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Comparative Analyses of the Neuron Numbers and Volumes of the Amgydaloid Complex in Old and New World Primates

A Dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Neurosciences

by

Cayenne Nikoosh Carlo

Committee in charge:

Professor Charles Stevens, Chair
Professor Andrea Chiba, Co-Chair
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Professor Katerina Semendeferi
Professor Lisa Stefanacci

2008

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2008

For my mother who has lead by example, encouraged, and supported me throughout all my academic pursuits.

We must walk consciously only part way

toward our goals, and then

leap in the dark to our success.

Henry David Thoreau, Journal, March 11, 1859

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LIST OF ABBREVIATIONS

AC	amygdaloid complex
V1	primary visual cortex
BNM	nucleus basalis of Meynert
AChE	aceytlecholinesterase
mc	magnocellular
pc	parvicellular
BLD	basolateral division of the amygdaloid complex
SLS	standard least-squares
NHP	non-human primate
CRF	corticotrophin-releasing factor
EEG	electroencephalography
SI	substantia innominata
AAA	anterior amygdaloid area
AB	accessory basal nucleus
ABmc	accessory basal nucleus, magnocellular subdivision
ABpc	accessory basal nucleus, parvicellular subdivision
AHA	amygdalohippocampal area
B	basal nucleus
Bi	basal nucleus, intermediate subdivision
Bmc	basal nucleus, magnocellular subdivision
Bpc	basal nucleus, parvicellular subdivision

CE	central nucleus
CEl	central nucleus, lateral subdivision
CEm	central nucleus, medial subdivision
COa	anterior cortical nucleus
COp	posterior cortical nucleus
I	intercalated nucleus
L	lateral nucleus
Ld	lateral nucleus, dorsal subdivision
Ldi	lateral nucleus, dorsal intermediate subdivision
Lv	lateral nucleus, ventral subdivision
Lvi	lateral nucleus, ventral intermediate subdivision
ME	medial nucleus
NLOT	nucleus of the lateral olfactory tract
PAC	periamygdaloid cortex
PL	paralaminar nucleus

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This dissertation, in full, is in preparation for submission to the Journal of Comparative Neurology. The dissertation author is the primary author and the secondary author is the dissertation advisor. The other two authors, Lisa Stefanacci and Katerina Semendeferi, are members of the dissertation author's committee.

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ABSTRACT OF THE DISSERTATION

Comparative Analyses of the Neuron Numbers and Volumes of the Amgydaloid Complex in Old and New World Primates

by

Cayenne Nikoosh Carlo

Doctor of Philosophy in Neurosciences

University of California, San Diego, 2008

Professor Charles Stevens, Chair
Professor Andrea Chiba, Co-Chair

The goals of this dissertation are to identify if neuroanatomical characteristics of the amygdaloid complex (AC) – nuclei volumes, cell numbers, and cell densities – vary across non-human primate (NHP) species and to consider which AC regions might influence the development of social and emotional behavior. NHP comparisons

at the level of individual amygdaloid nuclei have not been carried out in non-ape species.

Chapter I summarizes what we currently know about the structure of the AC in NHP and reviews the functions of individual nuclei. Thirteen Old and New World primates, representing five species, were analyzed using standard stereological methods for nuclei volume and neuron number in 26 AC subdivisions. Chapter II explains the methods used to evaluated volumes and measure cell numbers. This paper focuses specifically on four AC nuclei (accessory basal, basal, central, and lateral).

Chapter III presents my findings, which are three-fold: 1) the central nucleus volume is negatively allometric with the total amygdala volume while the accessory basal, basal, and lateral nuclei are isometric or changing at the same rate as the whole AC; 2) the central nucleus neuron numbers are increasing at a slower rate than the neurons in the three nuclei of the basolateral complex; 3) neuron density remains consistent across all nuclei evaluated. My data indicate that the central nucleus may have evolved differently from the three other amygdala nuclei I have evaluated. The isometric relationship between the neuron numbers and volume of the nuclei of the basolateral complex and the AC suggest that these nuclei evolved together. Chapter IV discusses some evolutionary interpretations of the data and speculates upon what these changes in AC structure could have on social behavior.

CHAPTER I

STRUCTURE AND FUNCTION OF THE AMYGDALOID COMPLEX

Affective disorders, conditions that manifest alterations of mood and emotion, afflict a large portion of the population. There is a 16.6% lifetime prevalence estimate for major depressive disorder alone (Kessler et al., 2005), and there are many other disorders that fall under the affective disorder umbrella. Such high occurrence rates for affective disorders put great economic and mental strain on primary caretakers, extended families and the general population. It is thus globally beneficial to understand the biological etiology of affective disorders. The neural circuits implicated in these disorders inevitably involve the limbic system (Papez, 1995), which is loosely defined as a variety of cortical and subcortical structures known to regulate our feelings and desires (LeDoux, 2000; Maclean, 1949; Maclean, 1952). Although the anatomical components of the limbic system differ somewhat from one definition of this system to the next, the amygdaloid complex is recognized to be a chief component.

The amygdaloid complex (AC) is a brain region that is critical for detection and interpretation of emotionally salient information. One of the most common functions attributed to this complex is the acquisition of the fear conditioned response, which is conserved across a wide variety of species (Antoniadis et al., 2007; LeDoux, 2000) making the amygdala an important region for possible species differences to be localized. Electrophysiological recording experiments from individual amygdala nuclei in Old World primates have isolated four main groups of functionally responsive neurons; the groups include neurons that respond to primary reinforcers of taste, visual stimuli (including those associated with primary reinforcement), novel stimuli, and faces (Rolls, 2000). Lesions of the adult, rhesus macaque amygdala have

been shown to increase affiliative behavior (Emery et al., 2001), reduce inhibition to novel stimuli (Mason et al., 2006), block the acquisition of fear-potentiated startle (Antoniadis et al., 2007), limit the use of species-typical aggressive behaviors (Bauman et al., 2006), induce lack of facial expression, and alter emotional responsivity (Stefanacci et al., 2003). In summary, the amygdala is in a position to integrate massive amounts of incoming sensory information and to subsequently alter important cognitive cortical and automatic subcortical systems. It follows that alterations in cytoarchitecture of the amygdaloid complex could contribute to the development of some human psychopathologies.

The human amygdala is active during tasks that require theory of mind (Stone et al., 2003), face and gaze processing, and interpreting emotional faces (Adolphs, 2002; Haxby et al., 2002; Vuilleumier and Pourtois, 2007). Amygdala volumes in humans, measured via magnetic resonance imaging, have been reported for a variety of disorders including, but not limited to: autism, Alzheimer's disease, major depressive disorder, schizophrenia (nonviolent and violent), antisocial personality disorder and bipolar disorder (Barkataki et al., 2006; Basso et al., 2006; Kalus et al., 2005; Palmen et al., 2006; Sheline et al., 1998; Teipel et al., 2006; Zetzsche et al., 2006). The findings of these studies demonstrate considerable variability in the size of the amygdala in both controls and patient populations (Pruessner et al., 2000). In addition, total amygdala volumes were not significantly different in any of the above disorders compared to controls. It is possible that such gross comparisons of the amygdala overlook the functional differences of the individual nuclei that make up the complex.

There are only a small number of studies reporting amygdala volume data in non-human primates (Barger et al., 2007; Emery et al., 2001; Stefanacci et al., 2003; Stephan and Andy, 1977; Stephan et al., 1981; Stephan et al., 1987). Stephan et al. (1987) completed an extensive quantitative comparison of amygdaloid complex volumes in insectivores and primates that was focused on breadth of species (see Stephan et al. 1977 and 1981). He explored the validity of an observation made by Crosby and Humphrey (1944) that the basolateral nuclei group (lateral, basal, accessory basal, and cortical nuclei) occupies a proportionally larger area of the complex in man compared to the shrew. Stephan and Andy (1997) found this to be true but also observed that the shrew had a larger centromedial nuclei group (central, medial, anterior hippocampal area, intercalated, and the nucleus of the lateral olfactory tract), specifically a larger medial nucleus (ME) and nucleus of the lateral olfactory tract (NLOT), than the human. Stephan (1977) determined that the amygdaloid complex, using the allometric method of comparison, increases from smallest to largest in insectivores, prosimians, and simians (Stephan and Andy, 1977). The allometric method is a form of nonlinear regression, such that the log of brain weight and log of body weight are inserted into the standard equation for a line (Moore, 2000; Smith, 1984). Often the parameter used in allometry is basal metabolic rate for each species.

