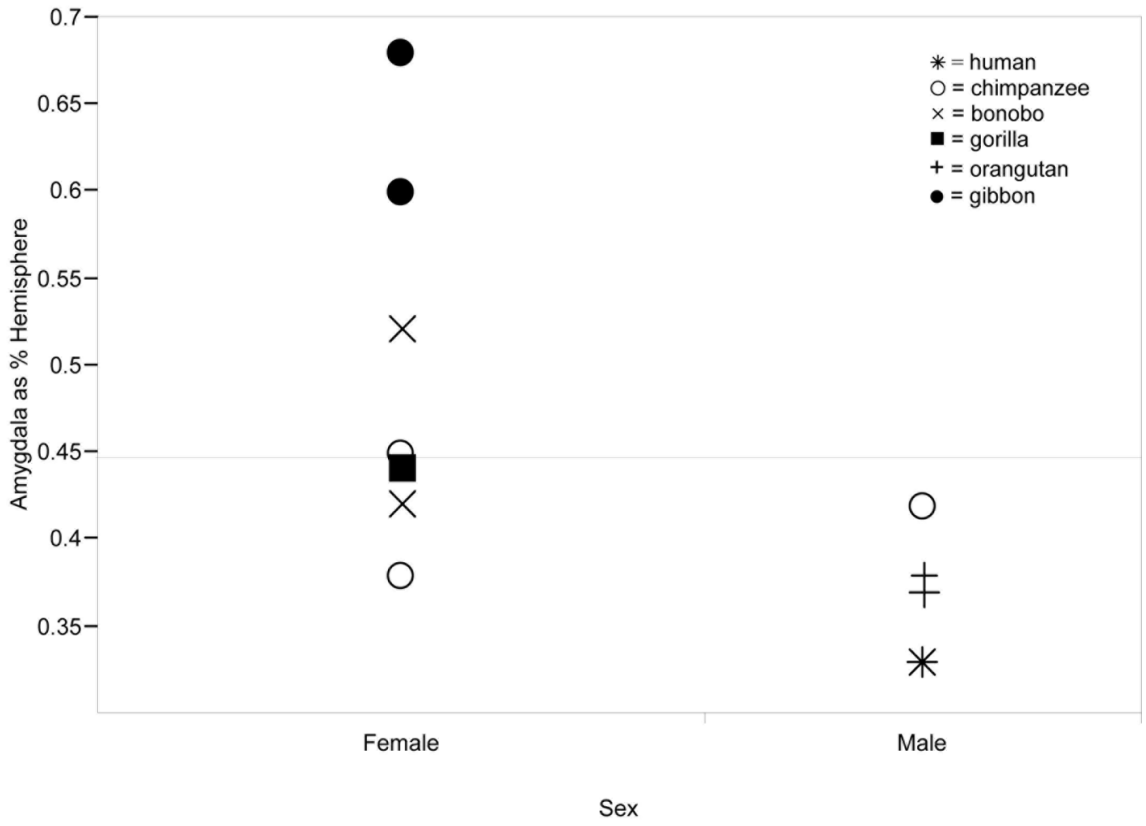

Figure 2.7. Range of relative volumes in male and female specimens. Amygdala (amygdaloid complex) volumes are reported as a % of the hemisphere volumes.

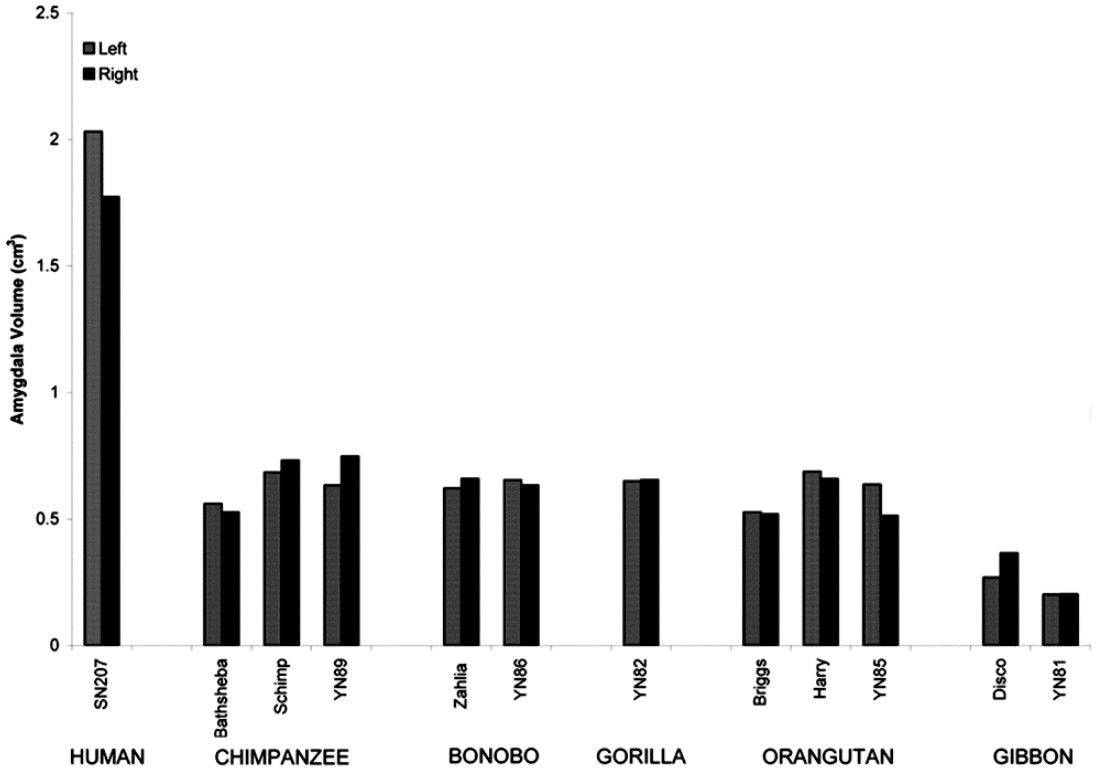

Figure 2.8. Graph of absolute right and left amygdala volume in cm^3.

Chapter 3

Neuronal populations in the basolateral nuclei of the amygdala are differentially increased in humans compared with apes: A stereological study[1]

ABSTRACT

In human and non-human primates, the amygdala is known to play critical roles in emotional and social behavior. Anatomically, individual amygdaloid nuclei are connected with many neural systems that are either differentially expanded or conserved over the course of primate evolution. To address amygdala evolution in humans and our closest living relatives, the apes, we used design-based stereological methods to obtain neuron counts for the amygdala and each of four major amygdaloid nuclei (the lateral, basal, accessory basal, and central nuclei) in humans, all great ape species, lesser apes, and one monkey species. Our goal was to determine whether there were significant differences in the number or percent of neurons distributed to individual nuclei among species. Additionally, regression analyses were performed on independent contrast data to determine whether any individual species deviated from allometric trends. There were two major findings. In humans, the lateral nucleus contained the highest number of neurons in the amygdala, while in apes the basal nucleus contained the highest number of neurons. Additionally, the human lateral nucleus contained 59% more neurons than

[1] The contents of Chapter 2 are currently in press.
Citation: Barger N, Stefanacci L, Schumann CM, Sherwood CC, Annese J, Allman JM, Buckwalter JA., Hof PR, Semendeferi K. 2012. Neuronal populations in the basolateral nuclei of the amygdala are differentially increased in humans compared with apes: A stereological study. *The Journal of Comparative Neurology*

predicted by allometric regressions on nonhuman primate data. Based on the largest sample ever analyzed in a comparative study of the hominoid amygdala, our findings suggest that an emphasis on the lateral nucleus is the main characteristic of amygdala specialization over the course of human evolution.

INTRODUCTION

The amygdala is comprised of numerous discrete nuclei with distinct cytoarchitecture, chemoarchitecture, and patterns of connectivity with other brain regions (Freese and Amaral, 2009). Given its integrative function, there is also a high degree of intranuclear connectivity within the amygdala (Pitkänen and Amaral, 1998; Freese and Amaral, 2009; Barton et al., 2003). Extrinsically, specific nuclei communicate with diverse neural systems such as the autonomic nervous system, the striatopallidal system, and neocortical sensory regions (Price et al., 1987; Heimer and Van Hoesen, 2006; Stefanacci and Amaral, 2002). As such, the amygdala is strategically positioned to bridge higher-order sensory information from the neocortex with brainstem and subcortical structures that facilitate the production of adaptive physiological and motor responses (Price et al., 1987; Freese and Amaral, 2009; Heimer et al., 1999). Across mammals, the amygdala has been shown to modulate emotional responses to external stimuli, especially fear-producing stimuli (LeDoux, 2007; MacLean, 1949). In human and nonhuman primates, the amygdala has been characterized as a detector of salience, ambiguity, value, and threat (Adolphs, 2010; Morrison and Salzman, 2010; Amaral et al., 2003; Bechara et al., 2003), and it has also been associated with social behavior and social affiliation (Adolphs, 2003; Brothers, 1990 b; Bickart et al., 2010).

While the gross anatomical structure of the amygdala is similar across primate species (Fig. 1) (Price et al., 1987; Schumann and Amaral, 2005; Carlo et al., 2010; Barger et al., 2007; Heimer et al., 1999), its internal organization has been shown to vary across species both qualitatively (Pitkänen and Kemppainen, 2002) and quantitatively (Barger et al., 2007; Stephan et al., 1987). In earlier comparative analyses of primates, Stephan and colleagues (1987) determined that a gross subcomponent of the amygdala, which included its basolateral division (i.e., the lateral, basal, and accessory basal nuclei) and some of its superficial cortical nuclei, increased at substantially greater rates relative to overall brain size than the rest of the amygdala, which primarily included the dorsalmost set of cortical nuclei and the central nucleus (Stephan and Andy, 1977). Barton and Aggleton (2000) extended these analyses to show that the basolateral division, in particular, is larger in humans than predicted by allometry, and that it correlates with (i) social group size and (ii) parvocellular visual pathway size.

We have recently investigated these early findings in more detail, anatomically, by targeting the evolution of discrete nuclei in the primate amygdala. Across Old World and New World monkey species, we established that the volumes and number of neurons in the lateral, basal, and accessory basal nuclei generally increase at the same rate as the volume and number of neurons in the whole amygdala. In contrast, increases in the volume and number of neurons in the central nucleus are hypometric, i.e., they do not keep up with increases in the whole amygdala (Carlo et al., 2010). In humans and apes, we have found that as brain size increases, amygdala volume expands at similar rates as

the whole basolateral division (Barger et al., 2007). To date, no comparable quantitative information is available for the central nucleus in apes.

Moreover, our previous volumetric data indicate that, in its internal organization, the human amygdala exhibits specializations that are unique to our species (Barger et al., 2007). Specifically, the human lateral nucleus is significantly larger than predicted for an ape of human brain size. Consequently, the lateral nucleus is the largest nucleus in the human amygdala (Schumann and Amaral, 2005; Barger et al., 2007), whereas the basal nucleus is the largest nucleus in the ape amygdala. Thus, human amygdala evolution is not necessarily characterized by passive increases in volume associated with increases in overall brain size, but rather by evolutionarily reorganization (Holloway, 1968) of its component nuclei, perhaps as a response to selection pressures in human evolution (Semendeferi et al., 2010). The number of neurons in the ape amygdala has never been investigated, though, leaving open questions about the relationship between increases in volumes and neuronal populations in the evolution of large-brained primate species.

The goal of this study was to determine whether the number of neurons in the amygdala and in each of four major amygdaloid nuclei (lateral, basal, accessory basal, and central) differ between humans and our closest living relatives, the apes. This study comprises the largest sample ever used in a comparative analysis of the hominoid amygdala (35 specimens). In addition to humans, the sample includes all of the large, “great”, ape species (chimpanzees, bonobos, gorillas, and orangutans), as well as representatives of the more distantly related smaller, or “lesser”, apes (gibbons). The present study builds on our previous comparative study of amygdala volumes (Barger et

al., 2007) in the following ways: First, we used assumption-free stereological methods to estimate neuron numbers. Second, we counted neurons in the central nucleus, to test the hypothesis that the central nucleus might be more conserved across hominoids than the basolateral nuclei. Third, we included a macaque monkey species in the sample to provide a phylogenetic outgroup. Based on our volumetric findings, we predicted that the number of neurons in the basolateral nuclei would increase at greater rates across primate species than in the central nucleus. Additionally, we predicted that the number of neurons in the basal nucleus would be higher in apes than in humans, while the number of neurons in the lateral nucleus would be disproportionately higher in humans than in nonhuman primates.

MATERIALS AND METHODS

Specimens. Our sample (Table 1) comprised 35 specimens including humans (n = 11), chimpanzees (n = 5), bonobos (n = 4), gorillas (n = 5), orangutans (n = 4), gibbons (n = 3), and long-tailed macaques (n = 3). The sample includes specimens from our collective libraries (CMS, KS, JA, and JB), as well as nine new ape specimens processed by NB (Table 1). Human and ape brains were extracted within 24 hours of the individual's natural death and were free of neuropathologies. Brains were subsequently immersion fixed in either 10% formalin, Bodian solution, or 4% paraformaldehyde. For each collection, specimens were either paraffin embedded and sectioned (KS collection) or stored at 4°C in a solution of phosphate buffered saline (PBS; pH=7.4) and 0.01% sodium azide (CCS, CMS, JA, JAB, JMA, PRH collections) prior to tissue processing. Macaque brains were perfused with a 4% paraformaldehyde solution and subsequently

submersed in a sucrose solution for cryoprotection (Buckwalter et al., 2008). Our sample included individuals spanning developmental periods from juvenile to adulthood. We did not anticipate that the inclusion of younger individuals would substantially influence our results, because it is broadly held that neurons in the amygdala complete migration by birth (Schumann et al., 2011). Although postnatal neurogenesis has been evidenced in the adult primate amygdala (Bernier et al., 2002), we found that, within each species, neuron numbers in juveniles fell close to or overlapped adult values and that age was not significantly correlated with neuron number. Little is known about the effect of aging on amygdala neuron number in humans, but MRI data suggest that limbic structures, including the amygdala, are largely preserved into the eighth decade of life (Grieve et al., 2005). Stereological analyses of amygdala aging have only been performed in rats and indicate that neuron numbers are relatively similar in adult and aged mice (von Bohlen und Halbach and Unsicker, 2002; Rubinow and Juraska, 2009). As such, the inclusion of juvenile and aged individuals should not substantially influence estimated mean neuron numbers within species.

Tissue processing. For this study, we produced nine new series of sections from ape brain tissue including three chimpanzees, four gorillas, one orangutan, and one gibbon (Table 1). Either an entire hemisphere or a 3-4 cm anterior temporal lobe block was prepared for cryosectioning by submerging and saturating the tissue in increasing grades of a sucrose and PBS (10%, 20%, and 30%). The block was then serially sectioned at 50 µm, except for one gibbon and one gorilla specimens, which were cut at 40 µm (Table 1). Every 10th section was mounted and stained for Nissl substance with thionin.

Processing parameters for series drawn from existing libraries are as follows. Ten human brains (CMS) were cryoprotected, sectioned at 50 µm, and stained for Nissl substance with thionin (Schumann and Amaral, 2005). Eleven ape and human specimens(KS) were paraffin embedded, sectioned at 20 µm, and stained for Nissl substance with a modification of the Gallyas silver stain (Merker, 1983; Semendeferi et al., 1998). Two bonobo brains (JMA) were cryoprotected, sectioned at 100 µm, and stained for Nissl substance with Cresyl Violet (Allman et al., 2010). Three long-tailed macaque brains (JAB) were cryoprotected, sectioned at 50 µm, and stained for Nissl substance with thionin (Buckwalter et al., 2008). All brains were sectioned in the coronal plane. We followed standard stereological procedures to estimate neuron counts, which are robust against variation in section thickness and processing techniques.

Anatomical delineation. The amygdala is a roughly ovoid structure located in the anteromedial temporal lobe (Fig. 1), containing at least 13 distinct nuclei in primates (Price et al., 1987). The anatomical borders of the primate amygdala and its nuclei can be reliably defined across species in Nissl stained material (Price et al., 1987; Schumann and Amaral, 2005; Carlo et al., 2010; Barger et al., 2007; Heimer et al., 1999). In particular, the nuclei chosen for this analysis exhibit boundaries that are clear in Nissl preparations and are easily distinguishable across all species analyzed (Fig. 1A-F). Borders for the hominoid amygdala and the lateral, basal, accessory basal, and central nuclei were defined using anatomical descriptions of the macaque (Price et al., 1987) and human amygdala (Heimer et al., 1999; Schumann and Amaral, 2005). Although each nucleus can be further parcellated into discrete subdivisions, all nuclear subdivisions do not show

consistent chemoarchitectonic homologies between macaques and humans (Pitkänen and Kemppainen, 2002) and could not be reliably defined in great apes without considerable further study. One investigator (NB) hand-traced the boundaries of the amygdala and the lateral, basal, accessory basal, and central nuclei in serial sections under 1x and 2x objectives (N.A. 0.4 and 0.06, respectively) of a Nikon Eclipse 80i (Melville, NY) microscope with the StereoInvestigator software suite (MicroBrightField,Williston, VT). The anatomical borders for each region were identified using the following criteria.

Amygdala. The rostral pole of the amygdala was marked by the first appearance of the basolateral nuclei (Schumann and Amaral, 2005). The external capsule borders the amygdala dorsolaterally, especially at rostral levels. The putamen also borders the amygdala dorsolaterally in caudal sections and can be differentiated from the amygdala by differences in cell structure, density, and organization (Fig. 2F). Dorsomedially, the amygdala is bounded by the substantia innominata, marked by the presence of the basal nucleus of Meynert (Fig. 2D-F). Ventromedially, the semiannular sulcus (Fig. 2A-E) separates the entorhinal cortex from the amygdala and can generally be used as a reliable landmark in addition to cytoarchitecture (Schumann and Amaral, 2005; Sorvari et al., 1995; Insausti et al., 1995; Amaral et al., 1987). At caudal levels, the lateral ventricle and hippocampus form the amygdala's ventrolateral borders (Fig. 2E,F), while, at rostral levels, temporal lobe white matter forms the ventral border (Fig. 2A,B). Within the amygdala, the longitudinal association fiber bundles (Price et al., 1987), also referred to as the meduallary laminae (Heimer et al., 1999), generally mark the boundaries between the major nuclei.

Lateral nucleus. The lateral nucleus is the most laterally positioned nucleus of the amygdala and has been divided into four subdivisions in macaques and two in humans (Pitkänen and Kemppainen, 2002). Its lateral, dorsal, and ventral borders are consistent with those of the lateral amygdala. Rostrally and dorsally, the lateral nucleus is in close proximity to the ventral claustrum which is distinguished by larger, more darkly staining cells. The medial border of the lateral nucleus is defined by the lateral medullary lamina. Cells in this region are smaller and more compact than cells in the adjacent basal nucleus. The ventral aspect of the lateral medullary lamina often terminates above the ventralmost extent of the lateral and basal nuclei, creating a notch (see arrows in Fig. 2A-E). This feature may be used as an additional landmark to distinguish between the two nuclei at ventral levels where the lamina is less prominent. Caudally, the comparatively larger cells of the lateral nucleus distinguish it from the dorsally adjacent putamen.

Basal nucleus. The basal nucleus is separated from the lateral, accessory basal, central, and intercalated nuclei by the medullary laminae. The human and non-human basal amygdala has been divided into 3 subdivisions: a large celled "magnocelluar" division, which is located dorsally, a small celled parvicelluar division, which comprises the rostral and ventral portions of the nucleus, and an intermediate division located between the two (Sorvari et al., 1995; Price et al., 1987). The basal nucleus contains the largest cells in the amygdala and is situated between the accessory basal and lateral nucleus (Fig. 2B-E and Fig. 1A-F). The lateral medullary lamina divides the lateral aspect of the basal nucleus from the lateral nucleus. The intermediate medullary lamina divides the medial aspect of the basal nucleus from the accessory basal nucleus (Fig. 2). The

basal and accessory basal nuclei are further distinguished from one another by differences in cell size. Thus, the presence of such large cells in the basal nucleus can generally be used to distinguish it from the medial aspect of the lateral nucleus and the ventrolateral aspect of the accessory basal nucleus.

Accessory basal nucleus. The intermediate medullary lamina and the large cells of the basal nucleus distinguish the lateral border of the accessory basal nucleus. The medial border is demarcated by the medial medullary lamina, which divides the accessory basal nucleus from the superficial cortical nuclei. Our definitions of the accessory basal nucleus included three recognized subdivisions (Sorvari et al., 1995; Freese and Amaral, 2009; Price et al., 1987; but see also de Olmos, 2004, for a different delineation scheme). The small celled parvicelluar division is located rostrally and laterally. The large celled magnocelluar division is positioned dorsally and runs from midrostrocaudal levels to the caudal extent of the nucleus. The ventromedial division comprises a small, compact, grouping of large sized, darkly stained cells on the ventromedial aspect of the nucleus. It runs for only a short extent through midrostrocaudal levels of the nucleus (Fig. 2B-D) and shows a slightly different histochemical profile than the immediately adjacent parvicellular division. For example, parvalbumin levels in this division are intermediate between those in the magnocellular and parvicellular divisions (Ichinohe and Rockland, 2005; Sorvari et al., 1995).

Central nucleus. The central nucleus is encapsulated and separated from the substantia innominata, dorsally, and the basolateral nuclei, ventrally, by fiber bundles (Fig. 2C-F). This feature, as well as its smaller, lighter staining and less densely packed

cells, distinguish it from the superior aspects of the adjacent basal and accessory basal nuclei and the ventromedial surface of the putamen (Fig. 2F). It lies caudal to the anterior amygdaloid area (Fig. 2A), which contains more darkly staining and diffuse neuronal populations than the central nucleus. Throughout much of its caudal extent, the central nucleus is often nestled between a few of the distinct, small, and darkly staining intercalated amygdaloid nuclei, which flank the white matter fibers surrounding the nucleus on its ventral border and further clarify its position (Fig. 2F). There are two recognized subdivisions of the central nucleus, a lateral and medial division, which are separated by fiber bundles (Price et al., 1987; Sorvari et al., 1995).

Data collection. Neuron numbers were estimated using the optical disector probe in combination with fractionator sampling (West, 1993) in the StereoInvestigator software suite (MBF Bioscience, Willistown, VT). For the majority of specimens, stereological analyses were performed using a Dell workstation which received live video from an Optronics MicroFire color video camera (East Muskogee, OK) attached to a Nikon Eclipse 80i microscope (Melville, NY) equipped with a Ludl MAC5000 stage (Hawthorn, NY) and Heidenhain z-axis encoder (Plymouth, MN). Sections were viewed through a 100x oil objective (NA 1.4) under Köhler illumination. The disector frame size was 60 x 60 µm with a height of 9 µm, which yielded an average of 1-3 neurons per counting frame across species. Section thickness was measured at every site. Section thicknesses varied from 11-17 µm. To determine whether guard zones were necessary, we performed z-axis counts on paraffin embedded and cryosectioned tissue (Andersen and Gundersen, 1999; Gardella et al., 2003; Carlo and Stevens, 2011). Both processing

techniques yielded sections with fewer neurons at the margins of the tissue than in the center, indicating that tissue processing may have produced artifacts which impacted the distribution of neurons in the z-axis (Andersen and Gundersen, 1999; Gardella et al., 2003). To ensure that these artifacts at the margin of the tissue did not influence our counts, we applied guard zones of 1-3 µm, depending on section thickness.

In many cases, every available section was sampled, but when the sample interval included more than one section, the starting section in the interval was chosen at random and subsequent sections were sampled at fixed intervals, as is standard procedure (West, 1993). The distance between sampled sections ranged between 0.4 - 1.2 mm, reflecting the diverse array of brain sizes in the sample. Due to these brain size differences and also to differences in volume across the nuclei, several different grid sizes were utilized for each nucleus in each species (Nonhuman primates: Table 2; Humans: Schumann and Amaral, 2005). A neuron was counted only if its nucleus first came into view within the counting frame or intersected the lines of inclusion located on the frame's top and right sides, but not the lines of exclusion to the bottom and left (Schumann and Amaral, 2005). A cell was marked as a neuron if it exhibited a large, clear, lightly stained nucleus, containing a single, distinct nucleolus, surrounded by darkly stained clumps of Nissl substance covering the remainder of the neuronal perikarya extending to the proximal portions of the dendritic processes (Fig. 3, arrows). Because the nuclei of the amygdala are generally regularly shaped, we report coefficient of error values using m=1 rather than m=0, the latter of which is more appropriate for irregularly shaped structures (Gundersen et al., 1999). In no case did the coefficient of error (Gundersen, m = 1)

exceed 8% for any region analyzed, indicating that the precision of stereological estimates was high. Sampling variance is unlikely to contribute more than 50% to observed group variance, a measure suggested to balance sampling precision and efficiency (West et al., 1991).

As in our previous analysis (Schumann and Amaral, 2005), post-processing section thickness was measured at each stereologic probe site so that mean measured section thickness could be used to estimate the disector's thickness sampling fraction when calculating neuron counts. Alternatively, the use of number weighted section thickness has been advocated to estimate neuron numbers when considerable deformation is present in the z-axis (Dorph-Petersen et al., 2001). Thus, we tested whether our choice of thickness measure would significantly influence our estimates. For each nucleus within each taxonomic group, estimates calculated with number weighted thicknesses varied less than 3% on average from values calculated with mean measured section thickness. These differences were not statistically significant when assessed within individual species or across the entire sample (student's t-test: $p>0.05$ for the amygdala and all nuclei).

We quantified data from one hemisphere in each specimen (Table 1) to maximize sample size. There was no influence of laterality on amygdala volume in our previous volumetric analysis (Barger et al., 2007) using many of the same specimens. Data for 10 of the 11 human amygdala were collected by CMS (Schumann and Amaral, 2005). In an interobserver reliability test performed on two of the 10 human specimens, NB produced neuron counts that were more than 95% concordant with previously published data

(Schumann & Amaral, 2005), confirming that data from the two analyses could be reliably combined.

Data analysis. Data from all structures passed the Shapiro-Wilk test for normality in all species; however, we opted to use nonparametric analyses when possible, as most distributions exhibited evidence of skewness and deviation for mesokurtosis likely due to the small intraspecific sample sizes. In addition to raw neuron numbers, we calculated the percent of total amygdala neurons contained in each amygdaloid nucleus to factor out the influence of total amygdala neuron number on interspecific comparisons. This measure was defined as the quotient of the neuron number in a nucleus divided by total amygdala neuron number (e.g., central neuron number/amygdala neuron number). Both raw neuron counts and percentage data were subjected to a Kruskal-Wallis test to determine whether means differed significantly across species. If significant variation was present, we further explored differences between individual species *post-hoc* using the Mann-Whitney U test (SPSS 17, SPSS, Inc).

We performed allometric regressions in two conditions: 1) with humans included to assess trends across primates and 2) with humans excluded to determine if observed human values were significantly greater than predicted by non-human primate values. To investigate allometric trends, species mean log-transformed data were entered into the phylogenetic independence contrasts program PDAP (Midford et al., 2003) in Mesquite 2.74 (Maddison and Maddison, 2010). Phylogenetic branch lengths (Purvis, 1995) were log transformed so that standardized contrasts did not correlate with their standard deviations (Garland et al., 1992). The number of neurons in each nucleus was regressed

against the total number of amygdala neurons minus the neuron number in that nucleus to eliminate statistical artifacts that results from regressing a structure against itself. Regression equations and confidence intervals obtained from PDAP were mapped back into the original data space, representing contemporary species data, for subsequent analysis. We chose to include all non-human primate species in the interest of increased statistical power. Although the macaque mean data point may be regarded as a possible statistical outlier that may influence the results of our analysis, the slopes of regression lines drawn through non-macaques fit well within the 95% confidence intervals of lines drawn through all species.

We tested for significantly positive or negative residuals to determine whether changes in neuron distribution reflected adherence to allometric trends across primates or derived features deviating from these trends. We also use this metric due to the tendency for PDAP to produce inflated prediction intervals (Midford et al., 2003). The value of each species' mean residual was subjected to a Student's one-sample *t*-test to determine whether residuals significantly deviated from 0. For more intuitive interpretation, we provide and report percent residuals for each species, which were calculated from untransformed values using the following formula: (observed-predicted value)/predicted value.

Photomicrograph production. Images were taken on either a Nikon Eclipse 80i microscope at 1x (Figure 2) or 100x (Figure 3) magnification or a Leica MZ6 stereomicroscope at 0.63x magnification (Figure 1) with an Optronics MicroFire camera (East Muskogee, OK) and the program Picture Frame 2.3 (Optronics, Inc, East

Muskogee, OK). The entire chimpanzee amygdala is too large to be captured at 1x, thus component images were montaged in Adobe Photoshop Elements 5.0 (Adobe Systems, San Jose, CA) to produce each panel in Figure 2. To ensure published images best approximated the clarity and contrast of slides as viewed under the microscope, brightness, contrast, and sharpness were manipulated in all images using GIMP 2.6.2 and Adobe Photoshop Elements 5.0 (Adobe Systems, San Jose, CA). Boundaries for published images were drawn with GIMP 2.6.2.

RESULTS

Neuron numbers. The total number of neurons in the amygdala of humans and great ape species (hominids) overlapped. All hominid amygdala exhibited approximately 12-14 million neurons. The absolute number of amygdala neurons in non-hominids was generally less than in hominids. Specifically, the amygdala of lesser apes, the gibbons, contained nearly half this number (6.6 million) and the amygdala of the long-tailed macaques, roughly a fourth (3.4 million) (Table 3; Fig. 4). This observation was statistically supported by the Kruskal-Wallis analysis which detected significant differences between species in the mean number of neurons in the amygdala and in most nuclei (Amygdala: $H_{(6)} = 14.10$, $p = 0.029$; Lateral: $H_{(6)} = 24.20$, $p < 0.000$, Basal: $H_{(6)} = 16.08$, $p = 0.013$; Central: $H_{(6)} = 20.44$, $p = 0.002$). Differences in the accessory basal nucleus approached significance ($H_{(6)} = 11.32$, $p = 0.079$).

Post-hoc comparisons confirmed that species differences in the number of neurons in each amygdaloid nucleus were largely split between large brained hominids

and smaller brained non-hominids (Table 3). That is, the amygdaloid nuclei of great apes and humans generally contained more neurons than those of gibbons and macaques.

Neuron numbers in individual nuclei stood out significantly in only two species. In humans, the lateral nucleus contained significantly more neurons (4.32 million) than all other primates analyzed (Fig. 4). Additionally, the human central nucleus contained significantly fewer neurons (0.37 million) than chimpanzee (0.50 million) and orangutan (0.44 million) central nuclei (Table 3; Fig. 4). The bonobo central nucleus also contained significantly fewer neurons (0.31 million) than the central nuclei of chimpanzees and orangutans, but not significantly fewer than humans (Table 3; Fig. 4). The average number of neurons in the gorilla central nucleus (0.42 million) was also greater than in bonobos or humans, but this difference did not reach significance.

Nuclei as percent of total amygdala neurons. There were significant species differences in the percent of total amygdala neurons distributed to each nucleus (Kruskal-Wallis: Lateral: $H_{(6)} = 20.52$, $p = 0.002$; Basal: $H_{(6)} = 19.13$, $p = 0.004$; Accessory basal: $H_{(6)} = 16.43$, $p=0.012$; Central: $H_{(6)} = 13.72$, $p = 0.033$). Species' mean values and the results of post-hoc analysis are presented in Table 4, while species mean values are presented graphically in Figure 4.

Post-hoc tests indicated that human amygdala contained a significantly greater percentage of neurons in the lateral nucleus than great apes. At 32.5%, the percentage of neurons in the human lateral nucleus was the largest of any nucleus analyzed in the human amygdala.

Ranging from 23.5-34%, the percentage of neurons in the basal nucleus of all ape species was the largest of any nuclei analyzed in the ape amygdala. Among the apes, the percentage of neurons in the orangutan basal nucleus (23.5%) was significantly smaller than in the other great apes(31.1-34%). Orangutans also had a significantly smaller percentage of neurons in the accessory basal nucleus than other apes (9.8-12%). Gorilla amygdala contained proportionately more amygdala neurons in the accessory basal nucleus than other great apes.

In long-tailed macaques, like humans, the largest percent of amygdala neurons was located in the lateral nucleus (Fig. 4). Nonetheless, the average percentage of neurons in macaque lateral nuclei (27.4%) was significantly smaller than in human lateral nuclei (32.5%). The long-tailed macaque amygdala contained a significantly greater percentage of accessory basal (15.4%) and central neurons (7.8%) than any other species.

Allometric analysis. The lateral nucleus fell very slightly below isometry with respect to number of neurons in the rest of the amygdala ($b = 0.937 \pm 0.539$ (95% CI), $R^2 = 0.800$, $p < 0.01$; Fig. 5A). Neuron numbers in the basal nucleus scaled with positive allometry ($b = 1.08 \pm 0.542$ (95% CI), $R^2 = 0.840$, $p < 0.01$; Fig. 5B). Because regressions for both the basal and lateral nuclei contain a slope of 1 in the 95% confidence interval, it is possible that both nuclei scale with isometry. Neurons in the accessory basal nucleus scaled considerably more negatively ($b = 0.642 \pm 0.243$ (95% CI), $R^2 = 0.902$, $p < 0.01$; Fig. 5C). The slope for central nucleus neuron numbers was low, but did not correlate significantly with total amygdala numbers ($b = 0.400 \pm 0.599$ (95%CI), $R^2 = 0.371$, $p = 0.147$; Fig. 5D).

Human departures from allometry. Human residuals for the lateral nucleus were significantly positive whether humans were included (residual = 0.174, $p < 0.000$) or excluded (residual = 0.202, $p < 0.000$) from the prediction equation (Table 5; Fig 6). When humans were excluded from the regression, the percent residual for observed human values was 59% (Table 5; Fig. 6). Additionally, human data points largely fell outside of the 95% prediction interval when they were excluded from the analysis, and the human mean clearly fell outside of this range (Fig. 7). Human residuals for the central nucleus fell 12% below predicted values when humans were excluded (residual = -0.061; $p = 0.028$) (Table 5; Fig. 6), but the regression equation did not reach significance ($b = 0.423$; $R^2 = 0.390$; $p = 0.185$). Neuron numbers in the human basal nucleus were nearly significantly smaller than expected when humans were excluded from the regression (residual = -0.037, $p = 0.067$), but the magnitude of this deviation was low, approximately 7% (Table 5; Fig. 6).

Allometric departures in nonhuman primates. Results are presented in Table 5 and graphically in Figure 6. Chimpanzees exhibited significant positive residuals for basal nucleus neuron number (residual = 0.16, $p = 0.033$). In contrast, orangutan mean residual neuron numbers for the basal nucleus were nearly significantly smaller than predicted by regressions drawn through other primates (residual = -0.11, $p = 0.059$). Because human residuals were low for this nucleus and may have a negative influence on the regression line, we also tested orangutan residuals in a regression that excluded human data points for the basal nucleus. In this case, the number of neurons in the orangutan basal nucleus was significantly smaller than predicted for a non-human

primate with a similar number of total amygdala neurons (residual = -0.13, p = 0.035). Orangutans' residuals were significantly negative for the accessory basal nucleus, as well (residual = -0.046, p = 0.035). Alternatively, gorillas' mean accessory basal neuron number residual was positive and approached significance (residual = 0.071, p = 0.080). When humans are excluded from the lateral nucleus regression, bonobo residuals for this nucleus are nearly significantly positive (residual = 0.05, *P* = 0.053).

Summary. Absolute neuron numbers in the amygdala and most nuclei generally overlapped in humans and great apes and were greater in these species than in gibbons and macaques. In one of the few deviations from this general observation, the human lateral nucleus contained significantly more neurons than the lateral nucleus of any other species in the analysis. When the number of neurons in each nucleus were considered as a proportion of total amygdala neurons, neuron numbers in the lateral nucleus were greatest in humans as well. Accordingly, humans exhibit 59% more neurons than predicted by allometric regression lines drawn through other primates. Together, the data provide robust evidence that a greater proportion of amygdala neurons are distributed to the lateral nucleus in humans when compared with our closest relatives.

The amygdala in apes contained a higher percentage of neurons in the basal nucleus than macaques and humans, and the human basal nucleus contained slightly fewer neurons than predicted by trends across nonhuman species. The chimpanzee basal nucleus contained more neurons than predicted, while gorillas distributed more neurons to the accessory basal nucleus. Neuron numbers in the basal and accessory basal nuclei are smaller in orangutans than predicted by trends across other non-human primates.

DISCUSSION

The goal of the present analysis was to examine the distribution of neurons in the amygdala of humans and apes. We quantified the number of neurons in the amygdala and its lateral, basal, accessory basal, and central nuclei in 24 nonhuman primate specimens representing all great ape species, gibbons, and macaques (Table 3). We found that the human amygdala is not simply an evolutionarily "scaled up" version of an ape amygdala. The human amygdala contained significantly and proportionately more neurons in the lateral nucleus than the ape amygdala (Fig. 4). This number was greater than expected based on trends across apes and macaques (Fig. 7). In contrast, neuronal populations in the ape amygdala were highest in the basal nucleus. The data indicate that, after the human lineage split from the last common ancestor we shared with great apes, a shift in amygdala organization occurred that resulted in increased neural populations in the lateral nucleus.

Evolutionary Scaling of Amygdaloid Nuclei across Species

The percentage and number of neurons found in each amygdaloid nucleus varied across species, with most of this variation accounted for by allometric scaling expectations. Because each nucleus exhibited a different scaling rate (Fig. 5), an increase in amygdala neuron number will have different, but largely predictable, consequences for the percentage of neurons distributed to any particular nucleus. In our sample, basal nucleus neuron numbers increased at a slightly greater rate than total amygdala neuron number (slope = 1.1). Thus, increases in total amygdala neuron number will lead to an increasingly larger percentage of neurons being distributed to the basal nucleus. In the

lateral nucleus, neuron number scaled with slight negative allometry (slope = 0.9), nearly keeping up with changes in total amygdala neuron number. As confidence intervals for the regression of both the lateral and basal nuclei contain a slope of 1, it cannot be discounted that neuron numbers in both nuclei scale isometrically with total amygdala neuron number. The accessory basal nucleus, in contrast, exhibited clear negative allometry with a slope of 0.6 (and upper confidence limit of 0.9), suggesting that neurons in this nucleus will only double for every tripling of total amygdala neuron number on average. Increases in central nucleus neuron populations did not show a strong relationship with total amygdala neuron number. The regression data suggest a trend for neurons in the central nucleus to double for every five-fold increase in total amygdala neuron number, although larger samples are needed to determine if this relationship is significant.

Evolutionary Specializations in Hominoid Amygdala

Human amygdala. The human lateral nucleus contained a disproportionately large number of neurons compared with other primates, especially the great apes. The human amygdala contained significantly more neurons in the lateral nucleus, both absolutely and proportionately, than was the case in apes (Tables 3 and 4), and this number was greater than expected based on trends across apes and macaques (Table 5; Fig. 6). Neuron numbers in the human lateral nucleus were nearly 60% greater than predicted by allometric trends, a degree of magnitude rarely seen in comparative analyses of human brain evolution (Sherwood et al. 2012). For example, the volume of the human neocortex is 24% larger than expected for a primate of our brain size (Rilling and Insel,

1999), while the frontal lobe, long assumed to be enlarged, is approximately the size expected for an ape of human brain size (Semendeferi et al., 2002; Semendeferi and Damasio, 2000). Increases in lateral neuron populations are perhaps balanced by decreases in neuron numbers in the central and basal nuclei, which exhibit subtle reductions in humans (Table 3; Fig. 6).

We previously reported that the volume of the amygdala is, on average, over 3 times larger in humans than in great apes (Barger et al., 2007). In contrast, we found that amygdala neuron number did not differ between the two groups. Given that great ape and human neuron numbers also overlap in area 13, a functionally and connectively related limbic structure in the posterior orbitofrontal cortex (Semendeferi et al., 1998), neuron numbers in hominid limbic structures may be characterized by evolutionary conservation. However, area 13 is less than twice as large in humans as it is in great apes (Semendeferi et al., 1998). Given this difference, one possibility that remains to be investigated is the potential importance of neuropil expansion in the evolution of the human amygdala.

Other hominids. Human share the phylogenetic classification of hominid with our closet living relatives, the great apes. These include chimpanzees, bonobos, gorillas, and orangutans, in order of their phylogenetic relatedness to humans. Even though neuron numbers were similar across hominids, the distribution of neurons across amygdaloid nuclei varied between humans and great apes indicating that the human amygdala is evolutionarily reorganized in relation to great ape amygdala. High rates of allometric scaling in the basal nucleus (Fig. 5b) may explain our related finding that neuron

numbers in great ape amygdala were highest in the basal nucleus absolutely and proportionately (Fig. 4)

In some cases, we found preliminary evidence that individual great ape species may exhibit neural specializations in the amygdala. The chimpanzee basal nucleus contained 38% more neurons that predicted for a species with a similar number of amygdala neurons, although the absolute number and percentage of basal nucleus neurons was not significantly greater in chimpanzees. We found that the amygdala of bonobos (or "pygmy chimpanzees") did differ from that of common chimpanzees, and this is consistent with a recent neuroimaging study (Rilling et al., 2011). Bonobo central nuclei contained the smallest number of neurons among hominids. They had nearly significantly fewer neurons in the bonobo central nucleus than most other great apes (Table 3). Additionally, bonobo lateral nuclei contained more neurons than all non-human hominids, although deviations from predicted values only approached significance in allometric regressions across nonhuman primates. Given this pattern, it is tempting to speculate that, of all the apes, bonobos might come closest to approximating human amygdala organization, but a substantially higher sample size would be needed to test that hypothesis. In gorillas, the accessory basal nucleus contained a larger percentage of neurons than any other hominid species, although residuals for this nucleus only approached significance in regression analyses (Table 5).

Among the great apes, orangutans are the most distantly related to humans. Although, like other great apes, the basal nucleus of orangutans contained more neurons than any other nucleus, the orangutan basal nucleus contains approximately 10% fewer

neurons than that of other great apes (Table 4) and neuron numbers in the orangutan basal nucleus are smaller than predicted when scaling rates in nonhuman primates are taken into account (i.e., when humans are excluded from the analysis). In addition, the proportion of neurons in the accessory basal nucleus of the orangutan amygdala was small compared with other primates and neuron numbers in this nucleus were 10% fewer than predicted by allometric regressions (Tables 4 and 5; Fig. 6). This was not the case for all basolateral nuclei, as the number and percent of neurons in the orangutan lateral nucleus were close to those of other great apes and residuals were not significantly different from 0.

Other hominoids. We are using the term hominoid to refer to the larger phylogenetic classification that includes humans, great apes, and lesser apes, the gibbons and the siamang. Gibbon amygdala contained fewer neurons than human and great ape amygdala, as a whole and in each nucleus analyzed (Table 3), but the organization of the gibbon amygdala followed the pattern present in great apes. Neurons in the gibbon amygdala were distributed predominantly to the basal nucleus (Fig. 4). In no case did the number of neurons in gibbons exceed predicted values for any nucleus analyzed (Fig. 6).

Gibbon neuron numbers exhibited a high degree of individual variation, which may increase the probability that our statistical analyses would produce negative results. An important feature of our gibbon sample is that it represented three distinct species. Traditionally, the social organization of all gibbon species was thought to be the monogamous pair bond; more recent data has challenged this presumption (Malone and Fuentes, 2009). In our study, the two gibbons with the highest numbers of amygdala

neurons (Fig. 5) are from two species, *Hylobates lar* and *H. concolor*, that have been reported to travel in groups of more than two individuals. *H. muelleri*, the gibbon species with the lowest number of amygdala neurons in this analysis (Fig. 5), has not been observed traveling in larger groups (Malone and Fuentes, 2009). Thus, it is possible that neuroanatomical variation in our sample might reflect behavioral variation among gibbon species, given social group size has been shown to correlate with amygdala volume (Bickart et al., 2010; Barton and Aggleton, 2000). Subsequent analyses with larger samples and a broader array of gibbon species would be needed to assess this hypothesis.

Cercopithecoids. One Old World monkey species, the long-tailed macaque, was added to our sample as a phylogenetic outgroup to contrast with hominoids. The number of neurons in individual nuclei of the long-tailed macaque amygdala did not deviate significantly from predictions based on allometric regressions. As such, it is most likely that differences between the organization of the ape and long-tailed macaque amygdala, i.e., a high percentage of amygdala neurons in the accessory basal and central nuclei, reflect the allometric relationships particular nuclei share with total amygdala neuron number rather than neural adaptations specific to this species (Table 5). A larger cercopithecoid sample would be needed to further explore this finding. Macaque values also appeared to cluster together more closely than great ape or human values. If the coefficient of variation is calculated (standard deviation/mean), macaques exhibit consistently lower values than hominoids.

Although the human amygdala clearly contained more lateral nucleus neurons than any species analyzed, we found that the human and long-tailed macaque amygdala

emphasized the lateral nucleus. This does not imply, however, that the human and macaque amygdala are more similar morphometrically than the human and great ape amygdala. Despite the fact that macaques in our study do distribute more neurons to the lateral nucleus that to other nuclei, the human lateral nucleus still contains proportionately more neurons than the macaque lateral nucleus. Additionally, the macaque amygdala contains a higher percentage of neurons in the accessory basal and central nuclei than the human amygdala. Our recently published study evidences a similar amygdala organization in long-tailed macaques; however, we found that rhesus macaques have more neurons in the basal nucleus than in the lateral nucleus, akin to ape amygdala organization (Carlo et al., 2010). Finally, from a phylogenetic perspective, the last common ancestor of humans and apes would share a similar amygdala organization that differs from those of cercopithecoids. Thus, based on the law of parsimony, human-specific increases in the lateral nucleus must have occurred after humans split with our most recent last common ancestor shared with apes and would not reflect the preservation of an ancestral cercopithecoid state (presuming long-tailed macaques represent that state). It may be the case that similarities in the amygdala organization of long-tailed macaques and humans reflect evolutionary parallelism related to functional adaptations. If the distribution of neurons does reflect amygdala function in closely related species, it may be important to consider issues of species-specific variation when investigating functional aspects of the primate nervous system and when using macaque species to model human disorders.

Comparison with Previous Volumetric Findings

Several of the findings from the present analysis are concordant with volumetric findings from our previous analysis (Barger et al., 2007). Specifically, the human lateral nucleus is significantly larger than predicted for a hominoid of our brain size, which is reflected in our findings for neuron numbers in this nucleus. We found that orangutans have significantly smaller accessory basal and basal nuclei than other great apes and this finding is also paralleled by reduced neuron numbers in both nuclei. Although the finding only approached significance, increased neuron numbers in the gorilla accessory basal nucleus would concord with our finding that volume is also increased in gorillas. In contrast, chimpanzees appear to have more neurons in the basal nucleus than predicted, but no such increase was indicated in our volumetric analysis.

Methodological Considerations

Given that many of the species in our sample are endangered and tissue samples are rare, we sought to maximize sample size by combining species from a variety of laboratories. Considerable debate has arisen concerning the influence of artifacts from tissue processing on stereological data collection. Counts from paraffin embedded tissue tend to be higher than from cryosectioned tissue (Ward et al., 2008), and we found this to be the case in our sample to some degree. Counts from paraffin embedded tissue were not significantly different from those obtained from cryosectioned tissue, though, for nearly all nuclei in all species (Mann-Whitney U Test: $Z>-1.80$, $p>0.05$, 2 tailed). The only exception was chimpanzee total amygdala counts. We tested whether counts from paraffin embedded or cryosectioned chimpanzee tissue were significantly different from

the combined mean to assess the potential impact of this difference. Counts from paraffin embedded tissue did not differ significantly from the mean (One-sample T-test: t=1.53, p=0.26), while counts from cryosectioned tissue did (t=-7.94, p=0.02), suggesting that counts from the former have a greater influence on the group mean.

Evolutionary and Functional Significance

Neural connectivity and amygdala evolution. Because the basolateral nuclei are strongly connected to the neocortex (Price et al., 1987; Freese and Amaral, 2009) and the central nucleus communicates mostly with brainstem and olfactory centers (Price et al., 1987; Stefanacci and Amaral, 2002), it has been hypothesized that high rates of neocortical enlargement in primate evolution influenced the more expansive development of the basolateral division, while conservation of the autonomic and olfactory systems resulted in the relative stabilization of the nuclei of other nuclei (Carlo et al., 2010; Stephan et al., 1987; Barton and Aggleton, 2000). Barton, et al., (2003) tested this hypothesis, finding that increases in neocortical volume are correlated with increases in the volume of the corticobasolateral amygdala (the lateral, basal, accessory basal, and more ventral cortical nuclei), but not the centromedial amygdala (the central nucleus, anterior amygdaloid area, and the more dorsal cortical nuclei). This link between neocortical enlargement and basolateral volume might be a response to increased processing demands from the neocortex, as the number of neurons in the basolateral nuclei rise concomitantly (Carlo et al., 2010). Buttressing claims that subcomponents of the amygdala evolve in a mosaic fashion (Barton and Aggleton, 2000; Stephan et al., 1987), our data provide further cellular evidence for evolutionary reorganization in the

primate amygdala, which occurs largely as a result of variation in the scaling patterns of individual nuclei.

In terms of cellular increase across hominoids, the basal nucleus appears to increase at the fastest rates as the total number of neurons in the amygdala increases. In primates, neocortex hyperscales with brain size, occupying increasingly larger proportions of total brain volume as brain size increases (Rilling and Insel, 1999; Stephan and Andy, 1969). Because the basal nucleus is the primary source of output to the neocortex, it may be the case that, as the cortical modulator (Freese and Amaral, 2009), the processing needs of the basal nucleus increase as brain, and, correspondingly, amygdala size increases.

A low allometric coefficient indicates that central nucleus neuron populations do not keep up with changes in total amygdaloid neuron numbers. In fact, we found only a weak relationship between increases in central neuron numbers and neuron numbers in the entire amygdala. Previous analyses suggest that the central nucleus, with its heavy projections to autonomic regions, is remarkably conserved across primates in terms of volume and neuron number scaling (Stephan et al., 1987; Carlo et al., 2010). This may reflect the fact that its major targets, hypothalamic and brainstem nuclei, are themselves quite conserved (Price et al., 1987; Stephan et al., 1987; Carlo et al., 2010).

In relation to great apes, the number of neurons in the human lateral nucleus was increased; this may also reflect its connectivity. Specifically, the lateral nucleus, as the primary recipient of cortical input, evaluates multimodal information about stimulus characteristics arriving predominantly from temporal lobe association cortices (Freese

and Amaral, 2009; LeDoux, 2007; Stefanacci and Amaral, 2002). The human temporal cortex is 23% larger than predicted based on trends in other primates, and the temporal lobe is the only major lobe that is known to be differentially expanded in humans in relative to apes (Rilling and Seligman, 2002; Semendeferi and Damasio, 2000). This elaboration of the temporal lobe includes not only increase in the temporal cortex but also in the subcortical white matter, which may have evolutionary and/or developmental consequences for the lateral nucleus (Rilling and Seligman, 2002; Schenker et al., 2005). It is conceivable that increased processing demands arising from the expanded temporal cortex may engender a disproportionate increase in the size of neuronal populations in the lateral nucleus. The fact that coordinated changes between the temporal cortex and amygdaloid nuclei are present only in humans suggests that these structures may have co-evolved as an integrated functional network as the human lineage split from our last common ancestors with great apes.

Complex social behavior and amygdala evolution. Many attempts have been made to explain the link between the conspicuously large size of the human brain and human behavioral complexity. An increasingly influential proposition has been the "social intelligence hypothesis", which asserts that complex primate cognition has arisen in the social, rather than material environment (Jolly, 1966; Humphrey, 1976; Byrne, 1996; Dunbar, 1993; Herrmann et al., 2007). It has been hypothesized that advanced cognitive capacities in primates arose in response to the demands of navigating complex and dynamic social environment that require an understanding of and adherence to somewhat arbitrary social rules, constraints, and conventions (Humphrey, 1976).