Stephan uses volume (size) as preliminary evaluation of a structure's importance in terms of function for each species. In human patient populations there does not seem to be a significant amygdaloid complex volume difference between normal controls and the schizophrenic, major depressive disorder, bipolar disorder or

autism populations (Bielau et al., 2005; Bowley et al., 2002; Chance et al., 2002; Schumann and Amaral, 2006). Measurements of volume alone do not exclude changes in cell number, layers, or connections, and for this reason I am investigating the absolute number of cells in each nucleus, not groups of nuclei.

Schumann et al. (2006) reported that while the amygdaloid complex and five of its subdivisions have consistent volumes in their autism spectrum disorder cohort (n = 9) when compared to controls (n = 10), there does exist a difference in the number of cells within some of the amygdala nuclei. This finding lends support to the idea that the distinct functional connectivity of each of the amygdala nuclei could be important for determining structural changes. I have evaluated four anatomically distinct nuclei for volume and cell number. While several studies indicate that volume does influence function (Barton, 1996; Joffe and Dunbar, 1997), it is unknown whether it is the connections made between neurons or an increase in neurons themselves that leads to increased neuronal processing power. To this end I have employed stereological counting techniques to determine absolute numbers of cells in each nucleus and provide insight into the question of how cortical processing in the amygdala changes among species. While I would like to put my neuroanatomical data into a functional framework, in the present study I will be providing the neuroanatomical data that is necessary to, in the future, make socioecological links across species. This dissertation greatly expands the scope of existing volume data and is the first study evaluating major individual nuclei of the amygdaloid complex in Old and New World primate species.

Brief Overview of the Amygdala: Intrinsic/Extrinsic Connections and Function.

The amygdala has three major functions that allow individuals to evaluate incoming, emotionally salient information. The amygdala inhibits our approach to novel stimuli, causes us to evaluate whether or not the novel stimulus is threatening and then activates our escape mechanisms, if need be. In addition, the amygdala is critical to our ability to function in social groups and consequently for survival. It is not surprising that such an important structure is present across the mammalian species.

Much work has been carried out to determine the extrinsic and intrinsic connections of the amygdala in non-human primates. Figure 1.1 summarizes the general flow of information through the amygdala from a common sensory input, the lateral nucleus, to the common sub-cortical output, the central nucleus, then onto downstream autonomic areas and the basal forebrain (Price, 2003). Massive cholinergic connections are sent from the nucleus basalis of Meynert to the rest of the cortex.

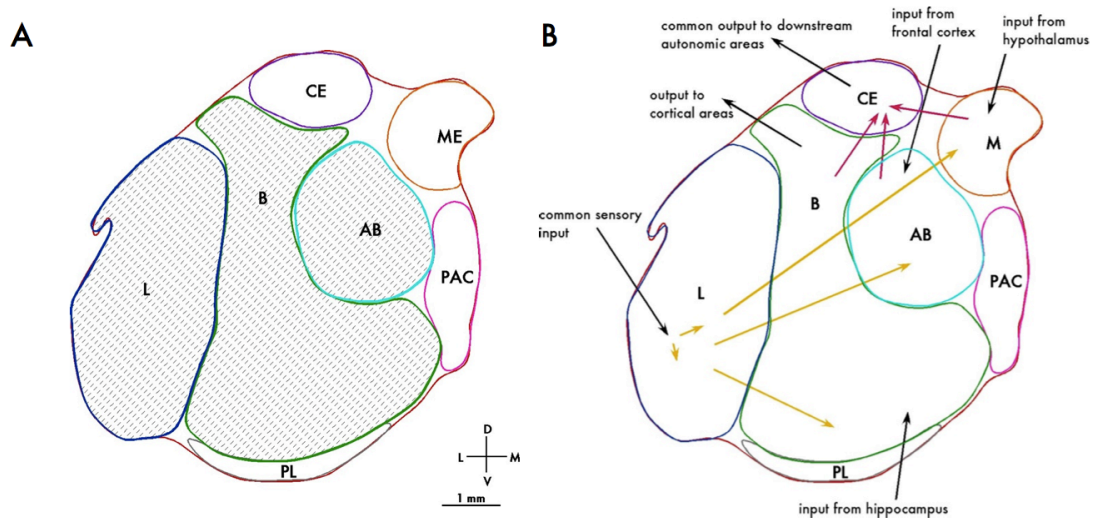


Figure 1.1 Amygdaloid complex and connections in nonhuman primates.
(A) Midrostrocaudal section showing major nuclei, filled areas denote the basolateral complex. (B) A summary of how information generally flows through the amgydaloid complex (adapted from Price and Amaral 1992). Abbreviations: AB, accessory basal nucleus; B, basal nucleus; CE, central nucleus; L, lateral nucleus; ME, medial nucleus; PAC, periamygdaloid cortex; PL, perilaminar cortex; orientation D, dorsal; V, ventral; L, lateral; M, medial.

Closer examination of the amygdala reveals that the connectivity with cortical and subcortical areas is extensive. Incoming information includes the following (Price, 2003): 1) primary olfactory information to the medial, cortical, and periamygdaloid cortex nuclei; 2) visual information to the dorsal subdivision of the lateral nucleus; 3) visceral information to the (dorsal) subdivision of the lateral nucleus; 4) posterior thalamic information to the dorsal intermediate subdivision of the lateral nucleus; 5) association sensory cortex information to the ventral and ventral intermediate subdivisions of the lateral nucleus. In turn, some of the amygdala nuclei themselves project out of the amygdala and these terminate in the following locations: 1) periaqueductal gray receives information from the central and basal nuclei; 2) the medial prefrontal cortex receives projections from the lateral, basal, and accessory

basal nuclei; and 3) the hypothalamus receives information from the central, accessory basal, and medial nuclei.

Brief Overview of Social Influences on Brain Region Volume.

Anatomical changes can be influenced by changes in function, evolution, and social behavior. It is important to consider the interactions that each species makes with their dominant and subordinate group members and how these social interactions differ when comparisons are made between species and across primate taxa, e.g. Old vs. New World primates. Social group size is thought to reflect the cognitive processing abilities of its members (Barton, 1996). Cognitive processing capabilities essentially put a ceiling on the size of the group that can be maintained as stable, coherent, functional units (Joffe and Dunbar, 1997; Kudo and Dunbar, 2001). Progression above this ceiling results in fission and a reduction of group size to a maintainable level. Additional support for the theory that volume is indicative of increased functional processing power comes from studies of the primate visual system.

The neocortex consists of about 50% visual areas and Barton (1996) found a correlation between the lateral geniculate nucleus' (LGN) parvocellular layer volume, cell number, and neocortex size (Barton, 1996). These parvocellular neurons are probably specialized for fine detail and/or color and lend support for the idea that visual areas have a role in social intelligence or the development of the social brain. Joffe and Dunbar (1997) explored this idea by correlating social group size with the

size of the whole brain, neocortex, primary visual cortex (V1), LGN, and amygdala across a number of non-human primate species. All of the volumes were obtained from Stephan's 1981 publication and were used to isolate the contribution of visual cortex to social group size. They found there to be a correlation between social group size, neocortex volume, and visual cortex volume. This supports the hypothesis that highly detailed visual information allows for greater discrimination of socio-visual interactions and thus changes in social behavior across species (Joffe and Dunbar, 1997).

Another critical finding was that total amygdala, corticobasolateral, and centromedial nuclei group volumes were not correlated with social group size. This finding clearly needs to be readdressed with an investigation of the individual nuclei volumes. It is probable that social behavior influences the volume of the neocortex. The functional differences associated with volume can be explored through the changes in the components that make up the neocortex. It follows that the data presented in this dissertation will provide, in part, the necessary measurements for future analyses considering social characteristics as variables or probable reasons why there are similarities or differences in amygdala volume or cell numbers across non-human primate species.