As the complexity of the social environment increases, cognitive systems dedicated to interpreting the identities, communicative signals, intentions and minds of social partners may become increasingly taxed (Jolly, 1966; Humphrey, 1976; Dunbar, 1993; Byrne,1996). Given the amygdala's role in social vigilance, its evolution may also be affected by these pressures. In gregarious, social mammals, like primates, the amygdala may be particularly involved in processing the emotional salience of stimuli that mark the relationships and the communicative intent of conspecifics as it is routinely engaged in processing emotionally communicative social signals (Yang et al., 2002; Sugiura et al., 2001; Adolphs, 2010; Sander et al., 2005). In support, increases in the size of the basolateral division correlate with larger social group sizes and higher frequencies of social play across species (Barton and Aggleton, 2000; Lewis and Barton, 2006). In both humans and macaques, within species comparisons indicate that amygdala volume correlates with social network or social group size (Bickart et al., 2010; Kanai and Bahrami, 2012; Sallet et al., 2011). Early analyses linked measures of social complexity to neocortical elaboration in primates (Dunbar, 1995). Because neocortical expansion is linked to basolateral expansion, it is not surprising that volumetric increase in both structures appears to be correlated with similar socioecological variables.

We provide preliminary evidence that two of the basolateral nuclei, the basal and accessory basal nuclei, are potentially reduced in terms of volume and neuron number in orangutans. Socially, orangutans are the most solitary of the apes, generally foraging in parties of one to two individuals (Delgado and Van Schaik, 2000). Previously, we found that orangutans also have reduced orbitofrontal cortex volumes (Semendeferi et al., 1997;

Schenker et al., 2005). This region is a major target of the basal nucleus and, to a lesser degree, the accessory basal nucleus in primates (Ghashghaei and Barbas, 2002), and both structures are central to the neural circuit subserving social affiliation in primates (Adolphs, 2003). The association between small social groups and reductions in functionally related neural structures is intriguing but our sample size precludes firm conclusions on the subject.

Although anthropoid primate social systems have been argued to be some of the most complex among mammals (Shultz and Dunbar, 2007), human social systems exhibit both quantitative and qualitative distinctions from those of other anthropoids. Although the maximum size of chimpanzee and bonobo social groups have been reported reach up to 150 individuals (Kano, 1992; Mitani and Amsler, 2003), human social networks, on average, exceed 120 individuals both in industrialized (Hill and Dunbar, 2003) and hunter-gatherer societies (Zhou et al., 2005). Qualitatively, humans are the only primates to form social groups comprised predominantly of non-kin of both sexes (Hill et al., 2011). The human social communicative repertoire is also extensive. The spontaneous use of spoken language unequivocally distinguishes human social communication from that of apes. While humans share a proficiency for other communicative acts, like facial or body gestures, with our closest living relatives, the great apes (Parr et al., 2005; Pika, 2008; Pollick and de Waal, 2007), great apes do not use their gestures in a referential or symbolic fashion (Pika et al., 2005). In contrast, human gestures can be iconic and metaphoric, accentuating spoken language (McNeill, 1996) and can essentially replace it as in the case of sign language (Poizner et al., 1990).

Across hominid species, we have found that the human amygdala, specifically, is specialized in emphasizing the lateral nucleus. Pathology of the lateral nucleus has been observed in several human neurological disorders. Autistic adults exhibit considerable reductions in the number of neurons only in the lateral nucleus (Schumann and Amaral, 2006) while volumetric reduction of the lateral nucleus has been suggested to be a feature of William's syndrome (Galaburda and Bellugi, 2000). Because both disorders are associated with impairments in social behavior, together they support the potential role this nucleus may play in social behavior. Additionally, reductions in volume and neuron number in the lateral nucleus characterize bipolar disorder, which may underlie the difficulties that patients have in assigning emotional significance to external stimuli (Berretta et al., 2007).

Most theories of human and non-human primate amygdala function are drawn from the expansive array of literature on amygdala connectivity in nonhuman primates. As previously mentioned the lateral nucleus is the primary recipient of cortical input in the amygdala and is the first stop for most cortical information, functioning as the primary "gateway" to the amygdala. Although many amygdaloid nuclei receive some cortical input, the lateral nucleus is the primary recipient of multimodal sensory information arriving from the temporal association cortices. Given the available evidence, we suggest that the volume and number of neurons in the human lateral nucleus have increased in response to a heightened need to process increased cortical input and emotional elements of the extensive human communicative repertoire and expansive human social networks.

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Chapter 3: Tables

Table 3.1. Specimens in sample. New series ([*]NB) were combined with specimens from the collections of [†]CMS, [‡]KS, [§]JMA, and [¶]JAB. [‖]CCS and PRH provided 40 µm thick tissue for 2 specimens.

Species	*Common Name*	*Sex*	*Age (Yr)*	*Hemisphere*
Homo sapiens[†]	Human	M	11	Left
Homo sapiens[†]	Human	M	14	Right
Homo sapiens[†]	Human	M	17	Left
Homo sapiens[†]	Human	M	18	Left
Homo sapiens[†]	Human	M	24	Right
Homo sapiens[†]	Human	M	25	Left
Homo sapiens[†]	Human	M	27	Right
Homo sapiens[†]	Human	M	27	Left
Homo sapiens[†]	Human	M	32	Left
Homo sapiens[†]	Human	M	44	Left
Homo sapiens[‡]	Human	M	75	Left
Pan troglodytes[*]	Common Chimpanzee	F	2	Left
Pan troglodytes[‡]	Common Chimpanzee	F	24	Left
Pan troglodytes[*]	Common Chimpanzee	F	27	Left
Pan troglodytes[*]	Common Chimpanzee	F	42	Left
Pan troglodytes[‡]	Common Chimpanzee	F	Adult	Left
Pan paniscus[‡]	Bonobo	F	2	Left
Pan paniscus[‡]	Bonobo	F	11	Left
Pan paniscus[§]	Bonobo	F	25	Left
Pan paniscus[§]	Bonobo	M	Adult	Right
Gorilla gorilla gorilla[*]	Western Lowland Gorilla	M	10	Right
Gorilla gorilla gorilla[‡]	Western Lowland Gorilla	F	20	Left
Gorilla gorilla gorilla[*‖]	Western Lowland Gorilla	M	22	Left
Gorilla gorilla gorilla[*]	Western Lowland Gorilla	M	34	Right
Gorilla gorilla gorilla[*]	Western Lowland Gorilla	F	50	Right
Pongo pygmaeus[‡]	Orangutan	M	17	Left
Pongo pygmaeus[*]	Orangutan	F	23	Right
Pongo pygmaeus[‡]	Orangutan	M	34	Left
Pongo pygmaeus[‡]	Orangutan	F	Adult	Right
Hylobates muelleri[*‖]	Müller's Bornean Gibbon	M	19	Left
Hylobates concolor[‡]	White-cheeked Gibbon	F	22	Right
Hylobates lar[‡]	White-handed Gibbon	F	Adult	Right
Macaca fascicularis[¶]	Long-tailed Macaque	M	4	Left
Macaca fascicularis[¶]	Long-tailed Macaque	M	5	Left
Macaca fascicularis[¶]	Long-tailed Macaque	M	5	Left

Table 3.2. Summary of stereological data collection for each nucleus in each nonhuman species.

	Species	*Grid Area (μm^2)*	*Neurons Counted (Average)*	*Sections Sampled (Average)*
Amygdala	*Chimpanzee*	$1{,}700^2$-$2{,}300^2$	223	11
	Bonobo	$2{,}300^2$	181	12
	Gorilla	$2{,}400^2$	218	10
	Orangutan	$2{,}400^2$	208	12
	Gibbon	2,000-$2{,}400^2$	216	10
	Macaque	$1{,}700^2$	288	10
Lateral	*Chimpanzee*	$1{,}000^2$-$1{,}200^2$	220	10
	Bonobo	$1{,}000^2$-$1{,}200^2$	179	11
	Gorilla	$1{,}000^2$-$1{,}200^2$	217	9
	Orangutan	$1{,}000^2$-$1{,}200^2$	243	11
	Gibbon	$1{,}000^2$-$1{,}200^2$	211	10
	Macaque	800^2	378	9
Basal	*Chimpanzee*	$1{,}200^2$-$1{,}500^2$	205	10
	Bonobo	$1{,}200^2$-$1{,}500^2$	173	11
	Gorilla	$1{,}300^2$	235	9
	Orangutan	$1{,}200^2$-$1{,}600^2$	164	10
	Gibbon	$1{,}200^2$	150	10
	Macaque	900^2	236	9
Accessory Basal	*Chimpanzee*	800^2	187	10
	Bonobo	800^2	171	11
	Gorilla	800^2-$1{,}000^2$	164	9
	Orangutan	700^2-$1{,}000^2$	173	10
	Gibbon	700^2	175	10
	Macaque	600^2	278	10
Central	*Chimpanzee*	500^2	202	10
	Bonobo	500^2	190	11
	Gorilla	500^2	187	9
	Orangutan	500^2	209	10
	Gibbon	400^2	173	9
	Macaque	600^2	178	10

Table 3.3. Average neuron numbers x 10^6 with standard deviations (SD) for each nucleus in each species . The final column illustrates post-hoc differences that were significant at **$p < 0.05$ in boldface** or that approached significance at $p < 0.08$ in italics. Abbreviations: Hu = Human, Ch = Chimpanzee, Bo = Bonobo, Go = Gorilla, Or = Orangutan, Gi = Gibbon, Ma = Macaque.

ROI	*(Hu)*	*(Ch)*	*(Bo)*	*(Go)*	*(Or)*	*(Gi)*	*(Ma)*	*Post-Hoc Comparisons*
Amygdala	13.27 (3.70)	12.05 (5.53)	12.28 (2.92)	11.68 (2.37)	13.99 (2.97)	6.61 (2.90)	3.35 (0.24)	Ma<**Hu**, **Ch**, **Bo**, **Go**, **Or** Gi<**Hu**, **Bo, Or,** *Go*
Lateral nucleus	4.32 (1.11)	2.87 (0.95)	3.22 (0.60)	2.79 (0.33)	3.09 (0.38)	1.43 (0.32)	0.92 (0.02)	Ma<**Hu**, **Ch**, **Bo**, **Go**, **Or** Gi<**Hu**, **Ch**, **Bo**, **Go**, **Or** Hu> **Ch**, **Bo**, **Go, Or**, **Gi**, **Ma**
Basal nucleus	3.59 (1.29)	4.10 (1.44)	3.82 (1.37)	3.86 (1.27)	3.29 (1.02)	1.78 (0.57)	0.77 (0.06)	Ma<**Hu**, **Ch**, **Bo**, **Go**, **Or** Gi<**Hu**, **Ch**, **Bo**, **Or,** *Go*
Accessory basal nucleus	1.36 (0.32)	1.28 (0.62)	1.20 (0.50)	1.40 (0.34)	1.22 (0.23)	0.78 (0.50)	0.52 (0.12)	*ns*
Central nucleus	0.37 (0.09)	0.44 (0.02)	0.31 (0.08)	0.42 (0.13)	0.50 (0.09)	0.24 (0.06)	0.26 (0.02)	Ma<**Hu**, **Ch**, **Go**, **Or** Gi<**Hu**, **Ch**, **Go**, **Or** Bo<**Ch**, **Or** Hu<**Ch, Or**

Table 3.4. Neuron number in each nucleus as a % of total amygdala neurons. The final column illustrates post-hoc differences that were significant at **p<0.05 in bold** or that approached significance at *p<0.08 in italics*. *Abbr*: Hu=Human, Ch=Chimpanzee, Bo=Bonobo, Go=Gorilla, Or=Orangutan, Gi=Gibbon, M=Macaque.

ROI	*(Hu)*	*(Ch)*	*(Bo)*	*Go)*	*(Or)*	*(Gi)*	*(Ma)*	*Post-Hoc Comparisons*
Lateral nucleus	32.5%	23.9%	26.2%	23.9%	22.1%	21.7%	27.4%	Hu>**Ch**, **Bo, Go, Or, Gi, Ma**
Basal nucleus	27.1%	34.0%	31.1%	33.0%	23.5%	27.0%	23.0%	Ma<**Ch**, **Bo**, **Go** Or<**Ch**, **Bo**, **Go** Hu< **Ch, Bo, Go**
Accessory basal nucleus	10.2%	10.6%	9.8%	12.0%	8.7%	11.8%	15.4%	Go>**Ch**, **Bo**, **Or** Or<**Ch**, **Bo**, **Go**, **Hu**, **Gi**, **Ma** Ma>**Hu**, **Ch**, **Bo**, **Or**
Central nucleus	2.8%	3.7%	2.5%	3.6%	3.6%	3.7%	7.8%	Ma>**Hu**, **Ch**, **Bo**, **Go, Or**, **Gi** Bo<*Ch, Go*

Table 3.5. Percent residuals derived from log-log independent contrast regressions. Residuals that were significant at **p<0.05 are in bold** and that approached significance at *p<0.08 are in italics*. *Human*$^{+}$=percent residual with all species included; *Human*$^{--}$= percent residual excluding human data from the regression.

	Human^{+}	*Human*$^{-}$	*Chimpanzee*	*Bonobo*	*Gorilla*	*Orangutan*	*Gibbon*	*Macaque*
Lateral nucleus	**48%**	**59%**	-3%	9%	-4%	-11%	-18%	7%
Basal nucleus	-2%	*-7%*	**38%**	20%	32%	*-20%*	3%	-12%
Accessory basal nucleus	5%	5%	6%	-3%	*19%*	**-10%**	-5%	0%
Central nucleus	*-10%*	**-12%**	12%	-23%	7%	19%	-22%	12%

Chapter 3: Figures

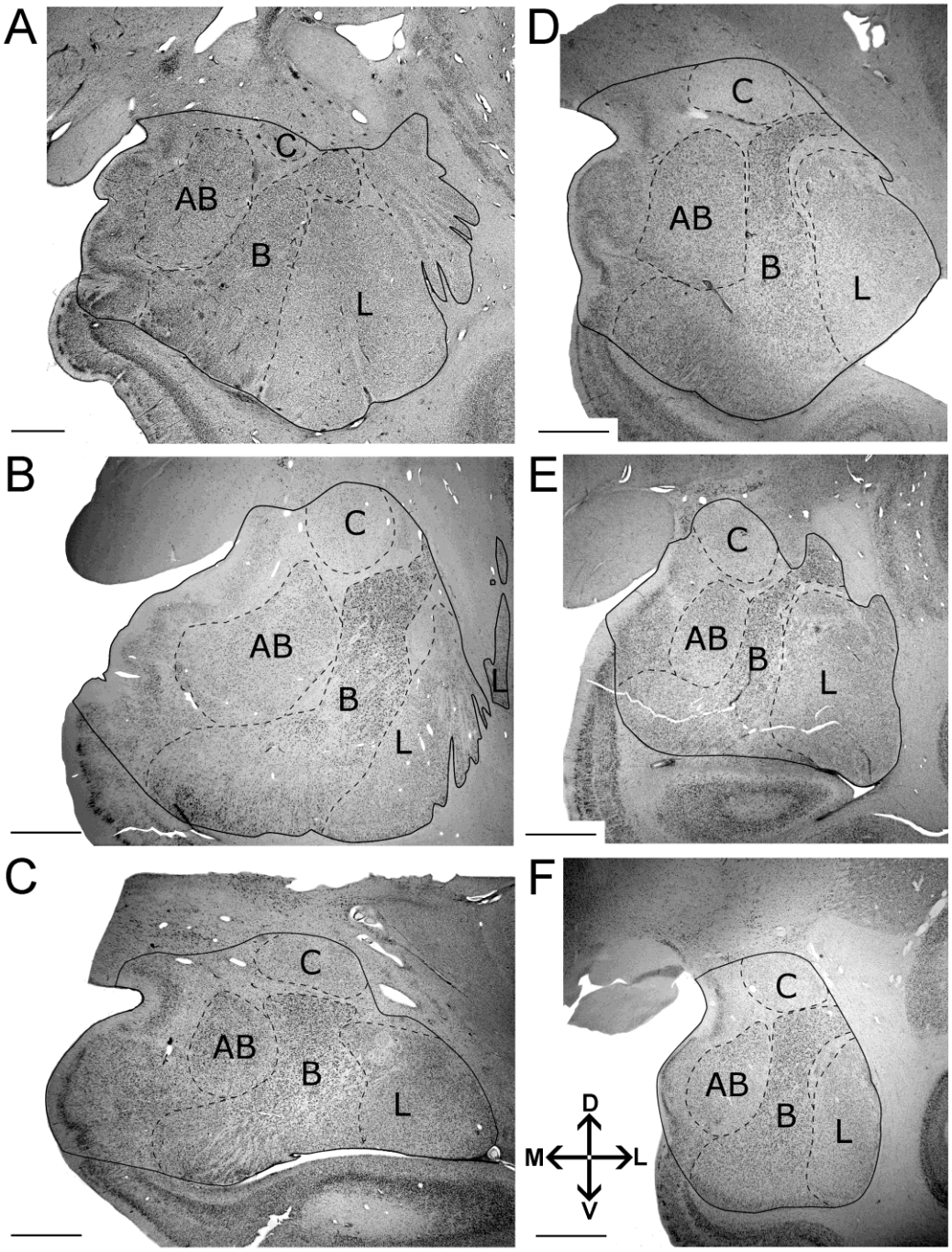

Figure 3.1. Delineation of left amygdala and nuclei included in this analysis illustrating the consistency of borders across species. Images were taken from midrostrocaudal levels in the following primates: A) human, B) gorilla, C) orangutan, D) chimpanzee, E) gibbon, and F) long-tailed macaque. Abbreviations: AB = Accessory Basal Nucleus, B = Basal Nucleus, C = Central Nucleus, L = Lateral Nucleus. Other amygdaloid nuclei are not represented in this comparative figure, but are highlighted in Figure 2. Scale bars represent 2 mm. The human image (Figure 1A) is modified from Schumann & Amaral (2005). Images follow radiological convention.

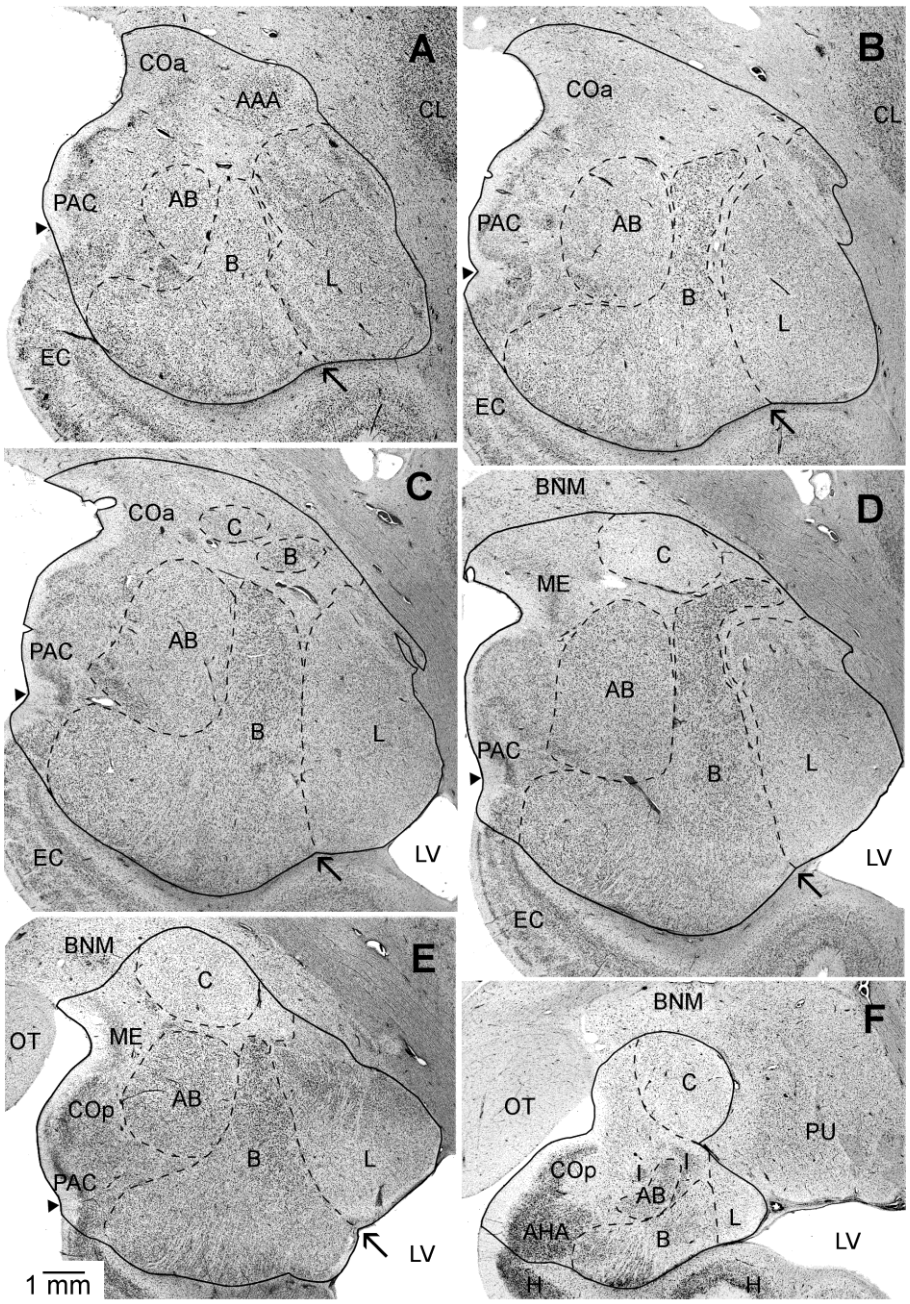

Figure 3.2. A series of brightfield photomicrographs illustrating the boundaries of the amygdala, lateral, basal, accessory basal, and central nuclei in coronal sections of the left hemisphere of a chimpanzee. Images are from rostral (A, B), midrostrocaudal (C, D), and caudal (E, F) positions in the amygdala. Arrows point to the "notch" that separates the ventral borders of the lateral and basal nuclei. Small arrowheads indicate the position of the semiannular sulcus used to mark the division between the cortical amygdaloid nuclei and the adjacent entorhinal cortex (anterior) or hippocampus (posterior). Abbreviations: AAA = Anterior Amygdaloid Area, AB = Accessory Basal Nucleus, AHA = Amygdalohippocampal Area, B = Basal Nucleus, BNM = Basal Nucleus of Meynert, C = Central Nucleus, CL = Claustrum, COa = Anterior Cortical Nucleus, COp = Posterior Cortical Nucleus, EC = Entorhinal Cortex, H = Hippocampus, I = Intercalated Nuclei, L = Lateral Nucleus, LV = Lateral Ventricle, ME = Medial Nucleus, OT = Optic Tract, PAC = Periamygdaloid Cortex, PU = Putamen. Scale bar = 1 mm in panel E (applies to A-F).. Images follow radiological convention.

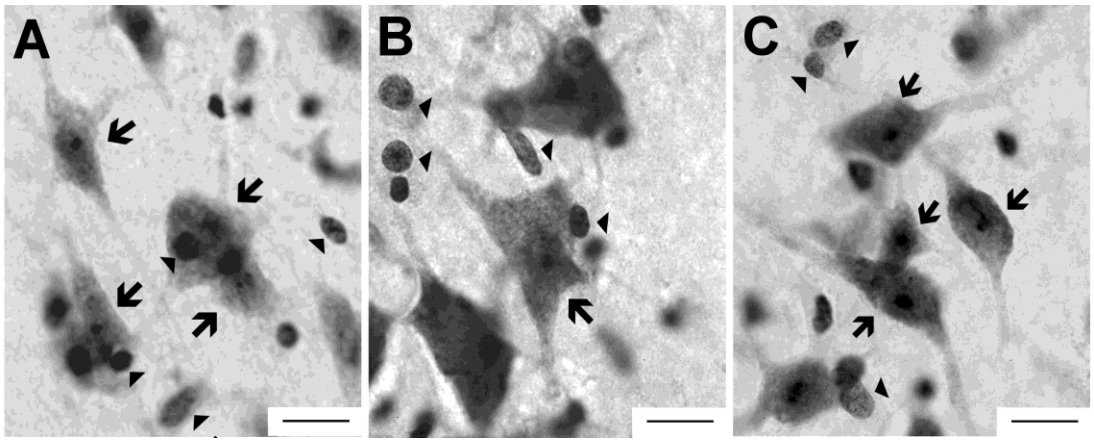

Figure 3.3. Tissue from the chimpanzee A) lateral nucleus, B) basal nucleus, and C) central nucleus as viewed through a 100x objective, the magnification used for data collection. Morphological features of neurons (arrows) and glia (arrowheads) can be distinguished at this magnification. Scale bar represents 15 µm.

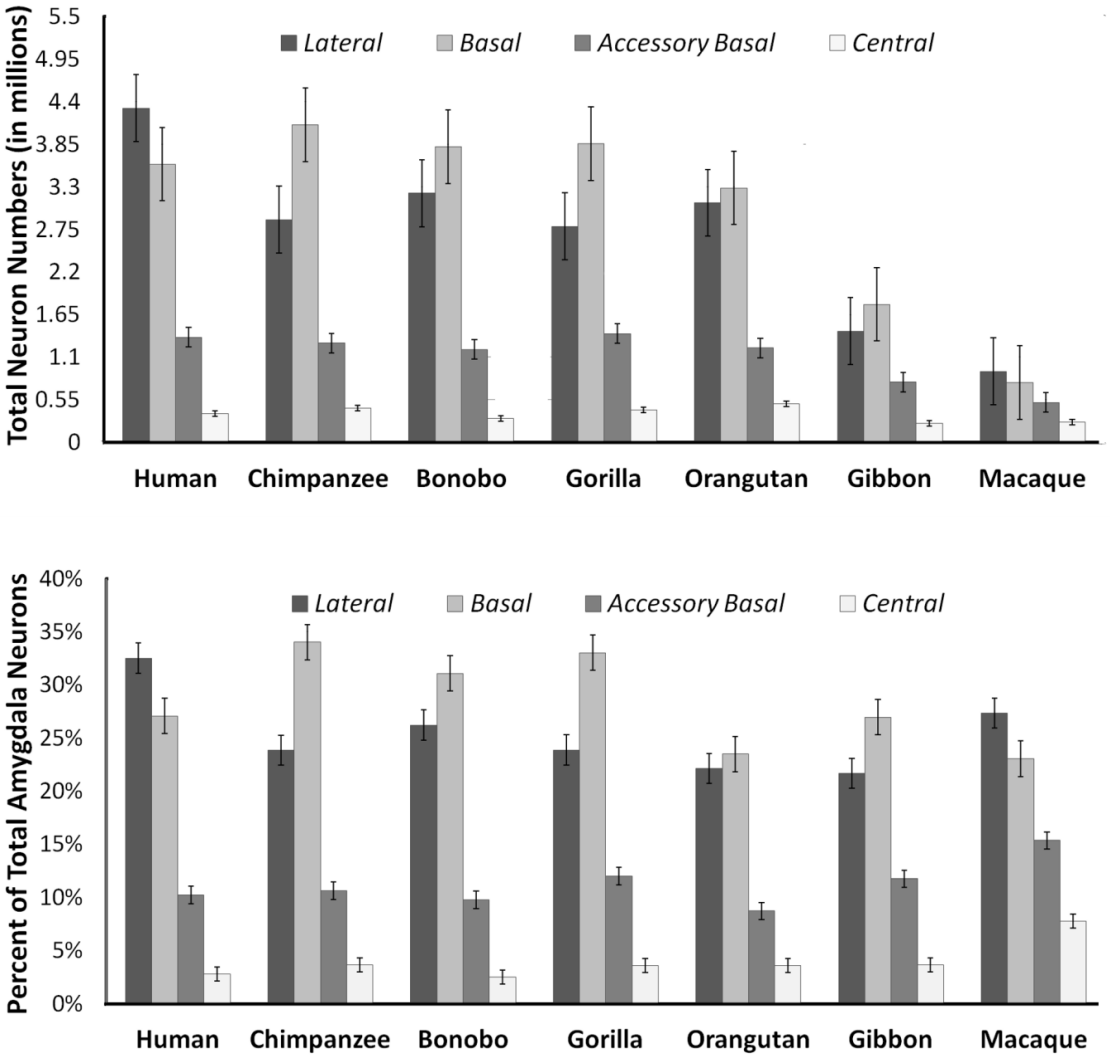

Figure 3.4. Histograms indicating the average number of neurons ($x10^6$) in the amygdala and four nuclei (top) and the average percent of total amygdala neurons distributed to the lateral, basal, and accessory basal nuclei across species(bottom) (n = 35). Error bars represent standard error.

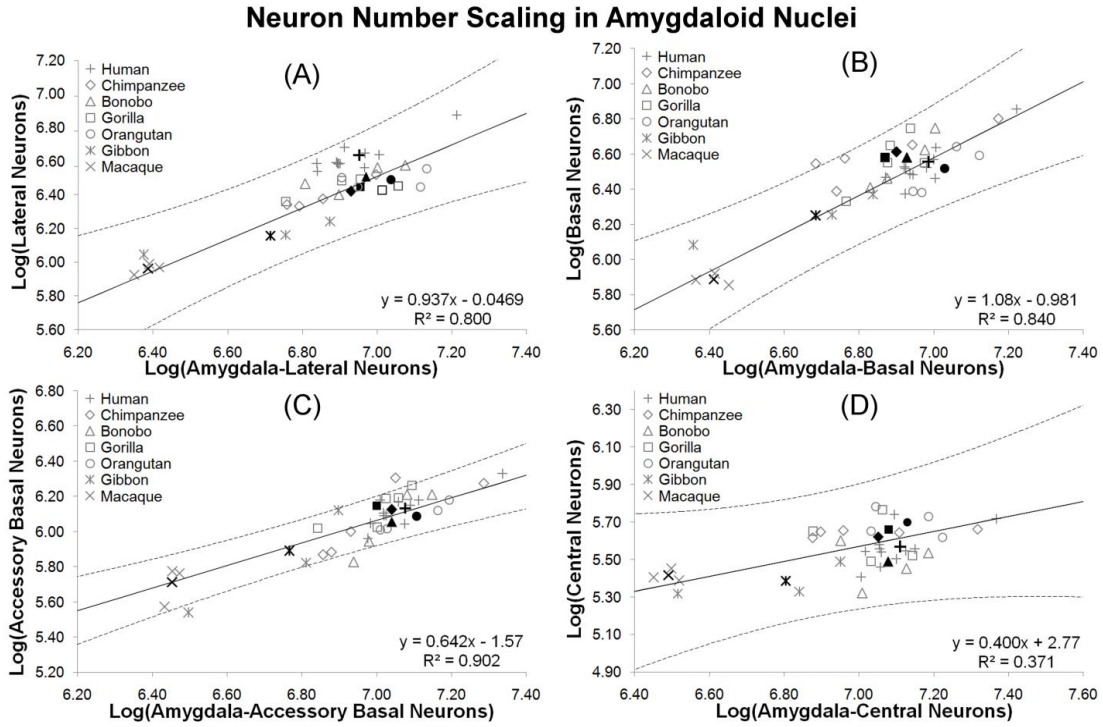

Figure 3.5. Independent contrasts regression plotting the log of total amygdala neuron number against the log of the neuron numbers in A) the lateral nucleus, B) the basal nucleus, C) the accessory basal nucleus, and D) the central nucleus with all species included in each regression. Individual data points are plotted as open, gray markers and species mean values are plotted as closed, black markers.

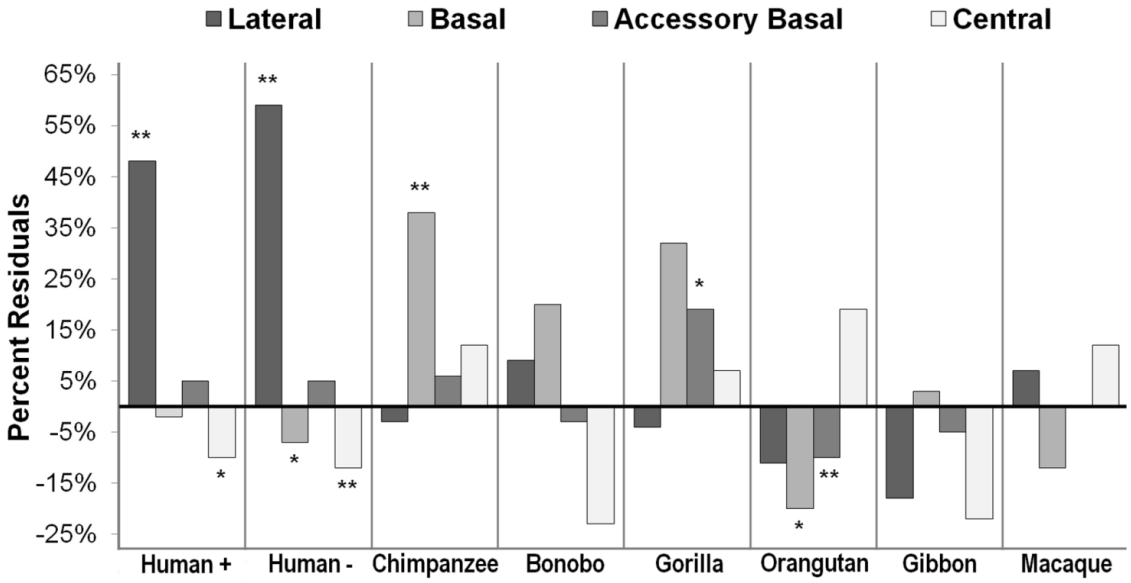

Figure 3.6. Average percent residuals from regression equations in each nucleus for each species. Starred bars represent values that were statistically significant (**) or close to statistically significant (*) from a residual of 0. Human+ = percent residual with all species included; Human- = percent residual excluding human data from the regression.

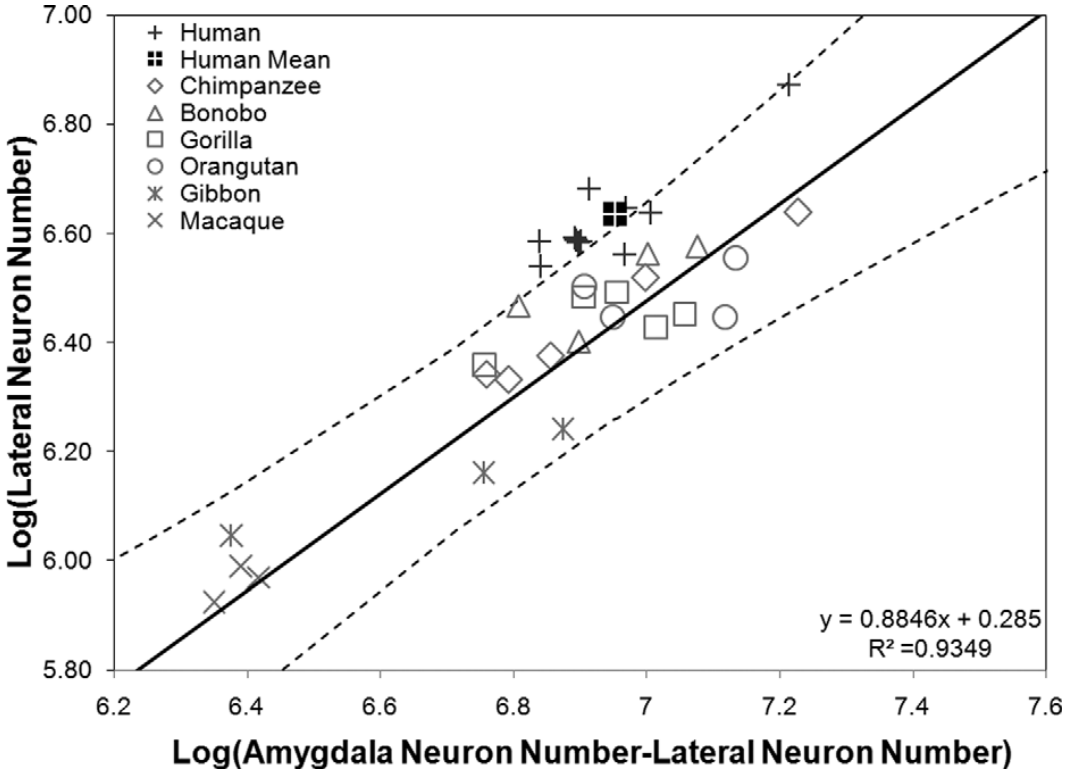

Figure 3.7. Independent contrasts regression plotting the log of amygdala neuron number against the log of neuron numbers in the lateral nucleus with humans excluded from the regression (n = 24).

Chapter 4

Emotion and human brain evolution: new data and a meta-analysis[1]

INTRODUCTION

Emotional behaviors, and the neural structures that subserve them, have traditionally been regarded as evolutionarily conserved across mammals. Nonetheless, many contemporary evolutionary theories emphasize the important role that socioemotional behavior may play in primate cognitive and behavioral evolution. Heightened tolerance towards, cooperation with, and knowledge sharing with conspecifics are theorized to be at the core of human problem solving and complex cognition (Hare, 2007; Herrmann et al., 2007, 2011). Attachment and bondedness to both related and unrelated conspecifics are suggested to influence fitness across primate groups (Dunbar and Shultz, 2010; Silk, 2007; Dunbar, 2009). As the only primate to form cooperative affiliative groups with predominantly non-kin, humans may exemplify an extreme manifestation of this trend (Hill et al., 2011). The contribution emotion makes to social-decision making and symbolizing is used to argue for the centrality of emotions to complex human behaviors (Allman et al., 2001; Barnard et al., 2007; Damasio, 1998). Additionally, emotion's role in social perception, mentalizing, and empathy has been integrated into theories emphasizing the importance of these capacities in human and

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non-human primate evolution (Aureli and Schaffner, 2002; Byrne and Bates, 2010; Parr et al., 2005; de Waal, 2008). Although behavioral changes are likely to reflect concomitant changes in neuroanatomy, few comparative investigations have addressed the evolution of structures that subserve emotional and motivational behaviors in primates.

A number of neural structures participate in emotion production and evaluation, and they have generally been grouped under the umbrella of the "limbic system". Paul Broca proposed segregating structures on the interior, medial "limen" of the brain from the surrounding cortex, suggesting that a c-shaped conglomeration of structures running from the subgenual anterior cingulate to the anterior parahippocampal gyrus and uncus constituted a distinct "great limbic lobe" (Broca, 1878; Lautin, 2001). In delineating a "corticothalamic mechanism of emotion", James Papez's provided a unifying functional thread tying many of these structures together (Papez, 1937). His circuit included the cingulate gyrus, hippocampus, hypothalamic mammilary bodies, and the anterior thalamus (Fig. 1). Later, Paul MacLean expanded Papez's circuit and formally established the concept of the "limbic system" proper (MacLean, 1949). In addition to Papez's circuit, MacLean complied evidence implicating the orbital frontal cortex, medial frontal cortex, temporal polar cortex, anterior insular cortex, and amygdala in emotional behavior (Fig. 2). While several other authors later stressed the important role that the ventrostriatopallidal system (including chiefly the ventral striatum, ventral globus pallidus, the septum (or septal nuclei), and striatal extensions of the amygdaloid nuclei) played in emotional and motivation behaviors (Lautin, 2001; Heimer and Van Hoesen,

2006). Despite some recent controversy regarding the utility of the term "limbic system" (LeDoux, 1996), it may be argued that many structures traditionally conceived as part of the limbic system are core components of a network of highly interrelated structures that subserve motivational and emotional behaviors (Heimer and Van Hoesen, 2006). As such, this model can, at the very least, serve as a useful heuristic for comparative evolutionary analysis.

Historically, views on the evolution of emotion-related structures *qua* the limbic system have been mixed. In its inception the limbic system concept was formulated as a response to experimental and psychiatric data (MacLean, 1949), but MacLean is perhaps best known for his evolutionary account of brain evolution, the triune brain (Fig. 3). In his formulation, the limbic, or "paleomammalian" brain intervenes both anatomically and evolutionarily, between the rudimentary components of the "protoreptilian brain" and the expansive "neomammalian" neocortex (MacLean, 1972, 1990, 1964). Comparative data have made it clear that the parts of the limbic system that more exclusively overlap with the "rhinencephalon", or olfactory brain, are especially conserved in primate evolution (Stephan and Andy, 1964; Finlay et al., 2001).

This perspective has been increasingly complicated as comparative data quantifying specific structures in the human and non-human primate limbic system become available. Although they are generally referenced for their neocortical data, Heinz Stephan and his colleagues focused considerable attention on several anatomical constituents of the limbic system in their extensive dataset including over 40 primate species (Stephan et al., 1981). They collected volumetric data for the hippocampal

formation (Stephan, 1983), amygdala (Stephan et al., 1987; Stephan and Andy, 1977), septal nuclei (Andy and Stephan, 1968; Stephan et al., 1981) and striatum (Stephan et al., 1981), further subdividing both the hippocampal formation (schitzocortex and hippocampus) and amygdala (corticobasolateral and centromedial amygdala). Their comparative findings converged on one argument: limbic structures are not evolutionary conserved or regressive, *sui generis* (Andy and Stephan, 1968; Stephan and Andy, 1964; Baron et al., 1987; Stephan, 1983). Unfortunately, their conclusions are rarely reported, likely due to the eccentric statistical analyses they used which do not translate to contemporary evolutionary methodologies. Comparisons of small samples of great apes and humans provide some additional support for this contention, however. Thalamic nuclei that process limbic information appear disproportionately expanded in humans when compared with apes, while sensory relay and "association" nuclei do not (Armstrong, 1980, 1981, 1986). Similarly, preliminary evidence suggest that the lateral nucleus of the amygdala, included in Stephan's corticobasolateral division, has undergone substantial increase over the course of human evolution (Barger et al., 2007). Thus, it cannot be assumed that limbic structures are necessarily conserved.

Further, increases in the volume of some limbic structures have been shown to correlate with behavioral variables operationalizing hypothesized evolutionary pressures. Measures of complex social behavior are chief among these. In humans, increased amygdala volume predicts larger online and real-world social network sizes (Bickart et al., 2010; Kanai and Bahrami, 2012). Additionally, orbital frontal and ventral medial frontal cortex volumes are associated with enhanced performance on intentionality tasks

(Powell et al., 2010). Across primates, the corticobasolateral portion of the amygdala scales with social group size, and the size of the whole amygdala and striatum correlate with rates of social play (Lewis and Barton, 2006; Graham, 2010). While hippocampal volume has been linked largely to executive function (Shultz and Dunbar, 2010; Frodl et al., 2006) and spatial memory (Maguire et al., 2000), affective disorders, like depression or post-traumatic stress disorder, are associated with decreases in hippocampal volume (Villarreal et al., 2002; Frodl et al., 2006). Although these findings do not provide explicit proof of a causal link between the variables measured, taken together they suggest that changes in the size of some limbic structures may be associated with behavioral variation within and across taxa.

In this analysis, we ask: Are neural structures associated with emotion processing necessarily de-emphasized in human evolution? Although comparative data are increasingly available, few studies have explicitly interrogated this question and fewer still have employed phylogenetically informed statistical analyses. To address these issues, we incorporate available information about primate limbic structures, and when possible discrete subdivisions of these structures, from all available, large datasets. Each is subjected to a novel analysis which firstly, assesses human deviations from allometry by comparing observed human values to values predicted from regressions drawn through nonhuman primates, secondly, uses statistical methods which assess the influence of phylogeny on trait values in individual species, and thirdly, regresses all brain components against the same variable, total hemisphere volume, in order to factor out the influence of increases in brain size on human departures from allometry. Given

the increased recognition of the interdependence of emotion and cognition and the central role that emotion may play in human adaptive behaviors, we hypothesize that limbic structures have not become decreased in human evolution beyond what would be predicted for a primate of our brain size.

MATERIALS

Data for this analysis are drawn from two primary sources, including data from: 1. our own laboratory and 2. Stephan and colleagues. Only datasets that included more than 4 nonhuman species were utilized, as datasets comprised of fewer species, e.g., thalamic data from Armstrong (1986), failed to produce significant regression equations. Additionally, we only analyzed datasets that also measured hemisphere volumes in order to utilize a common variable to standardize all comparisons. It must be emphasized that both datasets are not entirely comparable in their phylogenetic extent (Figs. 4 and 5). The Stephan dataset provides data suitable for contrasting human values with values across primates, but only 3 ape species (and individuals) are included. This makes statistical comparisons of humans and apes in the Stephan dataset unreliable due to low sample size and reduced statistical power. Our dataset, in contrast, focuses heavily on human comparisons with great apes, and thus cannot speak to evolutionary trends that may have significance for primates, more generally, across a broader evolutionary time span.

Human comparisons with great apes: Focusing on humans, all great ape species (bonobos, chimpanzees, gorillas, and orangutans), and several lesser ape species (lar, concolor, and Müller's Bornean gibbons) (Tables 1 and 3; Figure 4), our lab has collected relatively extensive volumetric data sets for: 1. the amygdala, 2. the lateral amygdaloid

nucleus, 3. the basal amygdaloid nucleus, 4. the accessory basal amygdaloid nucleus, 5. the central amygdaloid nucleus, 6. the hippocampus, 7. the orbital frontal cortex, 8. the medial frontal cortex, and 9. the insular cortex.

Data were obtained from a number of sources. New data for the amygdala and four of its nuclei, the lateral, basal, accessory basal, and central nuclei, are incorporated into the present analysis (Table 1; Figure 6) and were defined using previously published boundaries (Barger et al., 2007; Barger, in press). Additionally, we included previously unpublished hippocampal data collected in our lab (Teffer et al., 2004). Data for the insular cortex were previously published and were derived from structural MRIs; they have never been subjected to phylogenetic analysis (Semendeferi and Damasio, 2000). Data for the orbital, medial, and frontal cortices were also collected using structural MRIs (Fig. 7), but were previously analyzed principally as components of the frontal lobe (Schenker et al., 2005).

Human comparisons with anthropoid primates: The following structures were addressed across anthropoid primates (Tables 1 and 2; Fig. 6): 1. the amygdala (see Fig. 6 for delineation of subdivisions), 2. the corticobasolateral amygdala (lateral, basal, accessory basal, and ventral cortical nuclei), 3. the centromedial amygdala (the central, medial, and anterior cortical nuclei as well as the anterior amygdaloid area), 4. the hippocampus, 5. the schizocortex (entorhinal cortex and subiculum), 6. the hippocampal formation (hippocampus + schizocortex), 7.the septal nuclei, and 8. the striatum. To avoid statistical artifacts that may be related to commonly observed grade shifts between prosimian and anthropoid primates, we only used anthropoid data from the Stephan

dataset. For the amygdala, we utilized data from Stephan, et al., (1987). Data for other structures were taken from Stephan, et al., (1981).

METHODS

Data collection. Boundaries for the amygdala and amygdaloid nuclei were hand-traced on Nissl stained histological sections at 1x magnification as visualized through a MicroFire camera attached to a Nikon Eclipse 80i microscope. Volumes were calculated in the StereoInvestigator program (MicroBrightField, Inc.) using the Cavalieri estimator and yielded a Coefficient of Error (Gundersen, m=1) of < 5%. A correction factor was then applied to each volume to account for shrinkage that may occur during histological processing (Barger et al., 2007; Stephan et al., 1981).

Data analysis. We applied phylogenetic statistics to address the question: Are values for human limbic structures greater than predicted based on values available for 1) other hominoids and 2) other anthropoids. Consensus phylogenies for hominoids and anthropoids were obtained from the 10kTrees website (Arnold et al., 2010) and are displayed graphically in Figures 4 and 5. In all cases, species mean cerebral hemisphere volume (defined as the telencephalon + diencephalon) was used as the independent variable and the species mean value for the neural structure of interest served as the dependent variable. Because data for both hemispheres were not available for all subcortical structures, volumes for these structures represent the value for one hemisphere. When volumes of subcortical structures were reported in the literature as the sum of structures in both hemispheres, we halved the published values for the sake of

measurement consistency. In contrast, volumes for cortical territories are presented as the sum of structure volumes in both hemispheres.

Data were first subjected to phylogenetic generalized least squares (PGLS) analysis using the CAPER (V.0.4) (Orme et al., 2011) module in R (R Development Core Team, 2011) to determine whether allometric analyses exhibited a phylogenetic signal. In no case did CAPER produce a maximum likelihood value for lambda (ʎ) intermediate between 0 and 1. To determine whether human values were significantly greater than predicted, we performed several subsequent analyses. Datasets which indicated no phylogenetic bias (lambda *n.s*. different from 0) were analyzed in SPSS 17 (SPSS, Inc.), producing standard least squares regressions with 95% prediction intervals. Data sets which yielded evolutionary rates similar to Brownian motion (lambda *n.s*. different from 1) were analyzed using the PDAP module (Midford et al., 2003) of MESQUITE (Maddison and Maddison, 2010) producing least squares regression with 95% prediction intervals (PIs). In independent contrasts analysis, branch lengths were not transformed as contrasts were not significantly correlated with branch length. Regression lines and 95% PIs were procured from PDAP and then mapped back into the original data space to analyze human deviations from predicted values. In all cases, percent residual deviations from predicted values were also computed for each structure by inverting the predicted log value, subtracting the predicted value from the observed value and then dividing it by the observed value to allow for more intuitive comparisons across species (Sherwood et al., 2006). For cortical data, enough human data points were available to test species' average residual deviations from the line. Using SPSS 17, we tested whether residuals

varied significantly from zero and whether predicted values deviated significantly from observed values using one and two sided student's t-tests, respectively.

RESULTS

Regressions. Specific information for each structure is summarized in Table 4. The maximum likelihood value of lambda for most structures was not significantly different from 0. Lambda values for the orbital and dorsal frontal cortex were not significantly different from 1. In no case did CAPER produce lambda values that were intermediate between 0 and 1, indicating that the structures either did not show evidence of phylogenetic bias (based on an ultrametric tree) or that evolutionary change was explained by a basic Brownian model. For all structures, regressions against hemisphere volume were significant at $p<0.05$. R^2 values were high, ranging from 0.76-0.99, but tended to exceed 0.9 (Table 4).

Percent Residuals. Percent residuals for human data are listed in Table 4 and displayed graphically in Figure 8.

Compared with other anthropoids, humans exhibited positive residuals for the corticobasolateral amygdala, the whole amygdala, the septal nuclei, the schizocortex, and the centromedial amygdala from most to least positive. Humans exhibited negative residuals for the hippocampal formation, hippocampus proper, and striatum from least to most negative.

Compared with other hominoids, humans exhibited positive residuals for the hippocampus, lateral amygdaloid nucleus, orbital frontal cortex, and amygdala from most

to least positive. Humans exhibited negative residuals for the insular cortex, medial frontal cortex, basal amygdaloid nucleus, accessory basal amygdaloid nucleus, dorsal frontal cortex, and the central amygdaloid nucleus from least to most negative.

Analysis by Prediction Interval. Prediction intervals are listed in Table 4. Figure 9 illustrates the relationship between observed human values and the range of values predicted by the 95% PI.

Compared with other anthropoids, only the human striatum fell outside of predicted values, falling below the lower PI (regression presented in Fig. 10). Two other structures, the septal nuclei (regression presented in Fig. 11) and corticobasolateral amygdala (regression presented in Fig. 12), fell within the upper 5% of predicted values. For the septal nuclei, the upper limit of the PI was 0.14 or 1.38 cc, untransformed; the observed value was 0.11 or 1.31 cc, untransformed. For the corticobasolateral amygdala, the upper limit of the PI was 0.32 or 2.09 cc, untransformed; the observed value was 0.29 or 1.99 cc, untransformed.

Compared with other hominoids, the hippocampus and portions of the amygdala exceeded predicted values. The human residual for the hippocampus fell above predicted values (regression presented in Fig. 13). The human amygdala fell modestly (7%) above predicted values, and within the upper 5% of predicted values. The human observed value was 0.31, 2.03 untransformed; the upper limit of the PI was 0.33, 2.14 untransformed. In the amygdala, the value for the human lateral nucleus fell above predicted values (regression presented in Fig. 14), while the value for the central nucleus fell far below predicted values (regression presented in Fig. 15). The human basal

nucleus fell low, but was within the PI. The human observed value was -0.34, 2.19 untransformed; the lower limit of the PI was -0.35, 2.24 untransformed.