This anatomical study focuses on the amygdaloid complex and four of its subnuclei in the following species of Old and New World primates: *Macaca mulatta* (rhesus macaque), *Macaca nemestrina* (pig-tailed macaque), *Macaca fasicularis* (crab-eating macaque), *Callithrix jacchus* (white-ear-tufted marmoset), and *Cebus apella* (brown capuchin). My goals are to evaluate the morphology, neuron number

and volume, of the amygdala nuclei in order to determine what neuroanatomical aspects of the amygdaloid complex differ across non-human primate species, and to compare these data with those from the human brain. These findings will provide an important structural baseline and framework for the development of animal models with affective spectrum disorders and an evolutionary perspective on how the amygdaloid complex may contribute to changes in social cognition.

CHAPTER II

IDENTIFICATION OF AMYGDALA NUCLEI AND MEASUREMENT OF VOLUMES AND CELL NUMBERS

Histological Procedures

Brain tissue from 13 Old and New World monkey specimens were analyzed. Ten of these specimens were from the collection of Lisa Stefanacci, and were perfused with aldehyde fixatives and stored long-term in 10% formalin. In preparation for cutting, brains were submerged in solutions of 10% glycerol and 10% formalin until the brain sinks and then moved into 20% glycerol and 10% formalin until the brain sinks again—average time in each solution was 3-10 days. These cryoprotected brains were then cut coronally on a freezing microtome at a thickness of 40 or 50 μm. Every sixth or twelfth section was stained with thionin for visualization of Nissl bodies. In all available stored-sectioned brains, adjacent sections were stained for aceytlecholinesterase (AChE). See reference for staining protocol (Filipe and Lake, 1990). Three coronal series from adult marmosets were borrowed from Jon Kaas, Vanderbilt University. These specimens where perfused with aldehyde fixatives, cut at 50 μm on a freezing microtome (CJ5 cut at 32 μm) and stained for Nissl and AChE (no AChE sections available for CJ1) in Jon Kaas' lab at Vanderbilt University. Specific characteristics on all specimens are detailed in Table 2.1.

Table 2.1 Characteristics of the 13 specimens analyzed

TAXA		SPECIES	COMMON NAME	MONKEY	SEX	BODY MASS (kg)	Cut Section Thickness (μm)	Evaluated Series	Number of Amygdala Sections
Old World	CERCOPITHECIDAE	*M. fascicularis* (MF)	Crab-eating Macaque	MF1	M	2.4	40	1/12	10
				MF3	M	3.5*	40	1/12	10
				Mean MF		**2.95 ± 0.78**	**40 ± 0**	**–**	**10 ± 0**
		M. mulatta (MM)	Rhesus Macaque	MM1	F	9.35	40	1/12	14
				MM2	F	8.6	40	1/12	15
				MM3	M	11.89	40	1/12	15
				MM4	M	7.3	40	1/12	13
				Mean MM		**9.29 ± 1.93**	**40 ± 0**	**–**	**14 ± 1**
		M. nemestrina (MN)	Pig-tailed Macaque	MN1	M	8.1	50	1/10	16
				MN2	M	6	50	1/15	10
				Mean MN		**7.05 ± 1.48**	**50 ± 0**	**–**	**13 ± 4**
				Mean OW		**2.91 ± 7.14**	**42.5 ± 4.6**	**–**	**13 ± 2.5**
New World	CEBIDAE	*C. jacchus* (CJ)	White-ear-tufted Marmoset	CJ1	M	0.25*	50	1/4	15
				CJ2	U	0.25*	50	1/4	14
				CJ5	M	0.25	32	1/12	9
				Mean CJ		**0.25 ± 0**	**44 ± 10**	**–**	**13 ± 3**
		C. apella (CA)	Brown Capuchin	CA1	M	3.7*	50	1/6	12
				CA2	M	3.7*	50	1/6	22
				Mean CA		**3.7 ± 0**	**50 ± 0**	**–**	**17 ± 7**
				Mean NW		**1.63 ± 1.89**	**46.4 ± 8**	**–**	**14.4 ± 4.8**

*Average Body Mass from R.T. Smith, 1997

Data Collection

Left hemispheres were analyzed at ~0.5 mm intervals through the rostrocaudal extent of the amygdaloid complex. Nissl-stained digital images were acquired with a Leica MZ6 stereomicroscope (0.63 objective) and a SPOT Insight Color 3.2.0 digital camera system connected to a G4 Mac. Images were sharpened, adjusted for contrast and brightness in Photoshop7 and imported into CanvasX. Borders of the entire complex and the following nuclei were outlined in CanvasX on the Nissl-stained

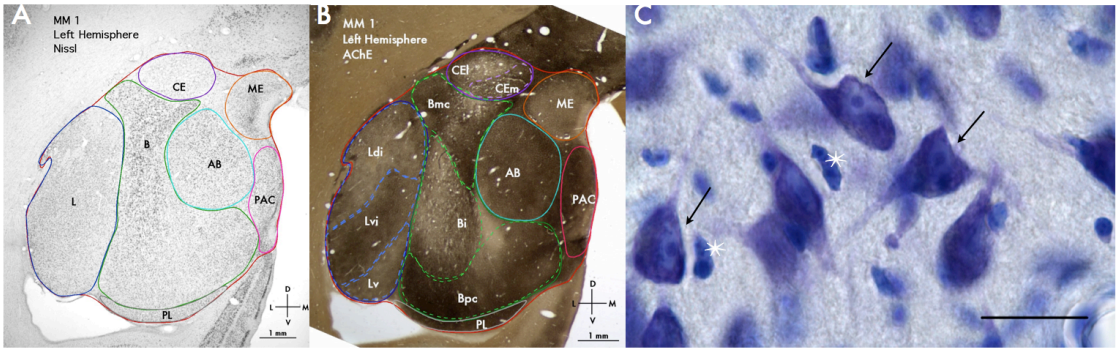


Figure 2.1. Nissl (A) and AChE (B) stained sections used to delineate amygdaloid complex nuclei and subdivisions. Example of neurons and glia (C) in the anterior hippocampal area at 100x with oil. Neurons (arrows) were identified by the presence of a nucleolus. Glial cells (stars) are much smaller and often have several darkly stained nuclei. Scale bar is 25 microns. All images are from a rhesus macaque and abbreviations are the same as in Figure 1.

images (Figure 2.1A), confirmed with the AChE-stained images (Figure 2.1B), and compiled to create a reference atlas for each specimen: anterior amygdaloid area, accessory basal nucleus, amygdaloid hippocampal area, basal nucleus, central nucleus, anterior and posterior cortical nuclei, intercalated nuclei, lateral nucleus, medial nucleus, periamygdaloid cortex, and paralaminar nucleus.

The data collection system consisted of an Olympus BX51 microscope with a Heidenhain motorized stage and Olympus U-CMAD3 digital camera connected to a Dell Optiplex G280 computer. AC borders and nuclei were redrawn, based on the

reference atlas, in Neurolucida v.7.52 using a WACOM Cintiq 214q tablet at low magnification, 2X objective. Nuclei subdivisions were delineated using 10x and 40x objectives on Nissl-stained sections and included the following regions: magnocellular and parvocellular divisions of the accessory basal nucleus; intermediate, magnocellular, and parvocellular divisions of the basal nucleus; lateral and medial divisions of the central nucleus; and dorsal, dorsal intermediate, ventral and ventral intermediate divisions of the lateral nucleus.

Amygdala neurons and glia were counted using Neurolucida software on available Nissl-stained sections at 100x with oil in approximately 10-15 sections per case (see Figure 2.1C). I employed a standard stereological sampling scheme called the optical fractionator method and an optical disector to mark neurons and glial cells. Neurons were identified by the presence of a nucleolus. This technique is the most commonly used unbiased estimation method partly because tissue shrinkage should not affect the estimates. The optical factionator technique is performed by counting nucleoli in a known fraction of your entire region of interest. Each part of the whole complex has an equal chance of being sampled. Optical disectors are randomly place on a series of sections and used to count the number of particles (nucleoli), which come into focus and fall within the acceptance lines of the disector (Howard and Reed, 1998; West and Gundersen, 1990; West et al., 1991). I used a 50 μm disector frame area in the Old World monkeys and the New World capuchin. The much smaller New World marmoset had a 35 μm counting frame. On average there were between two and seven disector counting frames per subdivision in each section of every case. Approximately 200 objects of interest were counted in each 3D region of interest

(Howard and Reed, 1998). Tissue thickness was measured twice in each nucleus on each section.

Guard zones are regions in the z-axis that form the upper and lower boundaries for the counting guard box, i.e. neurons or glial cells falling within these zones are not counted. I did not employ guard zones, opting instead to make the guard box extend through the entire z-axis of the tissue. Use of guard zones is a much-debated topic and several papers have compared the different embedding methods for their affects on differential distribution of nucleoli (Harding et al., 1994; West et al., 1991). Celloidin embedded- and cryo-sections are the only section types that have an even distribution of nucleoli in the z-axis (Gardella et al., 2003). Paraffin, glycolmethacrylate, and vibratome sections all demonstrated a symmetric inverse bell curve distribution of nucleoli (Gardella et al., 2003; Hatton and von Bartheld, 1999). I chose not to have guard zones because it reduces the potential bias to <1% (Gardella et al., 2003) and I did not see any split profiles in my initial stages of counting nor did I count in a brain region with small neurons that are subject to greater effects of compression in the z-axis (Bonthius et al., 2004). A recent paper by Berretta et al. (2007) confirmed my initial ideas on guard zones when they evaluated frozen sections from the human amygdala and systematically counted nucleoli with the use of 10 μm guard zones and without guard zones to find there to be no difference between the counts (Berretta et al., 2007).

Delineation of the Amygdaloid Complex and Nuclei

The amygdaloid complex is located in the medial temporal lobe, medial and anterior to the hippocampus. It consists of 13 nuclei, five of which can be parsed into discrete subdivisions. The medial border is defined by the periamygdaloid cortex (PAC) rostrally and the posterior cortical nucleus (COp) and amygdalohippocampal area (AHA) more caudally. The lateral border is the lateral nucleus (L) throughout most of the rostrocaudal extent of the amygdala. At more caudal levels portions of the central nucleus (CE), the COp, and the AHA form the lateral border. The paralaminar nucleus (PL) cups the more dorsal nuclei and is bounded ventrally by the subamygdaloid white matter. At more rostral levels the L, anterior amygdaloid area (AAA), and the anterior cortical nucleus (COa) form the dorsal border. In total, data was collected from 12 amygdaloid complex nuclei and their subdivisions. I will focus the boundary delineations, results, and following discussion on a subset of four major amygdala nuclei. See Tables 3.3 and 3.4 for complete data summaries for all amygdala nuclei.

At the mid-rostrocaudal level, the basal forebrain, identified by large, darkly stained cholinergic cells with low packing density, forms a more clearly defined border. Caudally, the CE and COp outline the dorsal border. These outlines are based upon knowledge of the rhesus macaque amygdala (Amaral and Bassett, 1989; Price et al., 1987) and the human amygdala (Schumann and Amaral, 2005). In the marmoset, capuchin, and pig-tailed macaque, these definitions were sufficient to create complete outlines of the amygdala (see Figure 2.2, 2.3, and 2.4). Where applicable, I will identify differences among these species. Recent articles from Schumann et al. 2005

and Barger et al. 2007 detail the amygdala complex outer borders and basolateral complex borders in the human and 5 species of great apes. I used similar parameters and will briefly review the anatomical boundaries used to define each nucleus discussed in this paper.

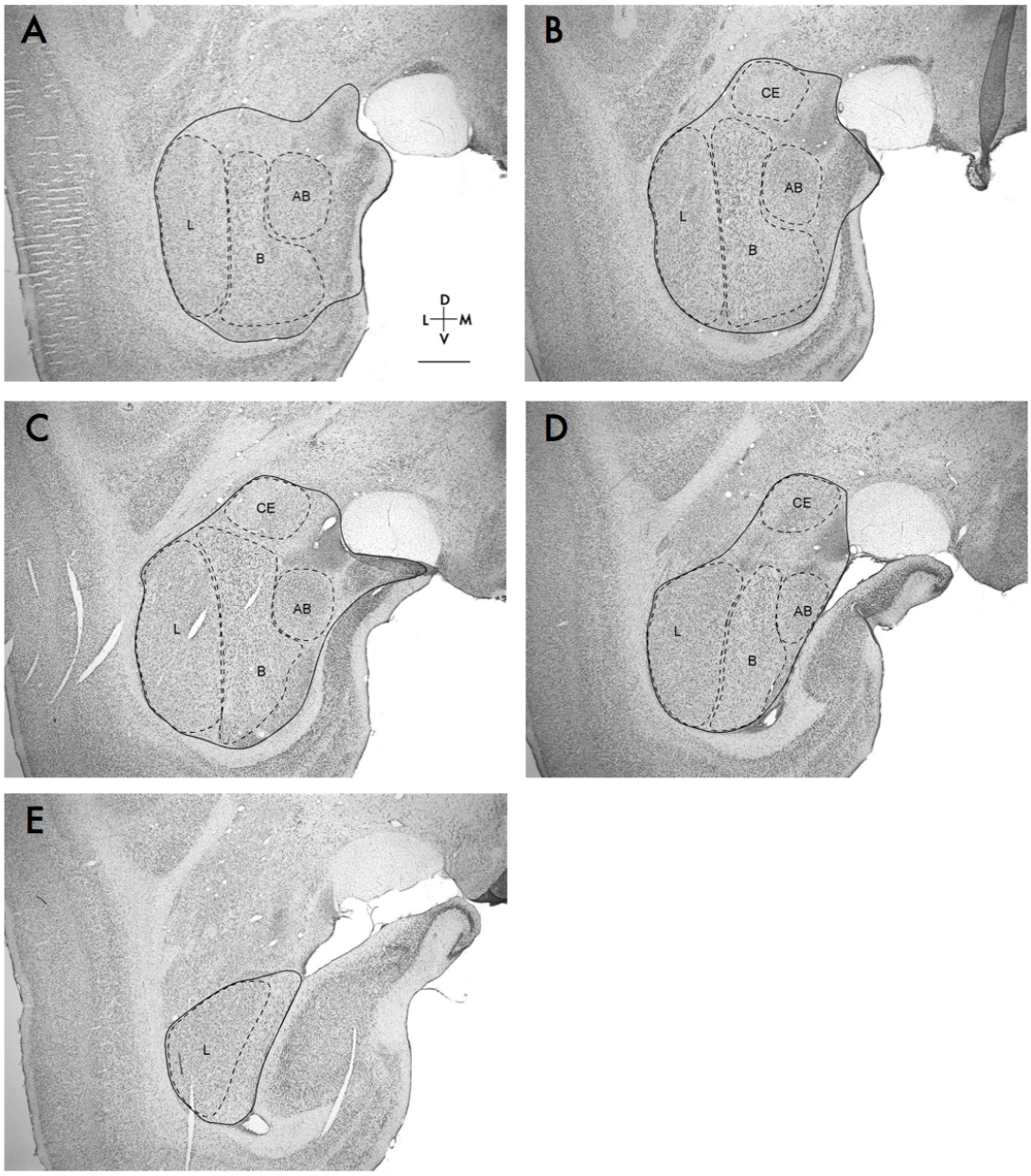


Figure 2.2 Coronal, Nissl-stained sections of the amygdaloid complex in the New World marmoset monkey. Sections begin at the (A) rostral level, continue to the (B-C) mid-rostrocaudal, and conclude with the (D-E) caudal level. Panel (A): Scale bar = 1 mm; Orientation D, dorsal; V, ventral; L, lateral; M, medial. Abbreviations: AB, accessory basal nucleus; B, basal nucleus; CE, central nucleus; L, lateral nucleus.