Test for Significant Differences in Means. For the cortical structures, we were able to test the significance of residual deviations from values predicted by the hominoid regression line due to large human sample sizes. The observed values for the human orbital frontal cortex were significantly positive ($t = 3.27$; $p = 0.01$). The mean human values for the dorsal frontal cortex ($t = -11.01$; $p < 0.001$) and medial frontal cortex ($t = -8.58$; $p<0.001$) were significantly negative. The mean observed value of the insula did not differ significantly from predicted values.

DISCUSSION

The volumes of many human limbic structures were contained within prediction intervals drawn through nonhuman primate values. As such, it does not appear to be the case that structures dedicated to emotion processing are necessarily decreased or de-emphasized in human evolution. In contrast, some structures appear derived in humans. Given that the cerebral cortex is generally considered the most expansive neural region, we would have expected to see cortical limbic structures exceed prediction intervals. Instead, we found the strongest evidence in favor of human-specific adaptations in two subcortical limbic structures, the amygdala and hippocampus. Also somewhat counter to expectations, these distinctions are more apparent when comparisons are made with our closest living relatives, the apes, rather than with a broader array of anthropoids.

Human comparisons with anthropoids. In relation to anthropoid primates (apes and monkeys), only one of the structures analyzed fell outside of predicted values, the striatum, and it was indicated to be significantly smaller in humans than expected. In contrast, the volumes of the human corticobasolateral amygdala, whole amygdala, and septal nuclei were around 20% larger than predicted, but were contained within the range of predicted values. Thus, it cannot be presumed that they are necessarily larger than predicted, especially given that the range of variation in humans is unknown. The septal nuclei and corticobasolateral amygdala presented interesting cases, however, which may merit further investigation. Both structures fell within the upper 5% of predicted values, nearly falling outside of the PI. Percent residuals for the human amygdala were slightly higher than for the septal nuclei, but human amygdala values were not so close to the upper limits of the prediction intervals. Predicted values for this structure were 0.46 or 2.91 cc, untransformed, while observed values were considerably smaller 0.42 or 2.64 cc, untransformed, falling within the upper 10% of values in the PI.

Human comparisons with hominoids. In comparisons with nonhuman hominoids, the apes, we found that human volumetric deviations from predicted values involved several structures, including the hippocampus, the amygdala, and possibly the orbital frontal cortex. At the most extreme, the volume of the human hippocampus significantly exceeded predictions by slightly over 50%. Although the volume of the human amygdala fell only slightly (7%) above predicted values but in the very upper limits of the PI, the intrinsic structure of the human amygdala appeared unequivocally distinct from that of apes, given several amygdaloid nuclei significantly fell outside of

predicted values. The human amygdala was dominated by the lateral amygdaloid nucleus, which was 37% larger than predicted for an ape of human hemisphere volume. In contrast the volume of the human central nucleus was substantially decreased, appearing over three times smaller than predicted. At the lower limit of the prediction interval, the volume of the basal nucleus was nearly significantly smaller than predicted by ape regressions.

Because multiple human data points were available for all cortical territories analyzed, we were also able to test whether mean human values for these structures deviated significantly from predicted values in human and ape comparisons. This was particularly advantageous in the case of the dorsal and orbital frontal cortices which produced lambda values near 1 in PGLS, necessitating the use of independent contrasts in regression analyses. The computer software running this program produces somewhat inflated prediction intervals (Midford et al., 2003), and calculating the significance of average residual deviations provides an important check on this method. We found that the average volume of the human insula was not significantly different from the average predicted volume, while the limbic medial frontal cortex and the non-limbic dorsal frontal cortex were, on average, smaller in humans than predicted. Only the average volume of the human orbital frontal cortex fell significantly above the value predicted for an ape of similar hemisphere volume. At the same time, all *individual* values fell within the range of values predicted. Thus, the mean volume of the human orbital frontal cortex appears 11% greater than predicted for an ape of our brain size, but we cannot anticipate

that individual values will entirely fall outside of the wide prediction interval for this structure (Fig. 9B).

An additional factor may be influencing human orbital residuals, however. We have previously noted that orangutans have particularly small orbital frontal cortices (Schenker et al., 2005); they may consequently influence the regression line. In this species, mean orbital frontal cortex volume fell below the regression line, and orangutan residuals were significantly lower than residuals for any other species, suggesting they may be a statistical outlier (One-way ANOVA: $F_{(6)} = 6.04$, $p < 0.01$; Tukey HSD, $p < 0.05$ for comparisons of orangutans and all other species). Thus, we also ran the independent contrasts regression omitting this taxon ($b = 1.06$; $R^2 = 0.98$; $p = 0.01$). In this case, the human residual was significantly negative and fell 25% *below* the prediction interval, near the value of the limbic medial frontal cortex. Given this evidence, it is difficult to say that the orbital frontal cortex is unequivocally increased in humans. Nonetheless, a non-limbic structure generally associated both with executive function and motor control, the dorsal frontal cortex, was also significantly smaller in humans and its residual was the most negative of all the cortical territories analyzed. As such, it cannot be said that regions containing limbic cortices are singularly decreased in humans relative to apes.

Comparisons between groups. Although the structures reported in our ape dataset and Stephan's anthropoid dataset do not entirely overlap, two structures, the hippocampus and amygdala, are present in both datasets. This allows us to contrast evolutionary findings for these structures in anthropoids and hominoids directly. The high human hippocampal residual in ape comparisons may in part reflect the fact that

scaling in the hippocampus is considerably decreased in hominoids compared with anthropoids. A slope, or allometric scaling coefficient, of 1 would indicate that a structure is increasing at the same rate as the total size of the hemispheres in a particular taxon. In apes, the allometric coefficient for hippocampal volume was 0.36, while in anthropoids, it was 0.76. Of course, some hominoids are included in the anthropoid regressions, potentially creating a problem for phylogenetic comparisons of the two taxa. However, removing hominoids from the anthropoid regression minimally influences the slope (slope = 0.777 without hominoids, slope = 0.755 with hominoids), and drawing a regression line through Stephan's apes closely approximates the slope obtained when a regression is run through our ape dataset (slope = 0.322 with Stephan data, slope = 0.371 with our data). Methodologically, concordant findings between analyses run on individuals in our dataset, who spent much of their life in zoos, and individuals in the Stephan data set, who were wild caught, provide some assurance that the results of our humans and ape comparisons are not simply artifacts of neural responses to captive environments.

When compared with anthropoids, the human amygdala residual is over twice that obtained from hominoid comparisons, and this may reflect a higher rate of allometric scaling in the hominoid amygdala. Again, we found that removing the apes from the Stephan dataset did not substantially influence the anthropoid regression (slope = 0.690 without hominoids, slope = 0.691 with hominoids). Running a regression line through the 3 ape data points in the Stephan sample produced a slope comparable to the slope run through our expanded ape dataset (slope = 0.767, with Stephan data, slope = 0.778, with

our data), although it did not reach significance likely due to the small sample size (n=3). This suggests that volumetric increases in the hemispheres and amygdala are more tightly coupled in hominoids than across their parent taxonomic group (anthropoids). Combining findings from cross-taxonomic comparisons of amygdala and hippocampal scaling, hemispheric increases in non-human hominoids appear more tied to amygdala rather than hippocampal increase.

In anthropoids, the corticobasolateral amygdala provided an interesting case for more in depth analysis, while the centromedial amygdala was only slightly volumetrically increased in humans. In apes, we quantified several individual amygdaloid nuclei that together comprise the majority of the corticobasolateral amygdala and may help to further explore our anthropoid findings. Because the lateral nucleus occupies the largest component of the human corticobasolateral amygdala, it is tempting to speculate that the high residual for the corticobasolateral division may be influenced by the expansion of the lateral nucleus in humans. (*N.B:* The Stephan group switched terminologies for the corticobasolateral division from "corticobasolateral amygdala" (Stephan and Andy, 1977) to "lateral amygdala" (Stephan et al., 1987). The grouping "lateral amygdala" should not be confused with the lateral amygdaloid nucleus, which is a constituent of Stephan's "lateral amygdala".) Residuals for the accessory basal and basal nuclei, two related structures included in the corticobasolateral amygdala, were not significantly positive, and are not likely to be driving the positive human residual for the corticobasolateral division. In contrast, human residuals for the central nucleus were considerably negative when humans were compared with great apes. This is intriguing, given that the

centromedial division is not substantially decreased across anthropoid primates. It may be the case that other components of the centromedial division, like the medial nucleus or anterior amygdaloid area, make up for this reduction. Of course, these arguments are premised on the assumption that these divisions do not scale differentially across primate taxa.

Comparison with Previous Analyses

Anthropoids. Prior analyses of subcortical limbic structures performed by the Stephan group have often been difficult to reconcile with contemporary evolutionary analyses due to the methodologies used. The methods developed by Stephan and colleagues were in many ways a reaction to previous studies that used proportional measures to assess the evolutionary importance of particular structures (Stephan and Andy, 1977; Stephan, 1983). Essentially, in primates, neocortex expands at a much faster rate than other structures, increasing with positive allometry in relation to the rest of the brain. This implies that the allometric relationship that many other regions share with the brain size will be negative. If simple ratios are used to assess evolutionary "advancement", these structures will appear to occupy a smaller proportion of the brain in larger brains, simply due to this scaling relationship, presenting the appearance of relative, but perhaps not real, evolutionary regression (Stephan, 1983). The Stephan group aptly argued that structures may fall in line with or even exceed allometric predictions, but this information is lost in a simple analysis of neural ratios. Similarly, structures that are positively allometric across primates, like the frontal cortex, will

appear to be more “advanced” in humans, though from an evolutionary perspective, this could be the simple end product of a general scaling law.

To deal with this problem, Stephan and colleagues employed the “progression index”, a measure of the degree of evolutionary expansion in a particular neural structure relative to a baseline “primitive mammalian” value, which they approximated using values from insectivores. At its core, the progression index essentially answered the question: how much larger is a neural structure in a particular primate of a particular body size in relation to what would be predicted by allometric trends across primitive mammals, i.e., insectivores? If a neural structure in a primate had a progression index of 2, for example, that structure is twice the value expected for an extrapolated basal insectivore of similar body size as the primate species in question.

Using this new analytical tool, Stephan and colleagues suggested that human subcortical and limbic structures were not necessarily regressive. Progression indices for the striatum are large across primates, second only to the neocortex. The human striatum appeared 16 times larger than predicted for a basal insectivore of similar body size while progression indices for the chimpanzee were slightly more than half that value (Stephan and Andy, 1969). The progression index for the schizocortex (entorhinal cortex and presubicular portions of the hippocampal formation) was 5.5 time greater in humans than predicted for basal insectivores and nearly twice that seen in nonhuman anthropoids (Stephan, 1983). The progression index of the human hippocampus was 4.2 (Stephan, 1983), while the chimpanzee index was less than half that value (Stephan and Andy, 1969). Similarly, the progression index of the human septum was 4.5, nearly twice that of

the chimpanzee (Andy and Stephan, 1968). Progression indices for the human amygdala ranged from 2.4 for the centromedial division to 4.4 for the whole amygdala to 6.2 for the corticobasolateral division; chimpanzee progression indices were one third to one half of these values (Stephan et al., 1987). Thus, it could be argued that many limbic structures appear to be particularly enlarged in humans even in relation to our closest relatives, the chimpanzees.

From this perspective one would predict that the striatum would show the greatest positive deviation in humans, followed by the corticobasolateral amygdala, the schizocortex, the septum, and the hippocampus. This is not exactly what we found using contemporary allometric methods. When comparing humans to other anthropoids, the striatum was the only structure which fell outside of and significantly below the prediction interval. Residuals for the corticobasolateral division and septal nuclei were high and nearly exceeded prediction intervals, but residuals for all components of the hippocampal formation were negative and contained within the prediction intervals in anthropoid comparisons. An explanation for these discrepancies may lie in the observation that a striking number of structures exhibit large human progression indices in Stephan's analyses. This may be due to the fact that, in the progression index, the size of a structure is essentially normalized for body size, but not brain size. Human brain size is exceedingly large for human body size, and overall brain size is one of the best predictors of brain component size (Finlay and Darlington, 1995). Thus, it would make sense that the particularly large human brain, when normalized for body size, would have particularly large brain structures in relation to other primates. Higher progression

indices, then, may predominantly reflect human departures from allometric scaling between brain and body size as opposed to adaptive deviations in the size of each individual structure analyzed.

Hominoids. Our previous analyses have indicated that the human frontal cortex is not expanded in human evolution, but rather, in the words of von Bonin (1948): "man has precisely the frontal lobe which he deserves by virtue of the overall size of his brain". In several studies, we have found that the frontal lobe, as a whole, does not show disproportionate volumetric increase in the hominin lineage (Semendeferi et al., 2002; Semendeferi and Damasio, 2000). The human frontal lobe occupies approximately 36-39% of the cerebral cortex, overlapping the ranges found in most great ape species. In fact, human frontal lobe volumes fall consistently below predicted values for regression lines drawn through nonhuman primate data, while ape and monkey values are distributed evenly above and below the line, lending further support to the idea that the frontal lobe, as a whole, is not a strong target of adaptive volumetric expansion in humans (Semendeferi et al., 2002). As such, it is not surprising that residuals for the medial and dorsal frontal cortex were low in humans. In contrast, the present analysis indicates that the human orbital frontal cortices are slightly and significantly larger than predicted for an ape of human brain size. When orangutans are excluded from the analysis, values for the human orbital frontal cortex fall below predicted values, though, making it conceivable that the orbital frontal cortex is also subject to overall frontal lobe conservation.

Previously, we found that values for the human insula tended to fall above the regression line, but not significantly (Semendeferi and Damasio, 2000); here we further substantiate that the size of the insula is as expected based on our shared ancestry with apes. We found that absolute values for the human insular cortex showed a modest, negative, insignificant deviation from predicted values. Our present analysis differed from our previous analysis in that we log-transformed data, assessed the potential influence of phylogeny on the regression, and statistically assessed human deviations from the prediction line. This may account for differences in the position of human residuals between the two analyses. Our current finding is consistent with a recently published phylogenetically informed volumetric analysis in postmortem specimens (Bauernfeind, et al. in press). In that study, the authors found that the size of the human insula fell close to allometric predictions.

Despite the fact that cortical structures appeared largely conserved, we were surprised to find that subcortical components of the limbic lobe were more likely to exhibit explicit evolutionary change in humans. Little prior information is available to compare with our findings for the hominoid hippocampus, but, as previously mentioned, regression lines drawn through hippocampal data from Stephan, et al., (1981) approximate our lines. Concordant with our findings, their human data point falls 40% above predicted values based on their ape data, although confidence intervals cannot be reliably computed due to the small sample.

We and others have argued that the amygdala is a target of evolutionarily reorganization in human and nonhuman primates (Barger et al., 2007; Semendeferi et al., 2010; Barton and Aggleton, 2000). The present data support our previous observation

that the basolateral division has become reorganized in human evolution (Barger et al., 2007), i.e., it exhibits a different organization of its intrinsic components compared with closely related species. Using this larger sample, we have further confirmed that the lateral nucleus is the largest nucleus in the human amygdala and has expanded beyond allometric predictions. As in our previous study, we found that the basal nucleus is the largest nucleus in ape amygdala, but the present analysis provides more substantial evidence that the basal nucleus may be decreased in human evolution, as it was only 0.05 cc removed from the lowest PI. In this analysis, we present the first comparative data on the volume of the central nucleus in apes, finding that that the human central nucleus is over three times smaller than predicted for an ape of human hemisphere volume. In sum, human amygdala organization is characterized by a volumetrically increased lateral nucleus and decreased central nucleus compared to ape amygdala. Ape amygdala, in contrast, emphasize the basal nucleus, and this emphasis is likely to decrease concomitantly with increased emphasis on the lateral nucleus in human amygdala evolution.

We have recently reported that neuron numbers tend to follow these trends (Barger, et al., in press). While the volume of the human lateral nucleus is 37% greater than predicted, the human lateral nucleus contains nearly 60% more neurons than predicted for an ape with a similar number of amygdala neurons. Despite its substantial decrease in size in human evolution, neuron numbers in the central nucleus were only 12% fewer in humans. Neuron numbers were also slightly decreased in the human basal nucleus, by 7%, but this difference only approached significance. As such, it may be the case that changes in neuropil, which comprises the non-neuronal fraction of the

amygdaloid nuclei, are slightly decoupled from changes in neuron number. Given neurogenesis in the amygdala is largely completed at birth but overall growth is not (Schumann et al., 2011), it may be worth investigating, firstly, the exact nature of changes in neuropil from an evolutionary perspective, and, secondly, the potential ontogenetic origins of this phenotype.

Functional and Evolutionary Significance

Certain components of the limbic system appear increased in humans when compared with our closest relatives, although this is dependent upon the taxonomic comparison made. In relation to anthropoids, broadly, humans show few significant deviations from predicted values. Nonetheless, when humans are compared with other hominoids, clear differences in limbic structures appeared, including the increased volume of the hippocampus and evolutionary reorganization of the amygdala. This suggests that adaptations have arisen in the human limbic system in recent evolution, after the human and ape lineage split.

The amygdala and anterior hippocampus share some functional attributes which could be argued to underlie coordinated evolutionary changes in these structures. Both are involved in circuits underlying implicit learning and memory, modulate emotional responses to external stimuli through connections with the hypothalamic pituitary axis (Freese and Amaral, 2009; Fanselow and Dong, 2010), and subserve cue dependent and context dependent fear conditioning (Fanselow and Dong, 2010; LeDoux, 2007). Evidence for coordinated changes in the amygdala and anterior hippocampus could

support theories which hypothesize that changes in neural systems modulating emotional responses to conspecifics are key features of human cognitive evolution.

However, this story is somewhat complicated by the fact that the hippocampus is also involved in a number of processes that are not explicitly emotional in nature. The posterior hippocampus is commonly associated with topographic memory, i.e., spatial navigation and mapping, while the hippocampus, generally, is heavily involved in declarative and episodic memory (Bechara et al., 1995; Thompson and Kim, 1996; Fanselow and Dong, 2010). While hippocampal volume has not been shown to correlate with measures of ecological intelligence (Barton, 2000), it does correlate with several measures of executive function across primates (Shultz and Dunbar, 2010). Certainly, measures of socially relevant affect have not been correlated with either amygdala or hippocampal volume, because the behavioral data are largely unavailable. Thus, without further study, it is difficult to determine which factors may drive hippocampal increase and whether human specializations in the amygdala and hippocampus are verifiably related, although this may be the most parsimonious explanation from an evolutionary perspective. Given the functional distinction of hippocampal subdivisions, comparative analyses of these separate functional territories may provide greater insight into the behavioral correlates of hippocampal expansion in human evolution.

Given the recent theoretical interest in bonding, affiliation, and cooperative behavior, it may be surprising that structures associated with pro-social behavior, like the striatum, orbital frontal cortex, and septal nuclei, were not unequivocally increased in humans. However, in regards to the striatum, only the ventral striatum is incorporated into the limbic system (Heimer and Van Hoesen, 2006). Thus, we cannot exclude the fact

that evolutionary reorganization within this structure may result in an emphasis on the ventral striatum in human evolution, especially given that we have found reorganization within the amygdala. It is also likely that the orbital frontal cortex has undergone a subtle increase in human evolution. Because we procured volumes from MRIs, we were not able to explicitly parcellate the orbital frontal cortex into its individual cytoarchitectonic areas. In our previous histological analysis utilizing a small number of ape specimens, we found that a posterior portion of the orbital frontal cortex, area 13, is particularly reduced in humans, whose values overlap ape values (Semendeferi et al., 1998). If the orbital frontal region is increased in humans relative to other hominoids, it is likely that more anterior portions of the orbital frontal cortex, like areas 47/12 and 11 (Ongür et al., 2003), are expanded. Even if orangutans are taken to be a true outlier influencing human deviations from the line, expansion of anterior orbital territories is likely, given the extreme reduction of posterior area 13. Anterior regions process reward and higher order social behavior and may be interesting candidates for subsequent analysis (Kringelbach and Rolls, 2004). As a caveat, this may additionally be true of the dorsal frontal cortex. The "executive" areas of the dorsal frontal cortex have yet to be dissociated from the motor areas for volumetric analysis, making it premature to conclude that prefrontal "executive" structures are more or less expansive relative to orbital "limbic" structures.

Finally, we found some evidence that the septal nuclei may be increased in human evolution, given this structure almost exceeded the upper limits of the prediction interval. The septal nuclei have not garnered much interest in comparative evolutionary analyses, but are incorporated into the oxytocin/vasopressin system (Heimer and Van Hoesen, 2006), and, consequently, are implicated in bonding and affiliation with conspecifics,

traits hypothesized to be under selection in human evolution. In humans, the septal nuclei appear to be activated in cooperative behaviors (Moll, 2009). Moreover, some experimental evidence indicates that the septal nuclei may mediate not only emotional attachment to conspecifics but also to abstract ideologies which may form the foundation of cultural affiliation (Moll, 2009). Like the amygdala and hippocampus, which are highly interconnected with this structure, the septal nuclei are involved in memory as well as emotional behavior. Specifically, the volume of the septal region has been shown to correlate with measures of the ability to recall the source of received information, e.g., "source memory" (Butler et al., 2012). Given the important role attributed to memory, affiliation, and emotional processing in human social cognitive evolution (Byrne and Bates, 2010; Hare, 2007), the evolution of the septal nuclei in human and nonhuman primate brains may be a fruitful object of further study.

CONCLUSION

Given historical perspectives on brain evolution, one might predict that cortical territories, especially the non-limbic dorsal frontal cortex, should be more expansive than limbic subcortical structures in human brain evolution. Strikingly, we found that cortical limbic and non-limbic territories, specifically the medial and dorsal frontal cortex, were among the least developed in humans when compared with other apes. In contrast, we found the strongest evidence for human evolutionary specializations in the size of the hippocampus and organization of the amygdala. These specializations were evident in human comparisons with apes but not with all anthropoids, suggesting that changes in the limbic system have occurred relatively recently, after the human lineage split from our shared common ancestor with apes. This analysis presents a preliminary investigation of

the role of emotion processing in human brain evolution, but our results are far from conclusive. In many cases, we were not able to find data which parcellated out meaningful subdivisions of the limbic structures analyzed. More pointed analyses of limbic structures implicated in important adaptive functions, like the anterior and posterior hippocampus, anterior orbital frontal cortex, ventral striatum, and anterior cingulate cortex may yield a more complex and refined understanding of the importance of limbic structures and emotional behavior in human evolution. We cannot exclude the possibility that, like the amygdala, other limbic structures may be targets of evolutionary reorganization, as well.

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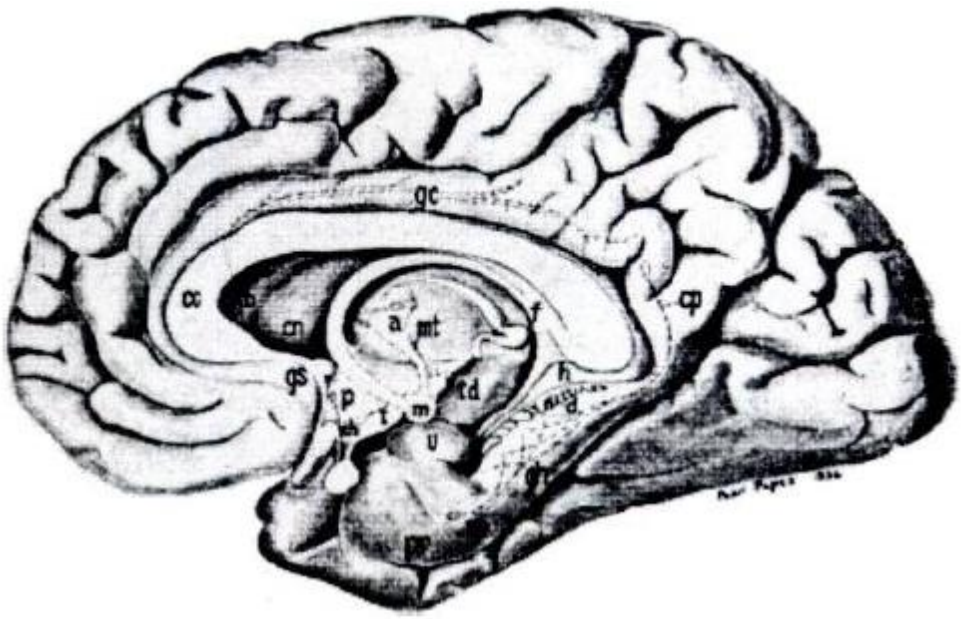
Chapter 4: Figures



Abbreviations

a	anterior nucleus	h	hippocampus nudus
ab	angular bundle	m	mamillary body
cn	caudate nucleus	mt	mamillothalamic tract
cc	corpus callosum	p	pars optica hypothalami
cp	cingulum posterius	pr	pyriform area
d	gyrus dentatus	sb	subcallosal bundle
f	fornix	t	tuber cinereum
gc	gyrus cinguli	td	tractus mamillotegmentalis
gh	gyrus hippocampi	th	tractus hypophyseus
gs	gyrus subcallosus	u	uncus

Figure 4.1. Papez's circuit. From Papez (1937).