Accessory Basal Nucleus

The accessory basal nucleus is the most medial nucleus in the basolateral complex and it has two subdivisions: The magnocellular (mc) portion is dorsal to the

parvocellular (pc) division. The former is present at midrostrocaudal levels, while the pc is present through most of the amygdala. The mc is easily distinguished by large, darkly, stained Nissl cells that also stain heavily for AChE. (Amaral et al., 1992)

Basal Nucleus

Lateral to the accessory basal nucleus (AB) is the much larger basal nucleus that extends throughout the entire rostrocaudal amygdala. The most noticeable cells in the basal nucleus (B) are the magnocellular cells (Bmc) that emerge around the time the medial nucleus (ME) and the central nucleus appear. These Bmc cells stain darkly for Nissl bodies and AChE and are often referred to as the dogleg of the basal nucleus. Bmc runs perpendicular to the rest of the vertically oriented basal nucleus and forms the dorsal border of the lateral nucleus. The lateral nucleus forms the lateral boundary of the B. The PL forms the ventral border of the basal nucleus. The anterior amygdaloid area, central nucleus, and lastly the cortical nucleus sit dorsal to the basal nucleus as you move in an anterior to posterior direction through the AC. There are two additional subdivisions within the basal nucleus: the intermediate division (Bi) and the parvocellular division (Bpc). Sandwiched between the Bmc and Bpc is the Bi. It has "intermediate" sized neurons and packing density and staining for Nissl bodies and AChE compared to Bmc and Bpc. Bpc is the largest basal subdivision and it extends throughout this nucleus. Bpc contains the smallest neurons, which have a low packing density and AChE staining. (Amaral and Bassett, 1989) Its shoe-shaped outline butts up against the periamgydaloid cortex (PAC) and is separated by the intermediate fiber bundle from the AB (Amaral et al., 1992).

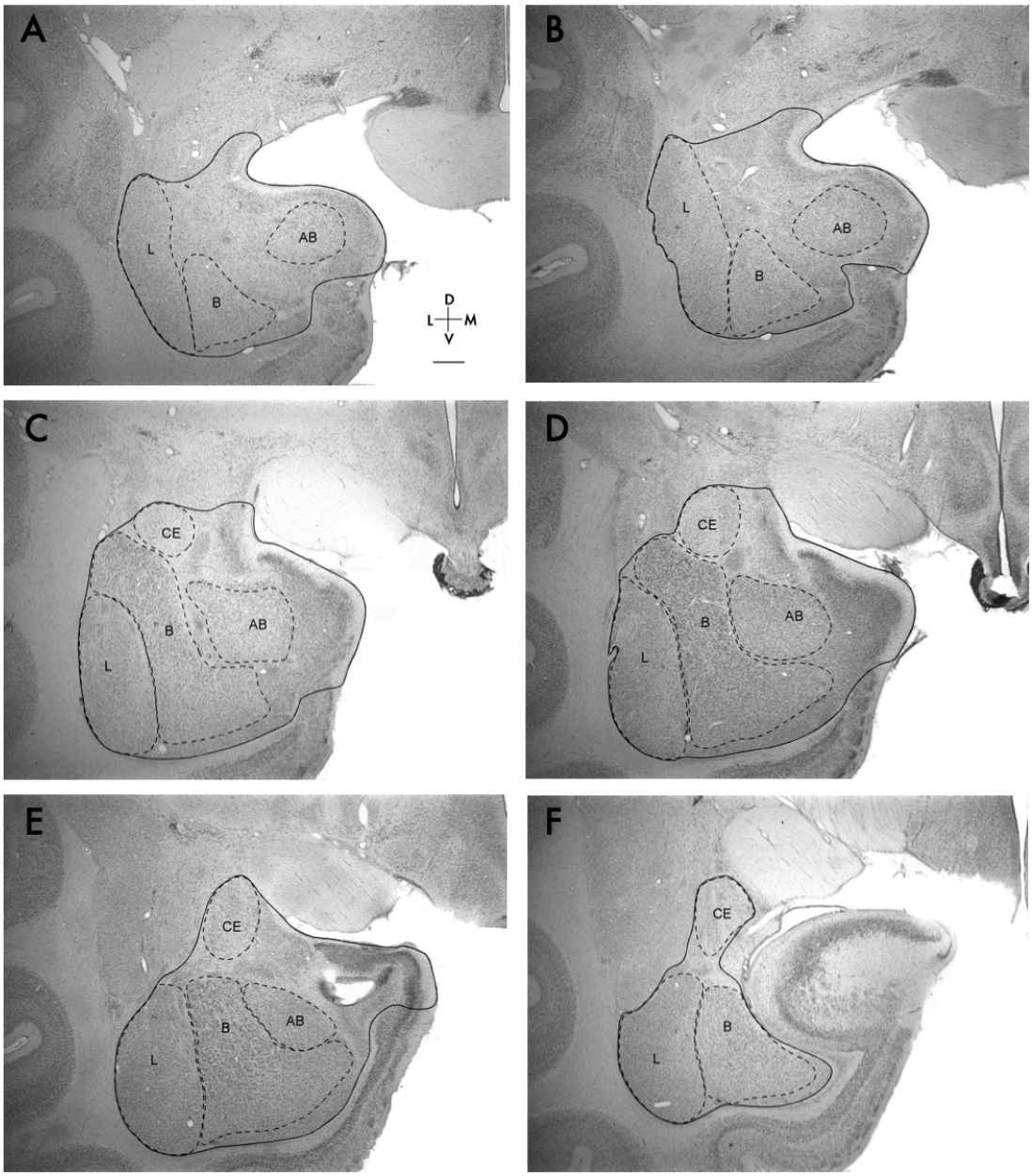


Figure 2.3 Coronal, Nissl-stained sections of the amygdaloid complex in the New World capuchin monkey. Sections begin at the rostral level (A-B), continue to the mid-rostrocaudal level (C-D), and conclude with the caudal level (E-F). See Figure 2.2 for abbreviations. Orientation and scale bar in (A) apply to (B-F).

Central Nucleus

The central nucleus (CE) appears with the 'dogleg' of the basal nucleus (Bmc subdivision) in the caudal third of the AC, and it continues almost until the AC

terminates. Most rostrally, the CE is medial to the external capsule and in more caudal sections it is medial to the putamen. Fiber bundles that are components of the stria terminalis surround the CE and these fibers also separate its medial (CEm) and lateral (CEl) subdivisions (Amaral et al., 1992). In Nissl stained sections, CEl has darker and more densely packed cells, in contrast to the small, pale cells of the CEm. The opposite is true in AChE stained sections; CEm is heavily stained for AChE (in particular the dorsal portions of CEm), while the CEl shows only light enzyme levels (Amaral and Bassett, 1989; Amaral et al., 1992; Pitkanen and Amaral, 1998). In the marmoset, CE shows up as soon as Bmc appears and COa ends. This is in contrast to the other species, when CE appears after the full dogleg of the Bmc is visible (see Figure 2.2B).

Lateral Nucleus

Forming the lateral AC border is the lateral nucleus (L). Immediately lateral to the basal nucleus, the L can be subdivided into four areas including the following: dorsal subdivision (Ld), dorsal intermediate subdivision (Ldi), ventral subdivision (Lv), and ventral intermediate subdivision (Lvi). The dorsal subdivision is most dorsal and located in the rostral portions of the AC with only light AChE staining and medium Nissl staining of its mostly pyramidal shaped cells (Pitkanen and Amaral, 1998). The Ldi appears almost at the same time as the Ld, but it later forms the dorsal border of the lateral nucleus after the Ld has disappeared. Like the Ld subdivision, Ldi also stains lightly for AChE and moderately for Nissl bodies. The biggest difference between the two subdivisions is the shape of the cells. The Ldi has a multitude of cell

shapes ranging from the more angular, like the pyramidal cells of the Ld, to rounded cells. Lv forms the ventral border of the L and runs the entire length of the L. This nucleus curves up and also forms the medial border of the L. It has a low density of neurons and it stains lightly for AChE compared to the more dorsal Lvi. Lvi often has a narrow oblique orientation and contains large, round neurons. It can horseshoe around the Ldi in the more rostral portions of the Lvi and can be differentiated from Ldi by a lower cell packing density and a heavy staining for AChE (Amaral and Bassett, 1989; Pitkanen and Amaral, 1998).

In the marmoset, the lateral nucleus extended much farther caudally then in the other species evaluated in the present study (see Figures 2.2E and 2.4E-F). L is bounded by B medially and the external capsule both laterally and along its dorsal border. As you move in a rostral to caudal direction, the dorsal border of the L consists of the following nuclei: the anterior amygdaloid area (AAA), the 'dogleg' of the basal nucleus (midrostrocaudal level), CE, followed by the caudate and putamen (Price et al., 1987). The ventral border is the lateral extension of the PL and subamygdaloid white matter. More caudally this area transitions to the lateral ventricle and the hippocampus.

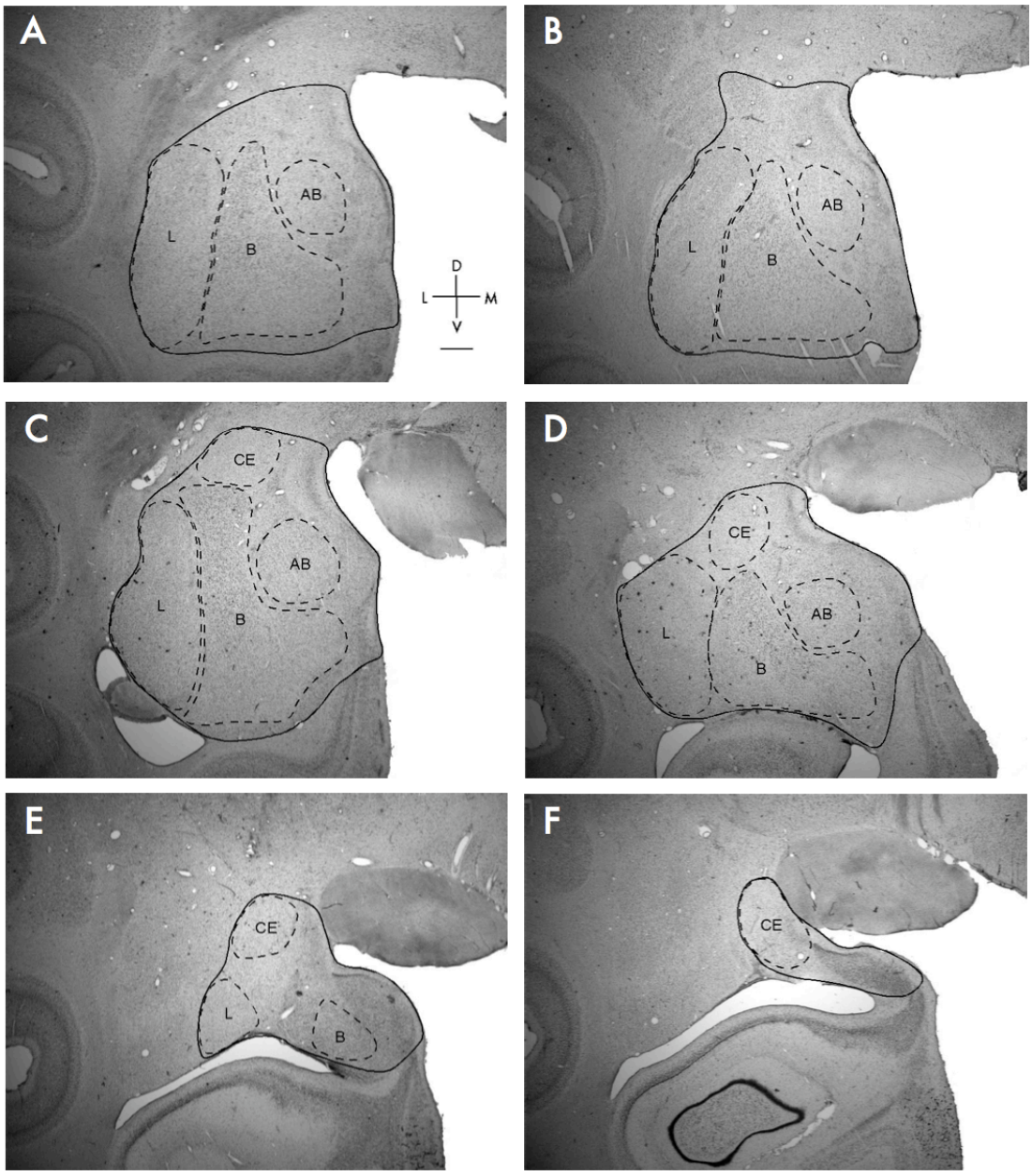


Figure 2.4 Coronal, Nissl-stained sections of the amygdaloid complex in the pig-tailed macaque an Old World monkey. Sections begin at the rostral level (A-B), continue to the mid-rostrocaudal level (C-D), and conclude with the caudal level (E-F). See Figure 2.2 for abbreviations. Orientation and scale bar in (A) apply to (B-F).

Cortical Nuclei, Intercalated Nuclei, Medial Nucleus, and Anterior Amygdaloid Area

Table 2.2 lists several additional nuclei volumes for comparison to a series of extensive volume papers by Stephan et al. (Stephan and Andy, 1977; Stephan et al.,

1981; Stephan et al., 1987) Even though these nuclei were not the focus of the present study, data was collected and they were identified according to the following parameters (Amaral et al., 1992; Pitkanen and Amaral, 1991; Pitkanen and Amaral, 1998): 1) Cortical nuclei (CO) – anterior nucleus (COa) located in the rostral half of the AC, has three layers with low levels of AChE staining. The cortical posterior nucleus (COp) is located in the caudal third of the AC and is bordered dorsally by ME and ventrally by the amygdalohippocampal area (AHA), has two layers, and stains darkly for AChE; 2) Intercalated nuclei (I) – are located between B and AB, or between B and L, or just below CE (Pitkanen and Amaral, 1991) or at the ventromedial tip of AB (Pitkanen and Amaral, 1998). These darkly Nissl stained pockets of cells have a high packing density and are often embedded amid white matter tracts and can have high levels of AChE staining; 3) Medial nucleus (ME) – located dorsal to COp at more caudal levels but dorsal to the nucleus of the lateral olfactory tract (NLOT) at rostral levels, has three layers, forms the dorsal border medially and is easily separated from the BNM by light AChE staining; 4) Anterior amygdaloid area (AAA) – located in the rostral portions of the AC and dorsal to Ld. AAA has small to medium-sized neurons that are larger and more homogeneous than its caudal extension CEm. Cells stain darkly for Nissl bodies and lightly to moderately for AChE. (Amaral and Bassett, 1989)

Table 2.2 Review of studies evaluating postmortem amygdala nuclei volume in non-human primates

		Total Amygdala[1]	Individual Nuclei Volume (mm^3)					Nuclei Group Volume (mm^3)			
STUDY	SPECIES	AMYG	L	B	AB	CE	REMAINING AMYG	L, B, AB, Co	LARGE-CELLED B[3]	SMALLER-CELLED L, B, AB, Co	AAA, CE, I, ME, NLOT[4]
OLD WORLD PRIMATES											
Present study	*Macaca fasicularis* (n = 2)	80.57	18.28	20.66	8.76	4.71	28.16	49.32	9.93	39.40	7.76
Stefanacci et al. 2003	*Macaca fasicularis* (n = 4)	234.10	47.40	57.30	27.70	14.70	87.00	–	–	–	–
Present study	*Macaca mulatta* (n = 4)	161.26	34.26	42.00	19.97	9.59	55.44	102.41	18.30	84.11	16.56
Emery et al. 2001	*Macaca mulatta* (n = 5)	277.98	57.15	64.53	37.89	15.30	103.11	–	–	–	–
Stephan et al. 1987[2]	*Macaca mulatta* (n = ?)	678.4	–	–	–	–	–	493.2	69.7	NA	185.2
Present study	*Macaca nemestrina* (n = 2)	260.61	53.91	73.81	18.99	9.33	104.57	155.21	29.54	125.67	18.97
NEW WORLD PRIMATES											
Present study	*Callithrix jacchus* (n = 3)	38.73	11.03	10.28	3.44	1.8	12.18	26.08	4.72	21.36	3.06
Stephan et al. 1981[2]	*Callithrix jacchus* (n = 3)	106.10	–	–	–	–	–	75.60	7.70	—	30.50
Stephan et al. 1977[2]	*Callithrix jacchus* (n = 1)	113.00	–	–	–	–	–	84.60	8.56	76.1	28.30
Present study	*Cebus apella* (n = 2)	210.08	45.5	58.1	19.49	8.17	78.82	130.94	24.96	105.99	15.70
Stephan et al. 1981[2]	*Cebus sp.* (n = 1)	502	–	–	–	–	–	384	41	—	118
Stephan et al. 1977[2]	*Cebus sp.* (n = 1)	535	–	–	–	–	–	404	43.1	361	131

AMYG = amygdala; L = lateral nuc.; B = basal nuc.; AB = accessory basal nuc.; CE = central nuc.; Remaining AMYG = [(L+B+AB+CE) - AMYG]; Co = cortical nuc.; AAA = anterior amygdaloid area; ME = medial nuc.