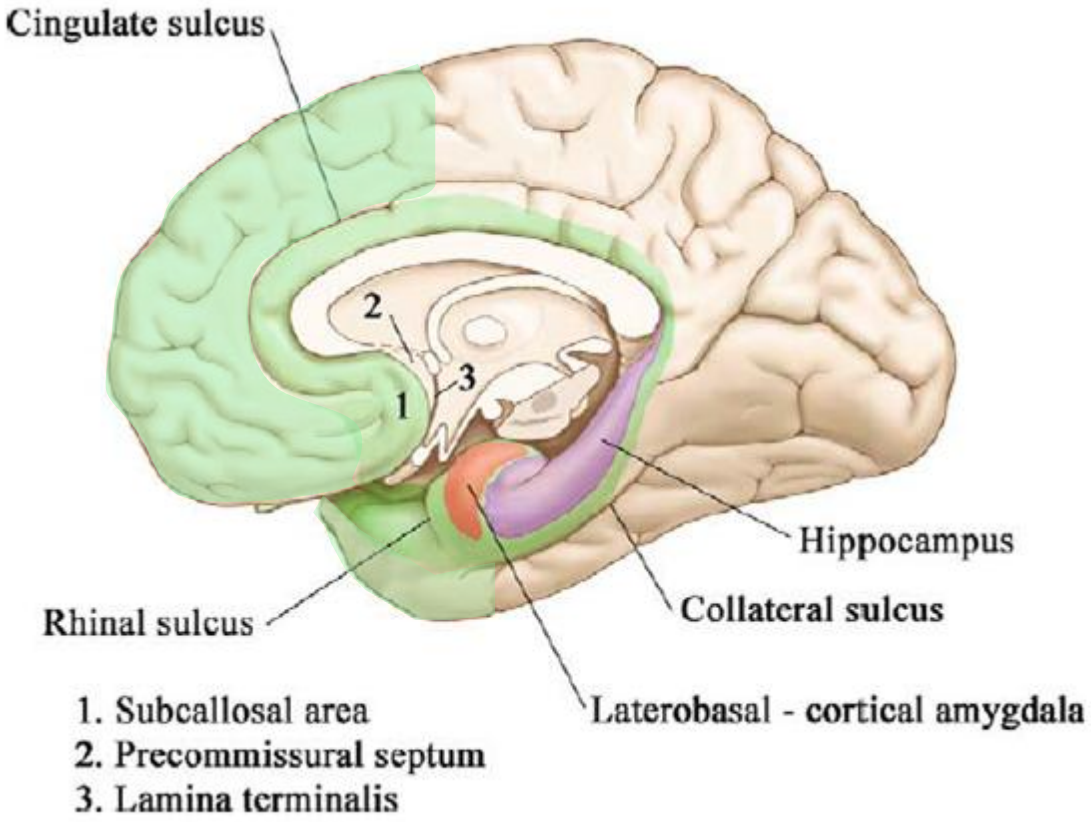


Figure 4.2. Limbic cortices (green) as defined by MacLean (1949). Modified from Heimer and van Hoesen (2006).

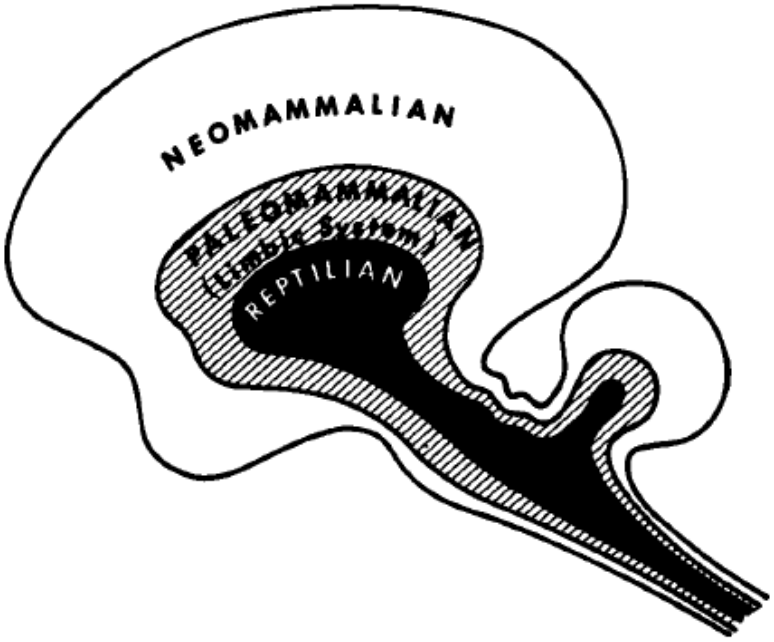


Figure 4.3. Maclean's "Triune Brain" concept illustrating the evolutionary "position" of the limbic, or emotional, brain.

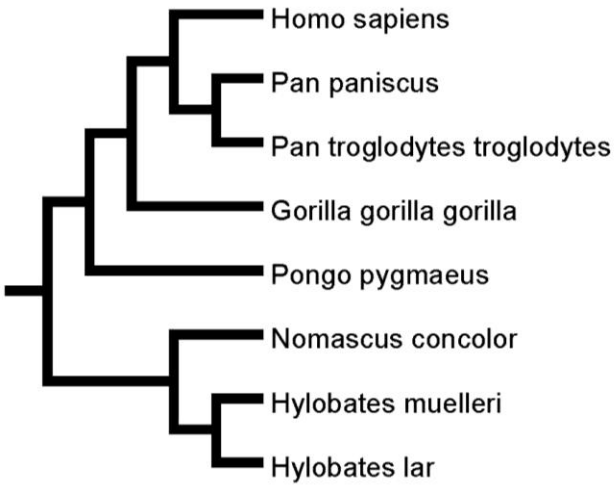


Figure 4.4. Consensus phylogeny for hominoids in the analysis.

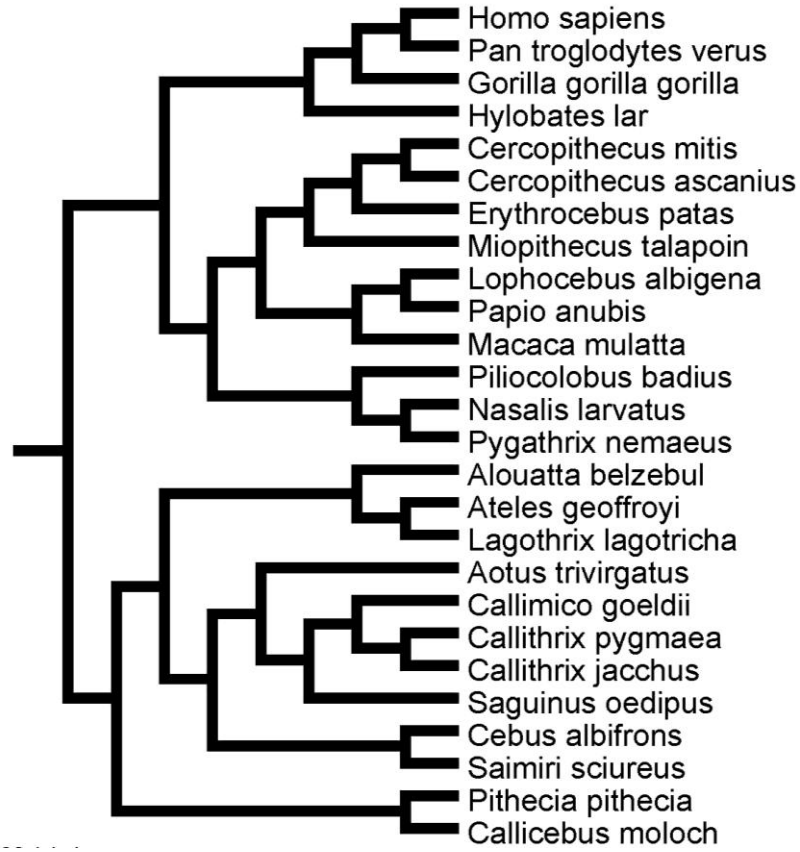


Figure 4.5. Consensus phylogeny for anthropoids used in the analysis.

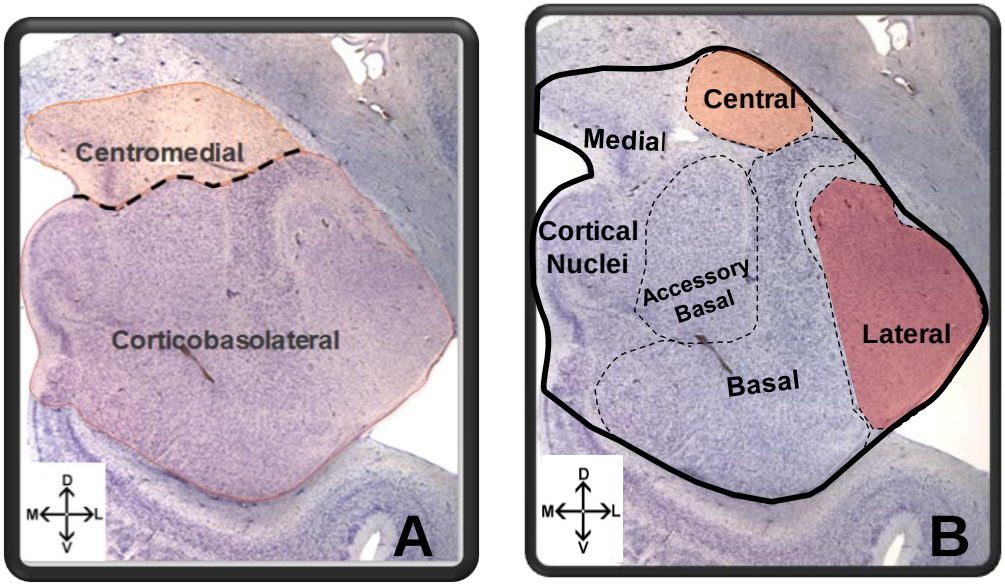


Figure 4.6. Subcomponents of the amygdala analyzed in A) Stephan, et al., (1977) and B) Barger, et al., (2007; in press).

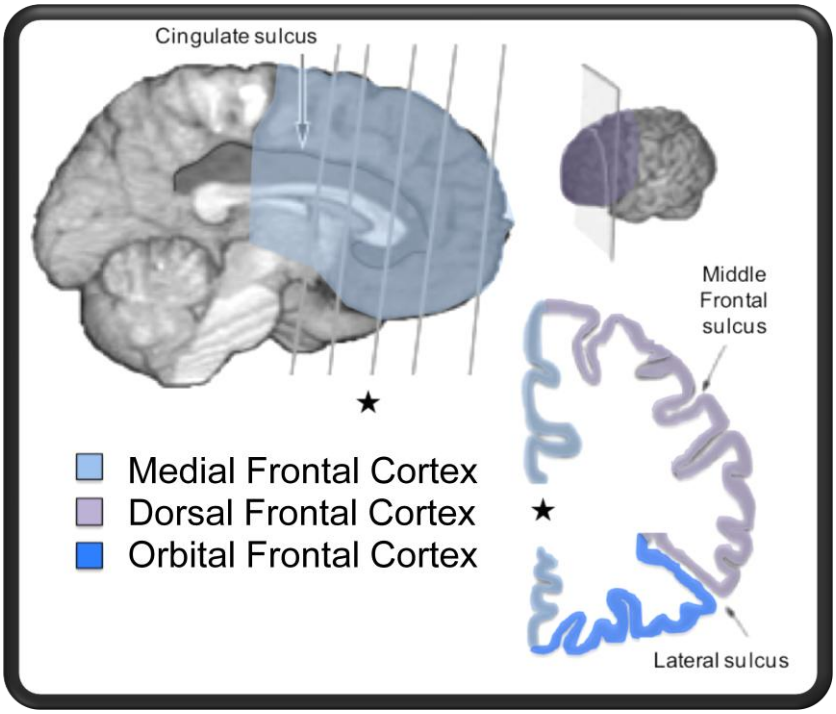


Figure 4.7. Divisions of the frontal cortex including limbic medial and orbital frontal cortex and non-limbic dorsal frontal cortex (modified from Courchesne, et al., 2011 using boundaries from Semendeferi, et al. 1997).

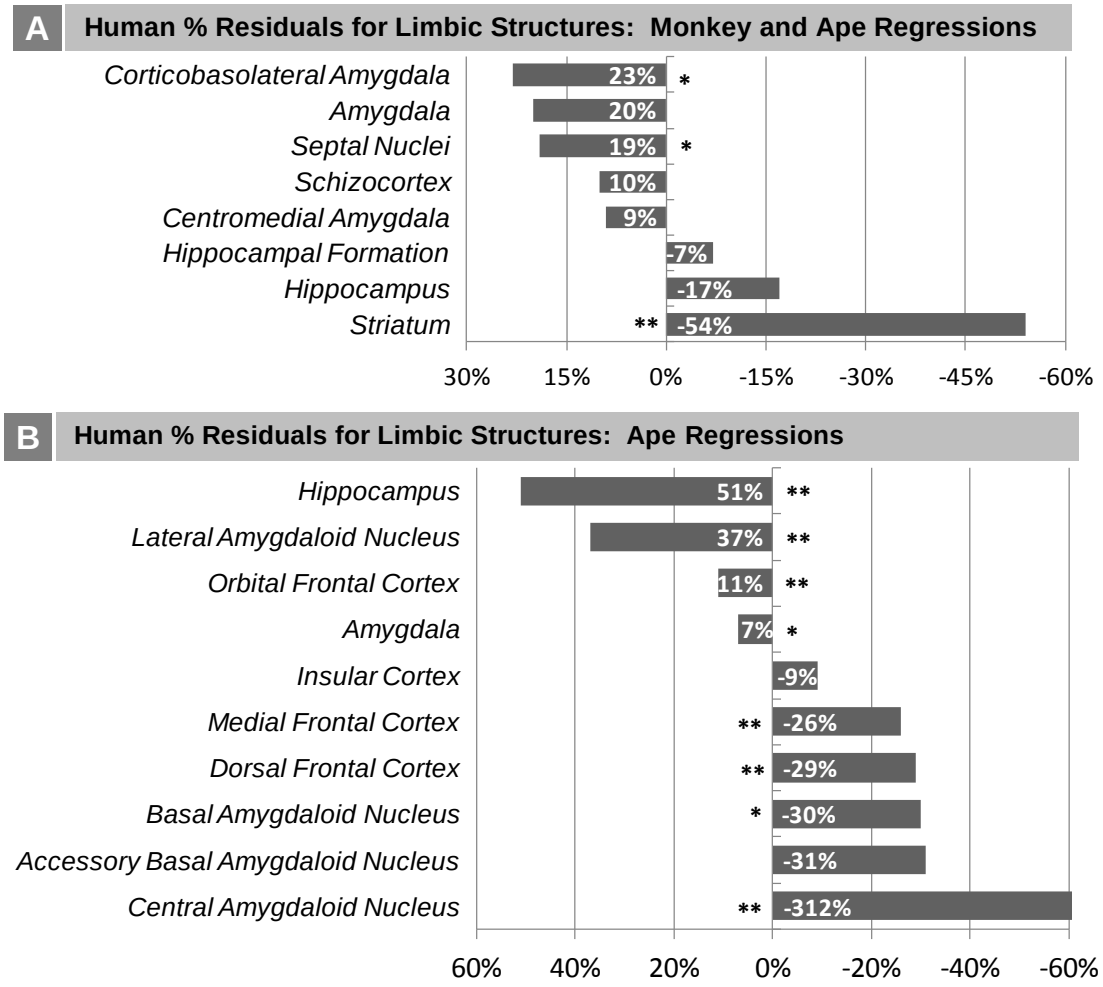


Figure 4.8. Human percent residual deviations from non-human regression lines for: A. Human comparisons with other anthropoids and B. Humans comparisons with other hominoids. *, residuals that approached significance at $p < 0.10$; **, correlations that were significant at $p < 0.05$ as determined either by prediction intervals (subcortical structures) or comparison of means (cortical structures).

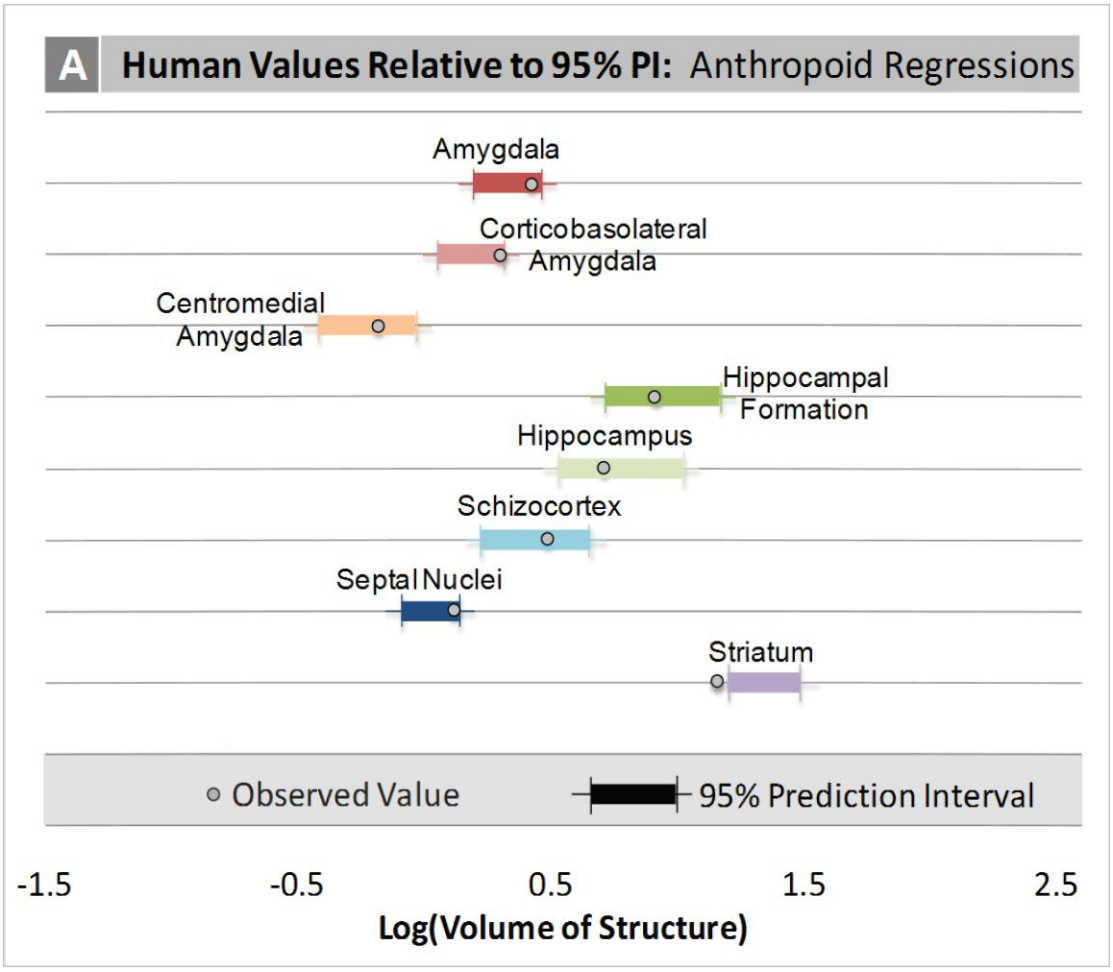


Figure 4.9. Observed volumes compared to prediction intervals for: A. Human comparisons with other anthropoids and B. Human comparisons with other hominoids.

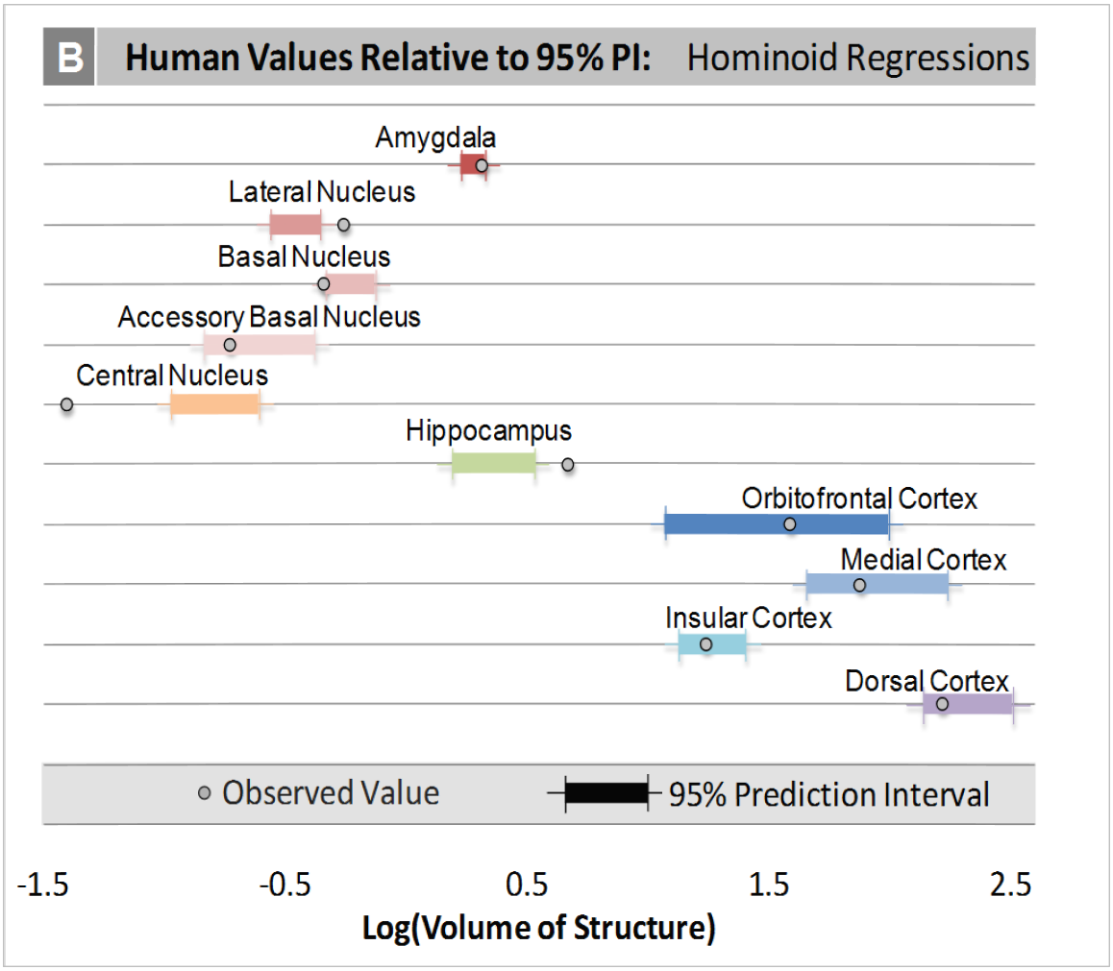


Figure 4.9. Observed volumes compared to prediction intervals for: A. Human comparisons with other anthropoids and B. Human comparisons with other hominoids, Continued.

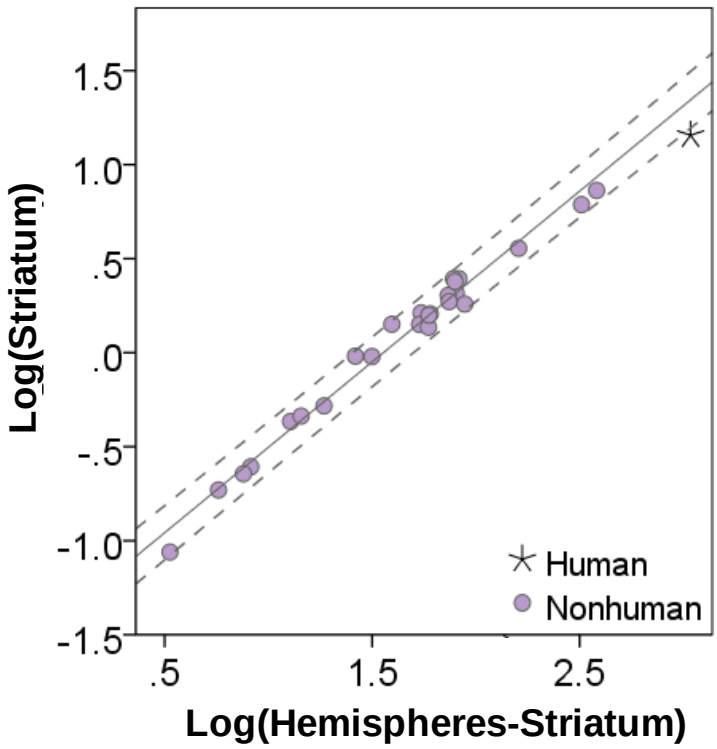


Figure 4.10. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the striatum drawn through nonhuman anthropoid species.

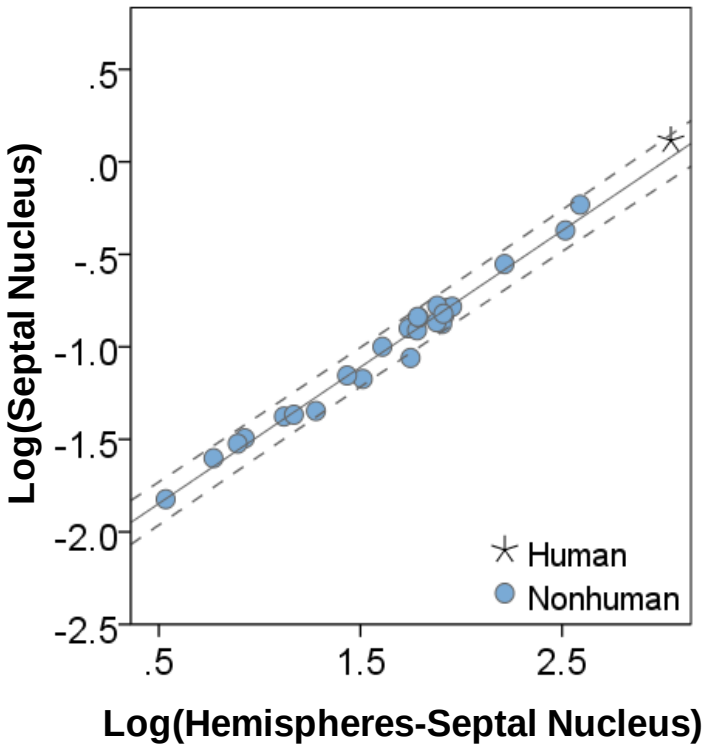


Figure 4.11. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the septal nuclei drawn through nonhuman anthropoid species.