[1] left hemisphere only unless otherwise stated. [2] All numbers reflect bilateral volumes. [3] Present study used Bi and Bmc. [4] Present study did not evaluate NLOT

Nuclei Group Classification

There have been several systems for classifying AC nuclei into groups. In general, I refer to the basolateral division (BLD) as a group consisting of the lateral, basal, and accessory basal nuclei (see Figure 1.1A). The BLD functions together to "detect" stimuli and they are part of the following nuclei group classifications: the corticobasolateral nuclei that also includes the anterior and posterior cortical nuclei (Stephan and Andy, 1977); the deep nuclei that also includes the paralaminar nucleus (Pitkanen and Amaral, 1998); and the "dorsal" amygdala mostly referenced in fMRI studies where spatial resolution limits distinction of individual nuclei (Whalen et al., 2001). The central and medial nuclei function to increase "vigilance" based upon the cortical feedback that returns to the lateral nucleus. They fall into the following nuclei group classifications: the centromedial nuclei that includes the anterior hippocampal area, intercalated nuclei, and the nucleus of the lateral olfactory tract (Stephan and Andy, 1977); the superficial nuclei that include the anterior and posterior cortical nuclei, and periamygdaloid cortex but excludes the central nucleus altogether (Pitkanen and Amaral, 1998); and the "ventral" amygdala (Whalen et al., 2001).

Calculations and Statistical Analyses

The estimated number of cells in each region of interest was calculated by taking "total cells" counted, summed from all counting frames in a nucleus, and put over the combined area of the counting frames. I then multiplied this ratio by the total area of each nucleus to obtain the estimated numbers of neurons and glia. Tissue

shrinkage has been reported to be ~70% in mouse neocortex and hippocampus (Bonthius et al., 2004), ~72% rat cerebellum (Goodlett et al., 1998; Thomas et al., 1998), 53% in rat spinal cord (Messina et al., 2000), 77% in rhesus prefrontal cortex (Dombrowski et al., 2001), and 60% in human amygdala (Berretta et al., 2007). Like Harding et al. (1994), I assumed uniform tissue shrinkage and thus did not apply a correction factor to my final estimates (Harding et al., 1994). Calculations were made using the cut thickness (see Table 2.1).

Standard least-squares (SLS) regression analyses were performed on LOG base 10 transformed data in Prism5 statistical software (GraphPad Software Inc., San Diego, 2007). All regression residuals were analyzed for normality using the Shapiro-Wilks test statistic ($W < 0.05$, reject null hypothesis that the data follows a normal distribution). Nuclei comparisons were made, based upon test of normality, with either the parametric student's t-test or the nonparametric Kruskal-Wallis test statistic. Significant nonparametric group mean comparisons were subject to multiple comparisons test, i.e. Tukey-Kramer test of means, to identify which group was significantly different from the other groups. I have included existing data on amygdala nuclei volumes where applicable. All graphs containing such data are clearly identified in the legend.

CHAPTER III

EVIDENCE FOR DIFFERENTIAL EVOLUTION OF THE BASOLATERAL COMPLEX AND THE CENTRAL NUCLEUS

Amygdaloid Complex and Nuclei Volume

Among the Old World primates in the present study, the pig-tailed macaque had the largest amygdaloid complex volume (260.61 mm^3, n = 2) and the crab-eating macaque had the smallest complex (80.57 mm^3, n = 2). The rhesus macaque fell between these values with a total amygdala volume of 161.26 mm^3 (n = 4, Table 3.1). The New World marmoset had the smallest absolute complex volume (38.73 mm^3, n = 3) and the capuchin had the largest volume (210.08 mm^3, n = 2). The basolateral division (BLD) relative to the total amygdala volume ranged from 56 – 64% across all species. The pig-tailed macaque had the smallest relative BLD volume, while the marmoset had the largest BLD. Out of the three nuclei in the BLD, the B was the largest nucleus in all but the marmoset. The marmoset L was the second largest nucleus, B = 45% and L = 41% (n = 3).

Table 3.1 Amygdaloid complex, nuclei, and medulla oblongata volumes (mm^3) for each specimen

ORDER		SPECIES	COMMON NAME	MONKEY	ACCESSORY BASAL	BASAL	CENTRAL	LATERAL	AMYGDALOID COMPLEX	MEDULLA OBLONGATA[1] (n)
Old World	CERCOPITHECIDAE	*M. fascicularis* (MF)	Crab-eating Macaque	MF1	7.42	18.37	3.99	14.43	68.14	–
				MF3	10.09	22.95	5.42	22.12	92.99	–
				Mean MF	**8.76 ± 1.89**	**20.66 ± 3.24**	**4.71 ± 1.01**	**18.28 ± 5.44**	**80.57 ± 17.57**	–
		M. mulatta (MM)	Rhesus Macaque	MM1	20.65	38.66	9.38	33.83	154.51	–
				MM2	21.49	50.69	10.12	41.56	189.21	–
				MM3	17.50	36.93	6.98	31.17	151.82	–
				MM4	20.23	41.72	11.86	30.46	149.51	–
				Mean MM	**19.97 ± 1.73**	**42 ± 6.12**	**9.59 ± 2.02**	**34.26 ± 5.08**	**161.26 ± 18.74**	**2329.14 ± 126.13 (2)**
		M. nemestrina (MN)	Pig-tailed Macaque	MN1	21.31	80.94	12.01	62.33	280.28	–
				MN2	16.66	66.67	6.65	45.49	240.94	–
				Mean MN	**18.99 ± 3.29**	**73.81 ± 10.09**	**9.33 ± 3.79**	**53.91 ± 11.91**	**260.61 ± 27.82**	–
				Mean OW	**16.92 ± 5.38**	**44.62 ± 21.06**	**8.30 ± 2.98**	**35.17 ± 14.76**	**165.93 ± 70.43**	–
New World	CEBIDAE	*C. jacchus* (CJ)	White-ear-tufted Marmoset	CJ1	3.41	9.86	1.58	12.39	38.80	–
				CJ2	3.72	12.36	2.87	11.98	45.34	–
				CJ5	3.18	8.61	1.05	8.73	32.05	–
				Mean CJ	**3.44 ± 0.27**	**10.28 ± 1.91**	**1.8 ± 0.94**	**11.03 ± 2.01**	**38.73 ± 6.65**	**318.24 ± 39.74 (4)**
		C. apella (CA)	Brown Capuchin	CA1	20.69	53.56	8.13	41.17	198.30	–
				CA2	18.29	62.64	8.20	49.82	221.85	–
				Mean CA	**19.49 ± 1.70**	**58.1 ± 6.42**	**8.17 ± 0.05**	**45.5 ± 6.12**	**210.08 ± 16.65**	**1738 (2)[2]**
				Mean NW	**9.86 ± 8.84**	**29.41 ± 26.42**	**4.37 ± 3.53**	**24.82 ± 19.17**	**107.27 ± 94.34**	–

[1] Average from Sherwood et al. [2] Average from Stephan et al. 1981

In order to compare total amygdala volumes across species I plotted amygdaloid complex volume against neocortex and medulla volume (Figure 3.1) and brain weight (Figure 3.2). The relationship of the amygdala volume with neocortex volume was negatively allometric (slope = 0.67, R^2 = 0.97**,** SE = 0.0213, $p < .0001$), medulla volume was isometric (slope = 1.09, R^2 = 0.94, SE = 0.0465, $p < .0001$), and brain weight was positively allometric (slope = 1.32, R^2 = 0.943, SE = 0.0535, $p < .0001$). My data follows previously established correlations, mainly that as the brain becomes larger so does one of its subcomponents, the amygdaloid complex. In addition, the largest nucleus was the B in all species except the marmoset, which has a larger L similar to findings in the great apes and humans. I conclude that the volume of the amygdaloid complex varies in an orderly way with entire brain size, and that this relationship extends to the human brain.