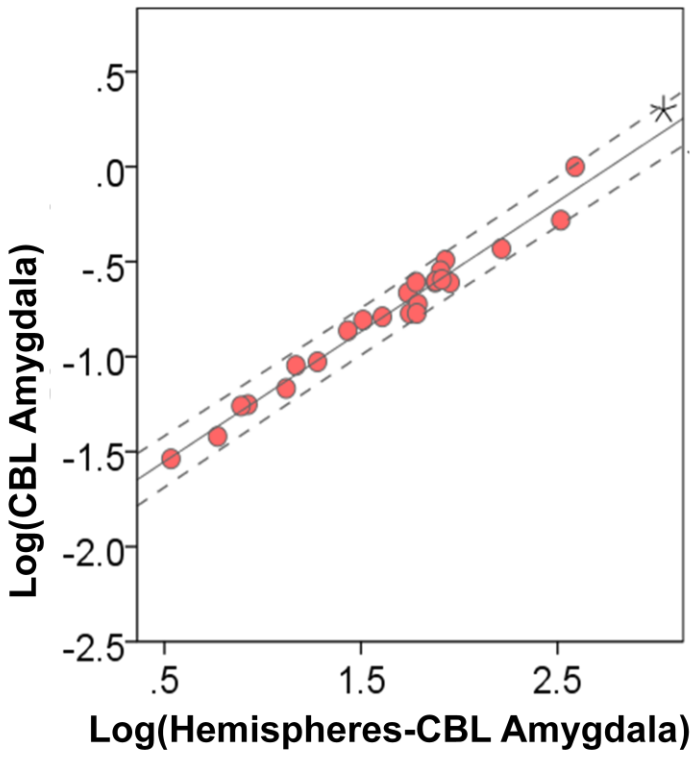


Figure 4.12. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the corticobasolateral amygdala (CBL) drawn through nonhuman anthropoid species.

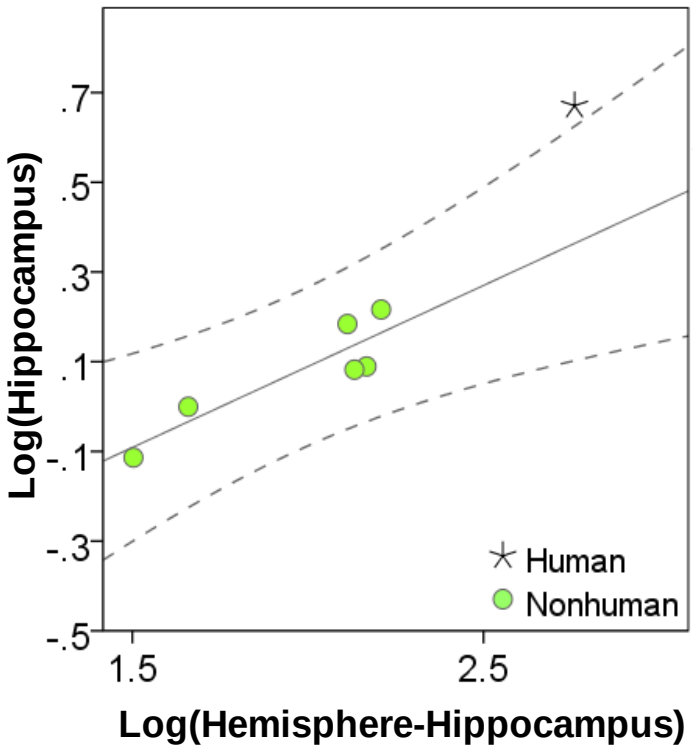


Figure 4.13. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the hippocampus drawn through nonhuman hominoid species.

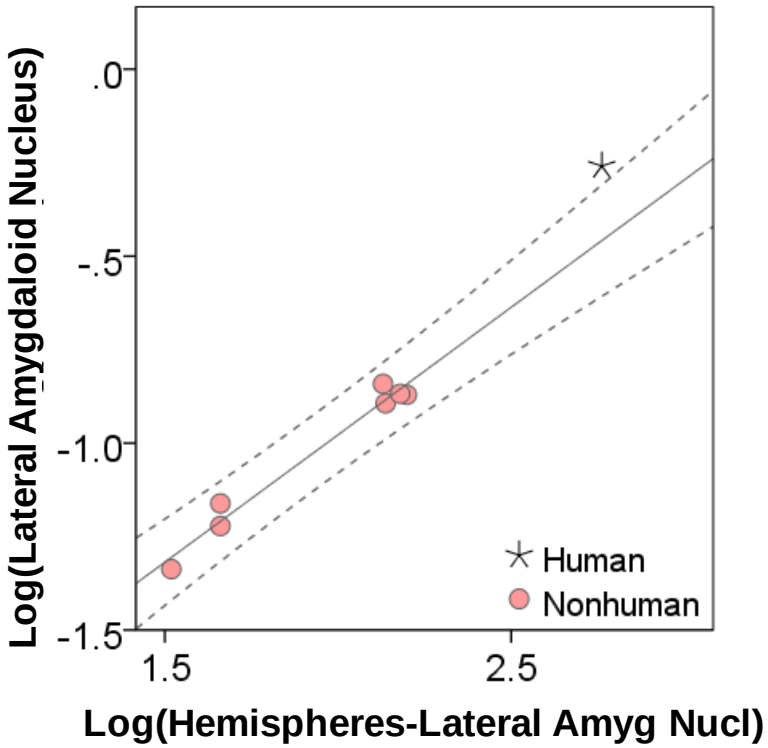


Figure 4.14. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the lateral amygdaloid nucleus drawn through nonhuman hominoid species.

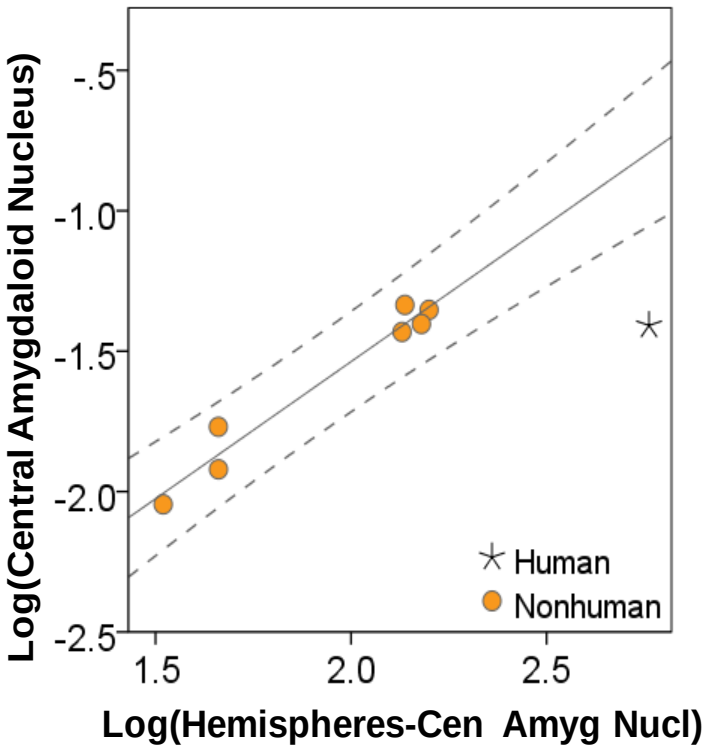


Figure 4.15. Log-log regressions and 95% prediction intervals (PI) of species average volumes (cc) for the central amygdaloid nucleus drawn through nonhuman hominoid species.

Chapter 4: Tables

Table 4.1. Subcortical limbic structures available in both datasets. Data from the current analysis (B12), Teffer, et al., 2004 (T04), Stephan, et al., 1981 (S81), and from Stephan, et al., 1987 (S87). Abbr: A = amygdala; Lat = lateral nucleus; Bas = Basal Nucleus; AB = accessory basal nucleus; C = central nucleus; CBL = corticobasolateral division; CM = centromedial division; Hippo = hippocampus; Schi = schizocortex.

		Amygdaloid Complex							Hippocampal Formation			Refs.
Specimen	Species	A	Lat	Bas	AB	C	CBL	CM	Hippo	Schi	Total	
SN207-84	*Homo sapiens*	2.031	0.551	0.452	0.188		--	--	4.685	--	--	B11, T04
--	*Homo sapiens*	2.643	--	--	--	--	1.990	0.653	5.144	3.071	8.215	S87, S81
Schimp 1	*Pan troglodytes*	0.685	0.138	0.224	0.070	0.065	--	--	1.772	--	--	B12
Bathsheba	*Pan troglodytes*	0.584	0.126	0.183	0.070	0.056	--	--	1.519	--	--	B12
C02	*Pan troglodytes*	0.571	0.115	0.176	0.066	0.037	--	--	--	--	--	--
C01	*Pan troglodytes*	0.417	0.095	0.117	0.047	0.024	--	--	--	--	--	--
C04	*Pan troglodytes*	0.749	0.165	0.263	0.064	0.049	--	--	--	--	--	--
--	*Pan troglodytes*	0.711	--	--	--	--	0.523	0.188	1.890	1.009	2.899	S87
YN86-137	*Pan paniscus*	0.634	0.164	0.191	0.066	0.042	--	--	1.557	--	--	B12
Zahlia	*Pan paniscus*	0.623	0.124	0.223	0.070	0.032	--	--	1.501	--	--	B12
YN82-140	*Gorilla gorilla*	0.651	0.100	0.247	0.096	0.037	--	--	1.229	--	--	B12
G01	*Gorilla gorilla*	0.867	0.167	0.266	0.112	0.053	--	--	--	--	--	--
G03	*Gorilla gorilla*	0.645	0.137	0.170	0.105	0.043	--	--	--	--	--	--
--	*Gorilla gorilla*	1.376	--	--	--	--	0.999	0.377	2.391	1.365	3.755	S87
YN85-38	*Pongo pygmaeus*	0.637	0.124	0.228	0.062	0.047	--	--	1.093	--	--	B12
Briggs	*Pongo pygmaeus*	0.520	0.105	0.151	0.052	0.036	--	--	1.325	--	--	B12
0I	*Pongo pygmaeus*	0.638	0.157	0.171	0.071	0.030	--	--	--	--	--	--
O02	*Pongo pygmaeus*	0.725	0.156	0.171	0.067	0.045	--	--	--	--	--	--
YN81-146	*Hylobates lar*	0.203	0.046	0.063	0.021	0.009	--	--	0.769	--	--	B12
Disco	*Hylobates concolor*	0.270	0.060	0.086	0.022	0.012	--	--	0.998	--	--	B12
GiCS	*Hylobates muelleri*	0.256	0.069	0.079	0.030	0.017	--	--	--	--	--	--
--	*Hylobates lar*	0.333	--	--	--	--	0.255	0.078	1.337	0.568	1.905	S87, S81

Table 4.2. Subcortical limbic structures available in both datasets (continued).

Specimen	Species	A	Lat	Bas	AB	C	CBL	CM	Hippo	Schi	Total	Refs.
--	*Papio anubis*	0.477	--	--	--		0.370	0.107	1.699	0.655	2.354	S87. S81
--	*Erythrocebus patas*	0.344	--	--	--		0.245	0.100	0.796	0.347	1.142	S87. S81
--	*Cercocebus albigena*	0.391	--	--	--		0.258	0.133	0.743	0.315	1.058	S87. S81
--	*Lagothrix lagothricha*	0.377	--	--	--		0.283	0.094	0.793	0.340	1.133	S87. S81
--	*Nasalis larvatus*	0.359	--	--	--		0.250	0.110	0.983	0.428	1.411	S87. S81
--	*Macaca mulatta*	0.339	--	--	--		0.247	0.093	0.677	0.320	0.996	S87. S81
--	*Colobus badius*	0.250	--	--	--		0.189	0.062	0.836	0.407	1.243	S87. S81
--	*Pygathrix nemeaus*	0.245	--	--	--		0.169	0.076	1.148	0.362	1.509	S87. S81
--	*Cercopithecus ascanious*	0.286	--	--	--		0.217	0.070	0.595	0.347	0.942	S87. S81
--	*Cercopithecus mitis*	0.352	--	--	--		0.247	0.105	0.683	0.309	0.992	S87. S81
--	*Cercopithecus talapoin*	0.207	--	--	--		0.156	0.051	0.353	0.130	0.482	S87. S81
--	*Callithrix jacchus*	0.053	--	--	--		0.038	0.015	0.045	0.111	0.156	S87. S81
--	*Cebulla pygmaeus*	0.038	--	--	--		0.029	0.009	0.067	0.041	0.107	S87. S81
--	*Callimico goeldii*	0.073	--	--	--		0.056	0.018	0.141	0.069	0.210	S87. S81
--	*Cebus sp.*	0.229	--	--	--		0.169	0.060	0.445	0.195	0.640	S87. S81
--	*Saimiri sciureus*	0.121	--	--	--		0.094	0.027	0.176	0.084	0.260	S87. S81
--	*Pithecia monacha*	0.183	--	--	--		0.137	0.046	0.417	0.145	0.562	S87. S81
--	*Allouata sp.*	0.213	--	--	--		0.162	0.051	0.660	0.255	0.915	S87. S81
--	*Ateles geoffroyi*	0.434	--	--	--		0.322	0.112	0.683	0.366	1.049	S87. S81
--	*Saguinus tamarin*	0.072	--	--	--		0.053	0.019	0.140	0.060	0.200	S87. S81
--	*Saguinus oedipus*	0.071	--	--	--		0.055	0.016	0.131	0.054	0.185	S87. S81
--	*Aotus trivirgatus*	0.097	--	--	--		0.068	0.028	0.270	0.122	0.391	S87. S81
--	*Callicebus moloch*	0.127	--	--	--		0.090	0.037	0.294	0.117	0.411	S87. S81

Table 4.3. Additional subcortical limbic structures (striatum, septum) (Stephan 1981).

	Septal Nuclei	Striatum
Homo sapiens	1.305	14.345
Pan troglodytes	0.426	6.123
Gorilla gorilla	0.587	7.284
Hylobates lar	0.151	2.392
Papio anubis	0.280	3.591
Macaca mulatta	0.136	2.016
Erythrocebus patas	0.165	1.812
Cercocebus albigena	0.147	2.073
Cercopithecus ascanious	0.126	1.414
Cercopithecus mitis	0.123	1.367
Cercopithecus talpoins	0.067	0.954
Lagothrix lagotricha	0.133	2.474
Colobus badius	0.144	1.609
Pygathrix nemaeus	0.145	1.583
Nasalis larvartus	0.167	1.868
Cebulla pygmaeus	0.015	0.087
Callimico goeldii	0.032	0.247
Cebus sp.	0.087	1.629
Saimiri sciureus	0.045	0.521
Pithecia monacha	0.070	0.959
Allouata sp.	0.100	1.415
Ateles geoffroyi	0.162	2.475
Callithrix jacchus	0.025	0.186
Saguinus tamarin	0.020	0.236
Saguinus oedipus	0.030	0.227
aotus trivirgatus	0.042	0.431
callicebus moloch	0.043	0.460

Table 4.4. Limbic and non-limbic frontal cortices. (Semendeferi 2001 and Schenker 2005).

		Limbic Cortex (cc)		
	Hemispheres	Orbital	Medial	Insular
Human1	1216.9	41.3	68.2	16.6
Human2	1218.4	41.3	83	18.8
Human3	1086.7	33.6	67.3	14.6
Human4	1115.9	31.2	66.9	17.7
Human5	1321.7	45.6	86	19
Human6	1318.4	44.7	81.7	18
Human7	1437.2	43.4	90.5	16.9
Human8	1238.6	30	74.6	16.5
Human9	1162.6	35.5	68.9	15.2
Human10	1221.7	41.6	67.4	20.3
Bonobo1	285.1	9	19.1	3.5
Bonobo2	309.2	13.4	18.2	4.1
Bonobo3	277.2	9.3	17.9	3.4
Chimpanzee1	280.2	9.5	15.8	2.6
Chimpanzee2	207.5	6.9	12.7	2.5
Chimpanzee4	269.8	9.6	15.7	3.5
Chimpanzee5	382.2	13.5	22.5	4
Chimpanzee6	303.4	8.7	17.7	3
Gorilla1	305	11.4	19.7	3.5
Gorilla2	406.7	14.7	26.3	7.1
Orangtuan1	395	9	30.1	5.9
Orangtuan2	455.8	12	33.1	4.3
Orangtuan3	387.7	10.8	28.3	5.3
Orangtuan4	326.1	7.1	22.7	3.7
Gibbon1	71	2.1	3.6	0.8
Gibbon3	68.5	2.4	3.7	0.7
Gibbon4	67.8	2.4	4.2	0.6

Table 4.5. Results of regression analyses run through: A. Anthropoid Values, B. Hominoid Value. † Student's t-tests were performed on mean observed human values for this structure. * Student's t-tests for this structure yielded significant results ($p<0.05$)**.**

A	ʎ	Regression Equation	R^2	P Value	95% Prediction Interval	Human Percent Residual
Amygdala	0	y = 0.69x-1.78	0.97	<0.01	0.19 – 0.46	20%
Corticobasolateral Amygdala	0	y = 0.68x-1.90	0.97	<00	0.05 – 0.32	23%
Centromedial Amygdala	0	y = 0.71x-2.34	0.95	<00	-0.42 – 0.03	9%
Hippocampal Formation	0	y = 0.76x-1.36	0.94	<00	0.72 – 1.17	-7%
Hippocampus	0	y = 0.76x-1.51	0.93	<00	0.53 – 1.03	-17%
Schizocortex	0	y = 0.77x-1.89	0.95	<00	0.23 – 0.66	10%
Septal Nuclei	0	y = 0.74x-2.22	0.98	<00	-0.89 – 0.14	19%
Striatum	0	y = 0.91x-1.41	0.98	<00	1.20 – 1.49	-54%

B	ʎ	Regression Equation	R^2	P Value	95% Prediction Interval	Human Percent Residual
Amygdala	0	y = 0.78x-1.87	0.99	<00	0.23 – 0.33	7%
Lateral Amygdaloid Nucleus	0	y = 0.68x-2.34	0.97	<00	-0.56 – -0.35	37%
Basal Amygdaloid Nucleus	0	y = 0.78x-2.37	0.98	<00	-0.33 – -0.13	-30%
Accessory Basal Amyg. Nuc.	0	y = 0.88x-3.04	0.91	<00	-0.84 – -0.38	-31%
Central Amygdaloid Nucleus	0	y = 0.98x-3.49	0.95	<00	-0.98 – -0.61	-312%
Hippocampus	0	y = 0.36x-0.63	0.76	0.01	0.19 – 0.54	51%
Orbital Frontal Cortex	1	y = 0.93x-1.32	0.92	0.01	1.07 – 1.99	11%†*
Medial Frontal Cortex	0	y = 1.12x-1.46	0.99	<00	1.87 – 2.10	-26%†*
Insular Cortex	0	y = 1.15x-2.27	0.98	0.01	1.12 – 1.46	-9%†
Dorsal Frontal Cortex	1	y = 1.17x-1.20	0.99	<00	2.14 – 2.51	-29%†*

Chapter 5

Conclusion

Increasingly, the idea that the production and mediation of complex behavior falls exclusively under the purview of the neocortex has been challenged by a perspective that stresses the importance of interactions between diverse neural territories. Particularly, the interdependence of "basic" emotion processing in cortical and subcortical limbic structures and "higher order" neocortical cognitive processing has been stressed (e.g., Damasio, 1998). The amygdala, or amygdaloid complex, is a subcortical structure that has traditionally been associated with emotional regulation but has also begun to receive greater scientific attention as a mediator of social cognition and affiliation (Kling, 1986; Brothers, 1990; Adolphs, 1999). The amygdala works to modulate emotional, neural, and bodily responses to external stimuli and direct an individual's attention based on the emotional significance of an external stimulus in order to facilitate coordination of a context appropriate response (Adolphs, 1999). Across primates, the evolution of the amygdala has been characterized by the differential expansion of its intrinsic subcomponents (Stephan et al., 1987). Increased volume in the most expanded subdivision has been shown to correlate with increased social group size, suggesting that this expansion results from social evolutionary pressures (Barton, et al., 2003; Barton & Aggleton, 2000). Given that subcomponents of the amygdala appear to be the targets of evolutionary change across primates, this dissertation investigated the potential importance of the amygdala and its constituent nuclei in human brain evolution,

specifically. Volumes and numbers of neurons in the amygdala and four of its major nuclei, the lateral, basal, accessory basal, and central nuclei were quantified using a large sample of specimens including individuals from all major hominoid genera. Additionally, we situated the evolution of the human amygdala within the broader context the evolution of the limbic system, undertaking a large scale investigation of human specializations in multiple limbic structures.

Summary Statement I: *Absolute amygdala size is much larger in humans than in great apes, although it is largely consistent with evolutionary trends across apes; amygdala neuron numbers, in contrast, appear conserved in humans.*

We found that the size of the human amygdala is over three times the size of the great ape amygdala. Volumetrically, the human amygdala is slightly larger than predicted for a non-human primate with similar overall hemisphere volume, i.e., residuals tended to be positive. However, in all volumetric analyses, the individual human amygdala datum was contained within the range of values predicted for an ape of similar hemisphere volume. Thus, the human amygdala is not unequivocally bigger than one might expect based on presumed evolutionary increase across apes. However, the amygdala is the only limbic structure that exhibited positive residuals when humans were compared both with anthropoids (Ch. 4) and with other hominoids (Ch. 2, 4). Given only one human data point was suitable for volumetric analysis, we are limited by the available data. This raises the possibility that increasing our human sample to allow for tests of species means may yield positive results.

The threefold increase in amygdala volume was not matched by increases in neuron numbers, which were considerably conserved in humans and overlapped with great ape values. These discrepant findings introduce the possibility that a profitable avenue for future research into human amygdala evolution would be to interrogate variation in neuropil as well as the somal and connective properties of individual amygdala neurons, e.g., soma size, dendritic branching, or oligodendroglia numbers.

Summary Statement II: *Evolutionarily, the gross volumetric and neuronal organization of the human amygdala sets it apart from that of other apes.*

The amygdala is a heterogeneous structure, comprised of numerous highly interconnected nuclei. While a significant number of these nuclei share connections with non-neocortical structures, the lateral, basal, and accessory basal nuclei are characterized by their strong connections with the neocortex (Price et al., 1987). These three nuclei are collectively referred to as the basolateral division of the amygdaloid complex. Because the neocortex is expanded in humans, we were interested in the potential development of these amygdaloid nuclei in human evolution. In contrast, a number of amygdaloid nuclei share connections with non-cortical regions like the olfactory cortex and brainstem (Price et al., 1987). As a contrast to the basolateral nuclei, we also addressed the evolution of the central nucleus in humans, which shares connections with more conserved regions like brainstem nuclei.

Despite the fact that they are all interconnected with neocortex, we did not find evidence for uniform expansion of the basolateral nuclei in human evolution. In humans, the lateral nucleus is clearly the largest of the basolateral nuclei, while the basal nucleus

is the largest in apes. The human lateral nucleus is also the largest of all the amygdaloid nuclei and contains the most neurons. Both the volume and number of neurons in the human lateral nucleus are significantly greater than predicted by allometric trends, indicating that they are evolutionarily derived compared to apes. Conversely, humans have especially small central nuclei with fewer neurons than most apes. There is some evidence that the human basal nucleus is decreased both in volume and neuron number, although these findings only approached significance. It should be noted that decreases in predicted central and basal nucleus volume are more substantial than the subtle decreases in central and basal neuron number, while increases in lateral neuron number exceeded increases in the volume of this nucleus.

Summary Statement III: *The data indicate that some subcortical limbic structures, like the amygdala, may have been important loci for neuroanatomical change over the course of recent human evolution.*

In humans, two subcortical limbic structures, the hippocampus and amygdala, are larger than expected when compared with apes, although only the hippocampus fell significantly outside the range of predicted values. However, volumes for subcomponents of the amygdala, including the "corticobasolateral" amygdala in comparisons of humans and other anthropoids and the lateral nucleus of the amygdala in comparisons with apes, were positioned at the upper limits of the 95% confidence intervals. Surprisingly, the evidence for volumetric increase in human cortical territories was not as strong, whether they were limbic or non-limbic in nature. Most regions of the frontal cortex, an area long theorized to be enhanced in human evolution (Semendeferi and Damasio, 2000), were

significantly smaller in humans than predicted. It may be the case that larger human sample sizes would yield more positive results for limbic structures, given we found that percent residuals were high for many limbic structures. Compared to other primates, human percent residuals for total neocortical volume have been reported to be approximately 24% (Rilling and Insel, 1999); compare this to residuals for the human hippocampus at 51%, septal nuclei at 19%, amygdala at 20%, corticobasolateral amygdala at 23%, and lateral nucleus at 39%. The largest percent residuals and the most significant deviations from nonhuman primate values resulted from human comparisons with apes rather than with anthropoids. Thus, the data point to important human adaptations in subcortical limbic structures occurring after the split between great ape and human lineages.

Proposition I: *The evolution of the human amygdala is largely defined by evolutionary reorganization; the nature of human amygdala organization may be particularly influenced by the discrete connectivity of individual nuclei, emphasizing the functional and anatomical interdependence of the basolateral amygdala and related cortical territories.*

Although very little is known about the evolution of the amygdala in primates, previous research suggests that a portion of the amygdala that is composed mostly of the basolateral division shows evolutionary increase in primates when compared with other portions of the amygdala (Stephan, et al., 1987). The authors attributed this differential change to the influence of neocortical expansion on the connected basolateral nuclei. One means of testing this assumption is to assess correlation coefficients across relevant

structures. If the average size of various amygdaloid nuclei correlates with that of highly interconnected cortical regions, then it would provide evidence as to the coordinated evolution of these structures (Barton et al., 2003).

The connective relationships between the basolateral nuclei and territories in the frontal and temporal cortices are summarized in Figures 1 and 2. The lateral nucleus primarily exhibits bidirectional connectivity with the temporal cortex. The basal nucleus projects to and receives considerable input from the orbital frontal and medial frontal cortices. The accessory basal nucleus shares reciprocal connections primarily with the medial frontal cortex, but also receives heavy projections form the superior temporal gyrus. Table 1 summarizes the results of a Pearson's correlation analysis (SPSS, 17) assessing the relationship between the volume of amygdaloid nuclei and the volumes of: 1) the temporal lobe and superior temporal gyrus (Rilling and Seligman, 2002) and 2) the limbic frontal cortex (Schenker et al., 2005). The volume of the medial frontal cortex correlated with the volumes of the amygdala, lateral nucleus, and accessory basal nucleus. The volume of the orbital frontal cortex correlated with the volumes of the amygdala, basal nucleus, and accessory basal nucleus. In the temporal lobe, only the volumes of the amygdala and lateral nucleus correlated with total temporal lobe volume and the volume of the temporal lobe minus the superior temporal gyrus. If the superior temporal gyrus is isolated, correlations with the accessory basal nucleus and lateral nucleus reach significance. The volume of the central nucleus showed no clear relationship to volumetric increase in any cortical territory. These volumetric correlations

were surprisingly consistent with general patterns of connectivity, although they are very preliminary in nature and do not adjust for phylogeny.

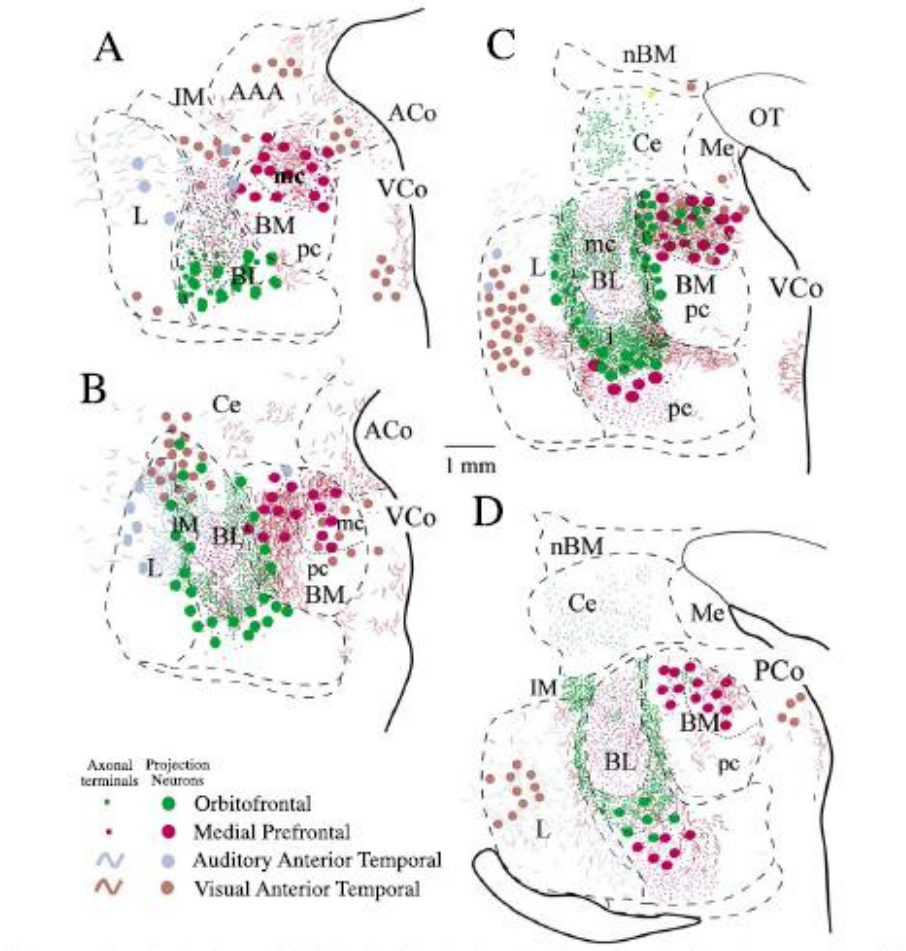


Figure 5.1. Illustration of the distribution of projection neurons targeting cortical territories and axon terminals targeting the amygdaloid nuclei in rostral to caudal (A-D) coronal sections. Abbr: AAA= anterior amygdaloid area, ACo = anterior cortical nucleus, BL = basal nucleus, BM = accessory basal nucleus, Ce = central nucleus, IM = intercalated nuclei, L = lateral nucleus, Me = medial nucleus, nBM = basal nucleus of Meynert, PCo = posterior cortical nucleus, VCo = ventral cortical nuclei, pc = parvicellular division of a nucleus, mc = magnocellular division of a nucleus. Figure modified from (Ghashghaei and Barbas, 2002).

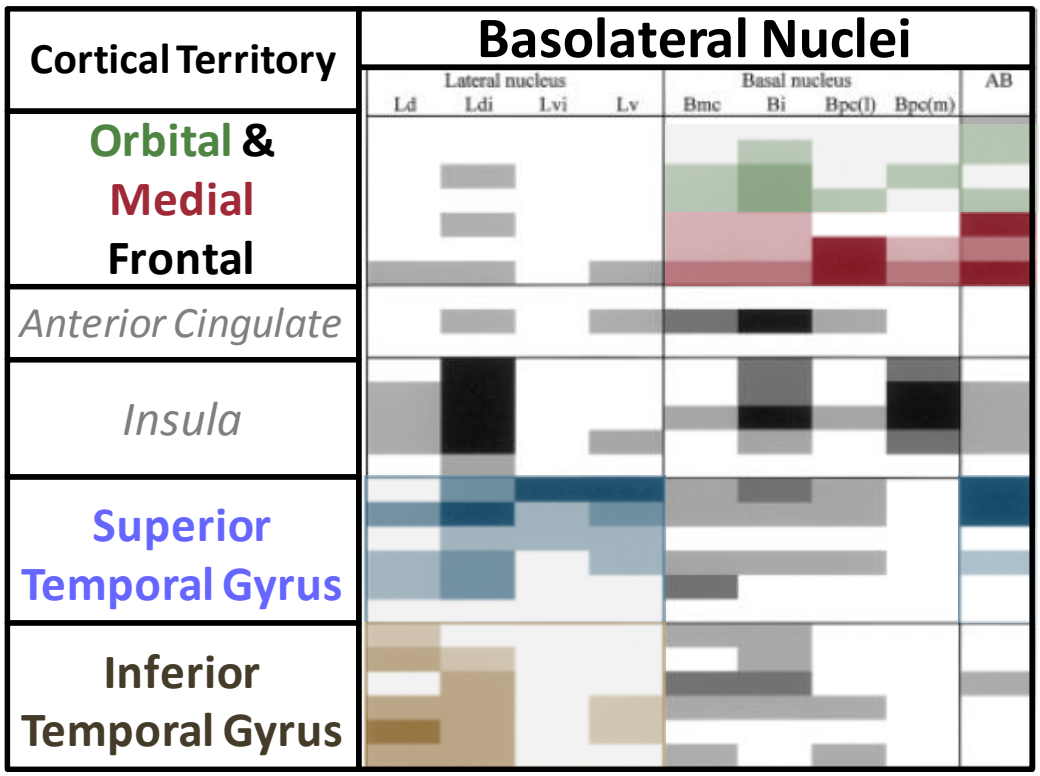


Figure 5.2. Projections from cortical territories to basolateral nuclei. The densest connections are noted by the darkest shade, less dense connections are marked by the medium shade, while the lightest shade indicates the lightest projections. The strongest connections between each cortical territory and nucleus are highlighted with colors coordinating with colors for these territories used in from Fig. 1. Image modified from (Stefanacci and Amaral, 2002).

Given these correlations, it is likely that the differential expansion of amygdaloid components is influenced by the differential elaboration of neuroanatomical regions which share strong connections with the nuclei, as has been previously suggested for larger subcomponents of this structure (Stephan et al., 1987; Barton and Aggleton, 2000). As the temporal lobe is the only major lobe shown to be differentially expanded over the course of human evolution (Rilling and Seligman, 2002; Semendeferi and Damasio, 2000), it may be evolutionarily significant that the lateral nucleus is the primary target of these temporal lobe projections. Given the functional and connective relationship between the temporal lobe and the lateral nucleus (Stefanacci and Amaral, 2002), it is

likely that the unique organization of the human basolateral division is driven by the influence of the information flowing from the enlarged human temporal lobe into the lateral nucleus (Rilling and Seligman, 2002; Semendeferi and Damasio, 2000).

Table 5.1. Correlation matrix presenting the relationship between the volumes of cortical territories and amygdaloid nuclei. Abbr.: A = amygdala, C = central nucleus, L = lateral nucleus, B = basal nucleus, AB = accessory basal nucleus, STG = superior temporal gyrus. *, correlations that were significant at $p < 0.05$; **, correlations that were significant at $p < 0.01$.

		A	**C**	**L**	**B**	**AB**
Medial Frontal	*Coefficient*	1.000**	0.371	0.943**	0.771	0.943**
	Sig. (2-tailed)	0.000	0.468	0.005	0.072	0.005
Orbital Frontal	*Coefficient*	0.829*	0.371	0.771	0.943**	0.943**
	Sig. (2-tailed)	0.042	0.468	0.072	0.005	0.005
Temporal Lobe	*Coefficient*	0.829*	0.371	0.943**	0.714	0.657
	Sig. (2-tailed)	0.042	0.468	0.005	0.111	0.156
STG	*Coefficient*	0.943**	0.600	0.829*	0.600	0.886*
	Sig. (2-tailed)	0.005	0.208	0.042	0.208	0.019
Non STG Temporal	*Coefficient*	0.829*	0.371	0.943**	0.714	0.657
	Sig. (2-tailed)	0.042	0.468	0.005	0.111	0.156

Proposition II: *Specializations in the human amygdala may be linked to specializations in behaviors subserved by the amygdala and related neural structures, like social perception, affiliation, and emotional modulation.*

Although general processes associated with the amygdala like implicit associative learning, attending to salient stimuli, memory consolidation (Phelps, 2005), and environmental appraisal (Emery, 2000) are undoubtedly central to many social cognitive skills, in the neuroimaging literature, amygdala activation is also associated with mediating and evaluating myriad, explicitly social stimuli. Some examples include the

processing of emotional vocal, facial, and full body expressions (Yang et al., 2002; Hadjikhani and de Gelder, 2003; Glascher et al., 2004; Sander et al., 2005), evaluating another's trustworthiness (Grezes et al., 2004; Singer et al., 2004), and deciding whether to conform to peers' suggestions (Berns et al., 2005). At the cellular level, neurons in the macaque amygdala are activated by both dynamic social behaviors like social interaction (Brothers and Ring, 1992, 1993) and social approach (Kling et al., 1979) and static representations like images of faces (Brothers, 1990). In the rapidly changing, complex social environments inhabited by primates (Humphrey, 1988; Whiten and Byrne, 1988; Dunbar, 2003), the sorts of processes subserved by the amygdala may provide an individual with essential tools for evaluating conspecifics and navigating the social *milieu*. Complex social cognition is hypothesized to be a possible "prime mover" in primate cognitive evolution, and it may be hypothesized to influence the evolution of neural structures subserving social behaviors, like the amygdala. Given that explanations for evolutionary change in the amygdala have primarily targeted the expansion of the neocortex and increased social complexity, one intriguing possibility is that the concomitant expansion of the human temporal lobe and lateral nucleus reflects the increased importance of the integration of emotion, decision making, and social perceptual pathways in human evolution.

Several lines of evidence support potential functional specializations in the human amygdala. In regard to qualitative distinctions in human social behavior, several higher order social evaluative behaviors that are regarded by some as characteristically, or even exclusively human, like moral judgment, empathy, resistance to social conformity, social

contract reasoning, and appraising the minds of others recruit the amygdala (Singer et al., 2004; Berns et al., 2005; Vollm et al., 2006; Stone et al., 2002; Adolphs et al., 1998; Stone et al., 2003). The fact that larger social network sizes are correlated with larger amygdala volumes suggests that social parameters may influence amygdala volume (Bickart et al., 2010; Kanai and Bahrami, 2012). Anthropoid primate social systems have been argued to be some of the most complex in the mammalian kingdom (Shultz and Dunbar, 2007), but human social systems exhibit both quantitative and qualitative distinctions from those of other anthropoids. While the maximum size of chimpanzee and bonobo social groups have been reported to reach up to 150 individuals (Kano, 1992; Mitani and Amsler, 2003), human social networks, on average, exceed 120 individuals both in industrialized (Hill and Dunbar, 2003) and hunter-gatherer societies (Zhou et al., 2005). Qualitatively, humans are the only primates to form social groups comprised predominantly of non-kin of both sexes (Hill et al., 2011).

In human evolution, the lateral nucleus has become emphasized, which, given its position in the flow of information processing across the amygdala, may reflect a general need for human amygdala to process the emotional significance of increased sensory input from the large human neocortex. The expansion of the corticobasolateral division across primates has long been linked to neocortical expansion in primates (Barton and Aggleton, 2000; Stephan et al., 1987). Of course, humans are characterized by their particularly expansive neocortices (Rilling and Insel, 1999). Because it receives the majority of neocortical input, the lateral nucleus is generally regarded as the “sensory gateway” to (LeDoux, 2007) and “evaluator nucleus” in (Freese and Amaral, 2009) the

amygdala. Arising largely in the temporal lobe, the majority of neocortical projections to the amygdala synapse on the lateral nucleus, providing multimodal information about stimulus characteristics (Freese and Amaral, 2009; LeDoux, 2007; Stefanacci and Amaral, 2002). Consequently, the human lateral nucleus may receive and evaluate a heightened amount of input from the expanded human neocortex, especially the expanded temporal cortices. In contrast, the basal and central nuclei provide primary output to regions involved in producing complex motor and instrumental responses, respectively, although they both do receive some cortical input (Freese and Amaral, 2009; Yaniv et al., 2004; LeDoux, 2007). For the most part, the majority of output nuclei are maintained in an inhibitory state to avoid initiating inappropriate emotional responses to irrelevant external stimuli (LeDoux, 2007). As such, the lateral nucleus is in a position to filter a considerable amount and diversity of information arriving in the amygdala from the temporal neocortex, prior to activating other nuclei, and may be particularly involved in modulating emotional responses to these stimuli.

Rodent models of fear inhibition provide a better understanding of the function of the lateral nucleus at the cellular level, implicating it in memory storage and consolidation. Excitatory sensory cortical afferents arriving in the lateral nucleus synapse on both inhibitory interneurons and projection neurons which, themselves, frequently send axon collaterals back to inhibitory neurons, providing for both feedforward and feedback inhibition (LeDoux, 2007). Novel stimuli activate the amygdala, but this extensive inhibitory network leads to response attenuation over time (LeDoux, 2007). However, if a stimulus is paired with an emotionally arousing, aversive event, a ”memory” of this stimulus pairing may be consolidated though synaptic remodeling, i.e.,

long term potentiation (LeDoux, 2007). If the stimulus occurs again without the aversive stimulus, the pontentiated synapses facilitate the flow of information through the rest of the amygdaloid nuclei, coordinating a fear response. Repeated pairings of a stimulus and aversive event strengthen synaptic remodeling, forming a representation in long-term memory (LeDoux, 2007). Given the lateral nucleus is not only the first stop in filtering cortical information but also participates extensively in emotional learning and memory consolidation, it may be the case that increased neocortical information about a complex array of sensory stimuli may further tax this structure.

The individual nuclei of the amygdala are difficult to isolate *in situ* in primates, thus there is little information about the exact function the lateral nucleus in humans. Some evidence can be drawn from studies of neuropathology. Autistic adults exhibit considerable reductions in the number of neurons only in the lateral nucleus (Schumann and Amaral, 2006), while changes in the anatomy of the lateral nucleus have been suggested to be a feature of William's syndrome (Galaburda and Bellugi, 2000). Both disorders are characterized by impairments in social behavior. Together, this evidence supports a potential role for the lateral nucleus in social behavior or at the very least a susceptibility to social pathology. Of course, concordant with common understanding of amygdala function, bipolar disorder, an affective but not explicitly social disorder, has been associated with reductions in the size and number of neurons in the lateral nucleus (Berretta et al., 2007). The authors posit that neuropathology of the lateral nucleus may underlie the difficulties that patients have assigning emotional significance to external stimuli. This may relate to social cognition in that the ability to rapidly and effectively appraise the emotional significance of relationships between and communication from

conspecifics is believed to be at the foundation of complex cognitive abilities (Byrne and Bates, 2007).

Evolutionarily, our findings may speak to the long held idea that social behavioral changes involving greater discrimination among complex social cues and increased affiliation between conspecifics may drive the evolution of human behavior and, consequently, neural systems (Holloway, 1972; Vilensky et al., 1982). As the primary recipient of temporal cortical input to the amygdala, the lateral nucleus receives a preponderance of information from territories in the temporal lobe that process social information like the temporal poles, the superior temporal sulcus, and, likely, the fusiform gyrus (Stefanacci and Amaral, 2002; Smith et al., 2009; Ghashghaei and Barbas, 2002). Although it also receives information from temporal regions not associated with social cognitive abilities, accumulating comparative data suggests that the most expansive portions of the temporal lobe are those that process complex social stimuli. Although it has yet to be investigated in a post-mortem comparative analysis, MRI data indicate that region of cortex surrounding the superior temporal sulcus is a likely candidate for expansion in human evolution (Hill et al., 2010; Rilling and Seligman, 2002). The temporal poles appear expanded in human endocasts relative to ape endocasts and in endocasts of the earliest hominin ancestors, gracile australopithecines (Hill et al., 2010; Rilling and Seligman, 2002; Falk et al., 2000). This feature is also present in the most recently discovered early hominin, *Australopithecus sediba* (Carlson et al., 2011), which has been argued to share affinities with early *Homo* and may serve as an evolutionary precursor to the *Homo* genus (Berger et al., 2010). Functionally, the

superior temporal sulcus and the temporal poles have been highlighted for their role in social perception, language understanding, and social categorization (Allison et al., 2000; Redcay, 2008; Olson et al., 2007). Given the available evidence, it is conceivable that the volume and number of neurons in the human lateral nucleus have increased in response to a heightened need to process the emotional salience of elements in the extensive human communicative repertoire and characteristics of individuals in expansive social networks that are processed in particularly expanded regions of the human temporal association cortices.

In order to better assess this hypothesis, more comparative histological data needs to be collected on these potentially specialized temporal regions. Moreover, although macaque data on amygdala connectivity is thorough and extensive, it cannot be assumed that macaque and human brains necessarily share the same patterns of connectivity. Recent diffusion tensor imaging studies are coming closer to addressing this question and may be able to provide more specific data in the future (Solano-Castiella et al., 2010; Smith et al., 2009; Saygin et al., 2011). In primate evolution, increasingly complex visual and auditory systems appear to have arisen in response to or been co-opted to support complex social information processing (Barton, 1998); our results suggest that humans are not unequivocally exempt from this trend.

Proposition III: *Shared functions of the amygdala and hippocampus may underlie cognitive specializations in emotional processing arising after the human lineage split from apes.*

The amygdala and hippocampus present the clearest cases of evolutionary specialization in the human limbic system. Although connectively and functionally linked, limbic structures interact with diverse neural systems and could contribute differentially to behavioral adaptations that emphasize particular aspects of emotional experience. Although there is considerable overlap in the connective and functional profiles of these two anatomically proximate structures, the amygdala has largely been conceived of as a structure subserving social cognition, while the hippocampus has received more attention for its role in topographic knowledge and ecological intelligence. This section presents an expanded, more speculative discussion of the potential shared sources of evolutionary change in the amygdala and the hippocampus.

Like the amygdala, the hippocampus is also somewhat heterogeneous, comprised of separate subdivisions which perform separate, but interrelated functions. Strikingly, many of these functions overlap with those of the amygdala. Both the hippocampus and amygdala mediate emotional responses to external stimuli (Freese and Amaral, 2009; Fanselow and Dong, 2010). Like the amygdala, the anterior hippocampus shares diverse connections with numerous neural systems, including the hypothalamic pituitary axis (Fanselow and Dong, 2010). Thus, it is also well positioned to modulate neuroendocrine responses to external stimuli (Fanselow and Dong, 2010). Also like the amygdala, the anterior hippocampus plays a role in fear processing and has been associated with cue and context dependent conditioning (Fanselow and Dong, 2010; LeDoux, 2007). Nonetheless, the hippocampus is involved in a number of functions that are distinct from amygdala function. The posterior hippocampus is widely known to be associated with topographic, i.e., spatial navigation and mapping, and episodic memory (Burgess, et al.,

2002). Although the amygdala and hippocampus participate in implicit conditioning , the hippocampus is known as the canonical structure subserving explicit, declarative and episodic memory (Bechara et al., 1995; Thompson and Kim, 1996; Fanselow and Dong, 2010). Unlike the amygdala, hippocampal volume correlates with several measures of executive function across primates (Shultz and Dunbar, 2010) and impairments in tests of executive function are associated with smaller hippocampi (Frodl et al., 2006). From an evolutionary perspective, it may be appropriate to ask how shared and distinct properties of the amygdala and hippocampus may relate, explicitly, to adaptive behaviors.

Given that certain functions of the hippocampus and amygdala are not consistent, it is possible that changes in these structures may be completely decoupled in human brain evolution. While amygdala and corticobasolateral amygdala volume have explicitly been linked to social behavioral measures (Bickart et al., 2010; Kanai and Bahrami, 2012; Barton and Aggleton, 2000; Lewis and Barton, 2006), little is known about the relationship between the hippocampus and measures of adaptive behavior in primates. The hippocampus has largely received attention in investigations of ecological, specifically topographic, intelligence due to demonstrated correlations between hippocampal volume and home range size in some birds and non-primate mammals (Suzuki and Clayton, 2000). In primates, however, hippocampal volume (normalized for a variety of variables like brain size, medulla size, and body size) has not been shown to correlate with ecological variables like range size (Barton, 2000). Among primates, London taxi cab drivers have provided the only positive correlation between hippocampal volume and spatial variables (Maguire et al., 2000). Nonetheless, this was only true of the posterior hippocampus, which participates in topographic memory, and occurred with a

concomitant reduction in the anterior hippocampus, which is associated with emotional processing (Fanselow and Dong, 2010). The strongest predictor of hippocampal volume have been measures of executive function, which would not exclusively participate in ecological or social intelligence (Shultz and Dunbar, 2010; Frodl et al., 2006).

From an evolutionary perspective, it may be most parsimonious to seek an adaptive explanation based on shared functional attributes of the amygdala and hippocampus. Complex primate social environments have been argued to require rapid social learning, selective attention mechanisms that allow for sustained focus on socially and biologically relevant behaviors, and efficient memory storage, all functions mediated by the anterior hippocampus and amygdala (Byrne and Bates, 2007, 2010). Moreover, several authors have linked emotional behaviors, in particular, to the management of complex social systems and relationships (Byrne and Bates, 2007; Aureli and Schaffner, 2002; Stiller and Dunbar, 2007; Dunbar, 2009). At their core, social relationships among anthropoid primates, and especially humans (Hill et al., 2011), are hypothesized to be uniquely cognitively challenging in that they frequently involve relationships between non-kin that appear as abstracted versions of kin-based relationships in other mammals (Dunbar, 2009; Kummer, 1967). Aureli and Schaffner (2002) have suggested that, in complex social systems, social emotions function to facilitate "social bookkeeping". Emotions are hypothesized to provide a rapid means of assessing relationships with both related and unrelated individuals because they can intrinsically summarize the frequency and quality of past interactions with conspecifics. What is particularly striking, and perhaps somewhat counterintuitive, is the degree to which anxiety appears to play a role in assessments of relationship quality (Aureli and Schaffner, 2002). As modulators of

endocrine responses to external stimuli, the hippocampus and amygdala are intimately tied to anxiety and may subserve rapid emotional assessment, at the same time processing complex information from the neocortices. A possible adaptive explanation for the emphasis on the amygdala and hippocampus in human evolution is an increased need to attend to the behavior of and effectively modulate emotional responses to large numbers of unrelated conspecifics, while at the same time rapidly recruiting of memories of past contexts in order to select "appropriate" behaviors in complex social environments that provide myriad possible behavioral choices.

As mentioned in the introduction, some evidence links emotion and emotional modulation to human social adaptations (Hare, 2007). The fact that we found distinctions in limbic structures only in comparisons of humans and great apes indicates that changes in emotional processing may be a recently evolved, human adaptation. Human infants exhibit heightened anxiety responses to novel objects when compared with adult great apes, which has been used to argue that humans have evolved a distinct temperamental profile (Herrmann et al., 2011). A marker of advanced human social cognition (Herrmann et al., 2007; Byrne and Bates, 2007; Hare, 2011), complex theory of mind develops earliest in children who exhibit shy, withdrawn temperaments and perceptual sensitivity to social cues, but is inversely related to aggression (Wellman et al., 2011). It has also been hypothesized that human emotional behavior has changed over the course of evolution to emphasize increased perceptual and emotional sensitivity to conspecifics which allowed for human-specific forms of social cooperation and problem solving, given that the best evidence for chimpanzee theory of mind has been obtained only in competitive contexts (Hare, 2007). Literature addressing the value of emotion to human

cognitive evolution is burgeoning. If these findings hold through further experimentation, it would provide more convergent evidence linking emotion, cognition, and human brain evolution.

It will be difficult to disentangle the possible evolutionary (or environmental) sources of human hippocampal increase until several gaps in the literature are addressed. Evolutionary investigations of the functional subcomponents of the hippocampus could provide insight into the potential adaptive pressures influencing hippocampal expansion by assessing whether differential expansion is occurring in the anterior hippocampus associated with emotional modulation or the posterior hippocampus associated with topographic knowledge. Alternatively, both regions could evolve in a coordinated fashion. Further, investigations of socioemotional behavior are largely limited by the paucity of evidence assessing emotional and temperamental distinctions in primates. Recently, increased attention has been given to the task of operationalizing emotional variables for comparative analysis, which may provide additional means for assessing the relevance of emotion to hippocampal and amygdala expansion in human and nonhuman primate evolution (Dunbar and Shultz, 2010; Herrmann et al., 2011; Clarke and Boinski, 1995).

Conclusion

Overall our findings largely support hypotheses of amygdala evolution that highlight the importance of functional networks within the brain and point to the importance of interconnected networks that may be influenced in a “mosaic” fashion characteristic of evolutionary reorganization. We found that variation in limbic structures is present among hominoids and that parts of the human limbic system might prove to be

highly specialized. These factors reinforce the idea that human emotional processes are not primitive relics of our evolutionary past but instead are highly evolved systems that are not superseded by but, rather, compliment higher order cognitive processes. Moreover, the associations between temporal lobe and amygdala expansion potentially point to the importance of social information processing in recent human brain evolution.

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