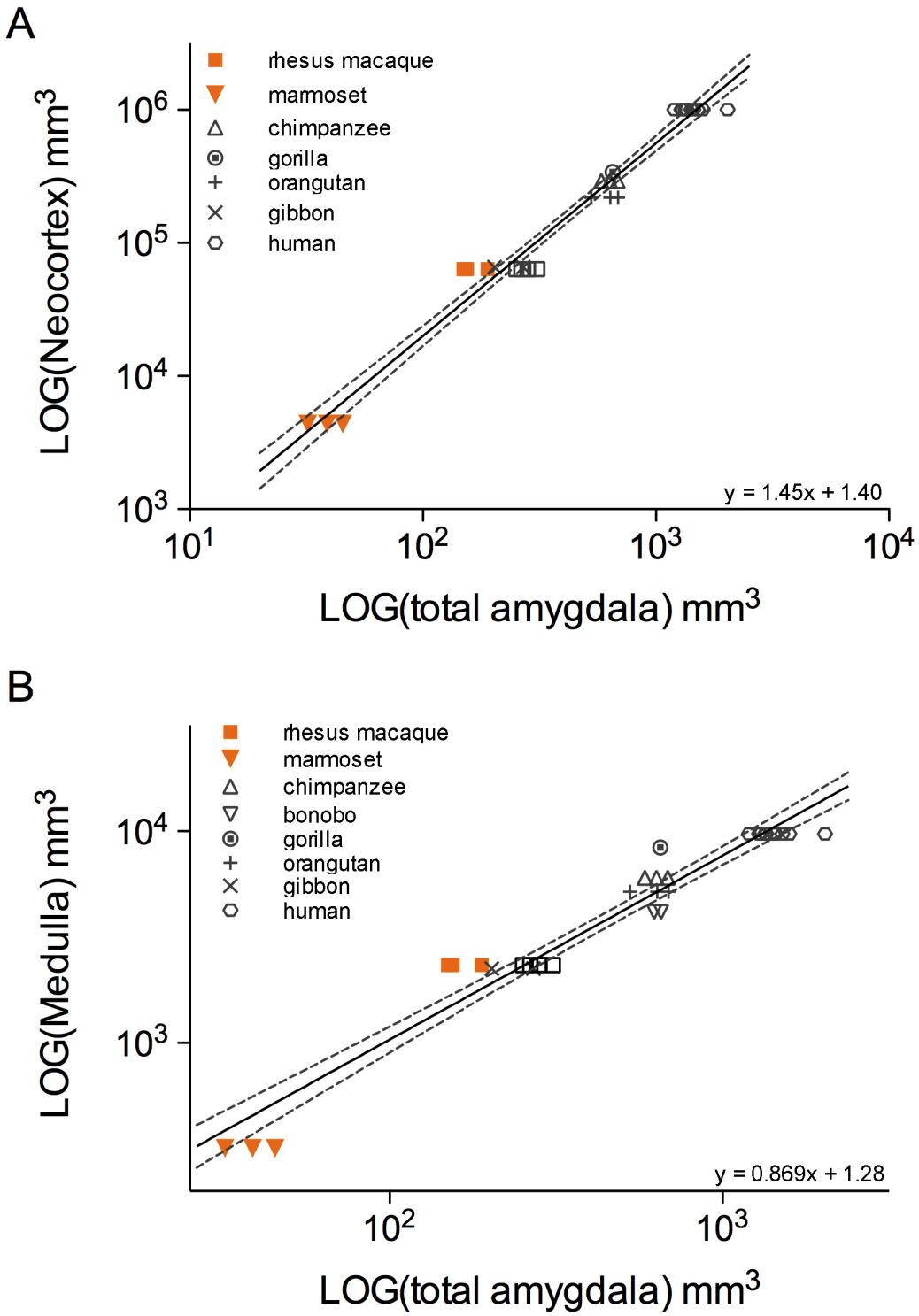


Figure 3.1 Amygdaloid complex volume (mm^3) regression against the neocortical volume in 7 species (A) and the medulla volume (B) in 8 species (includes the bonobo) representing 4 taxa of primates. Neocortical volume data for marmoset, rhesus, and gibbon from Stephan et al. 1981, all others from Sherwood et al. 2003. Medulla volume data for marmoset from Stephan et al. 1981, all others from Sherwood. et al. 2005.

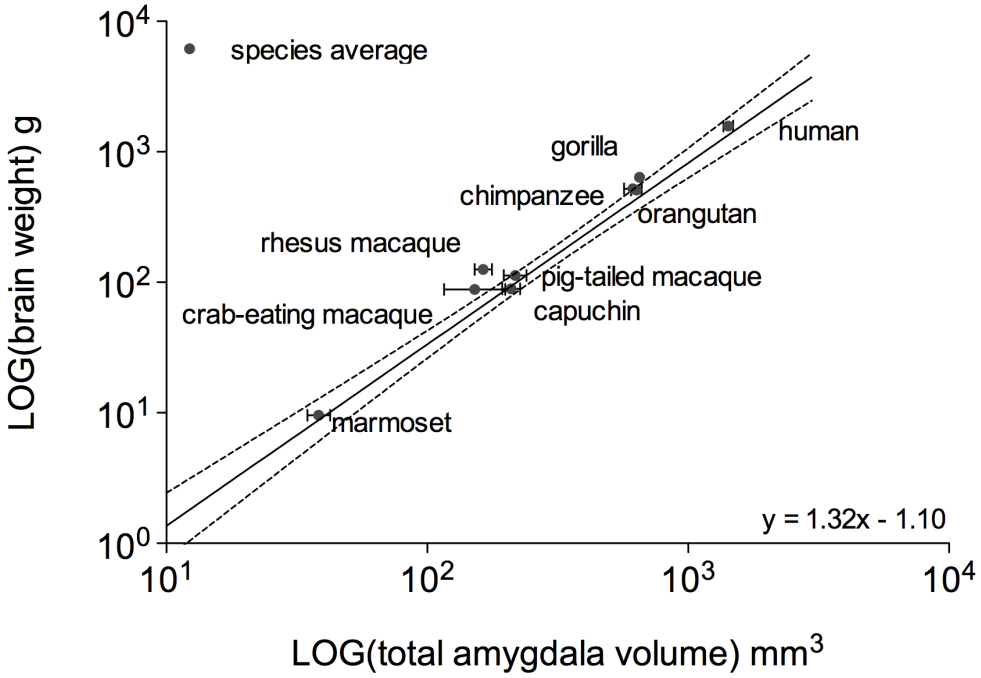


Figure 3.2 Amygdaloid complex volume (mm^3) plotted against the brain weight (g) in 9 species representing 4 taxa of primates. Brain weight data can be found in the following papers: capuchin, crab-eating and rhesus macaque from Bronson et al. (1981); marmoset from Stephan et al. (1981); orangutan, gorilla, chimpanzee, and human from Sherwood et al. (2003).

Relative size of individual nuclei

A recent study by Barger et al. (2007) evaluated the basolateral division of the amygdaloid complex (lateral, basal, and accessory basal nuclei) in six hominid species including: human, chimpanzees, bonobo, gorilla, orangutans, and gibbon. Interestingly, the L was found to be enlarged in humans but remained stable within the other apes evaluated (Barger et al., 2007). To provide a complete view of how the amygdaloid complex has changed across primate species I evaluated the accessory basal, basal, central, and lateral nuclei volumes by plotting their volumes against the total complex volume minus the nucleus of interest (Figure 3.3). These results focus on only these four nuclei, yet data was collected from 26 anatomically distinct regions

within the amygdaloid complex. See Table 3.3 for volumes of all subdivisions evaluated. This analysis of comparing relative volumes allows us to independently evaluate how a particular nucleus changes in relation to the rest of the amygdala (Barger et al., 2007). Volume analyses include published data from Barger et al. (2007) who evaluated 12 great apes and Schumann et al. (2005) who evaluated 10 human specimens.

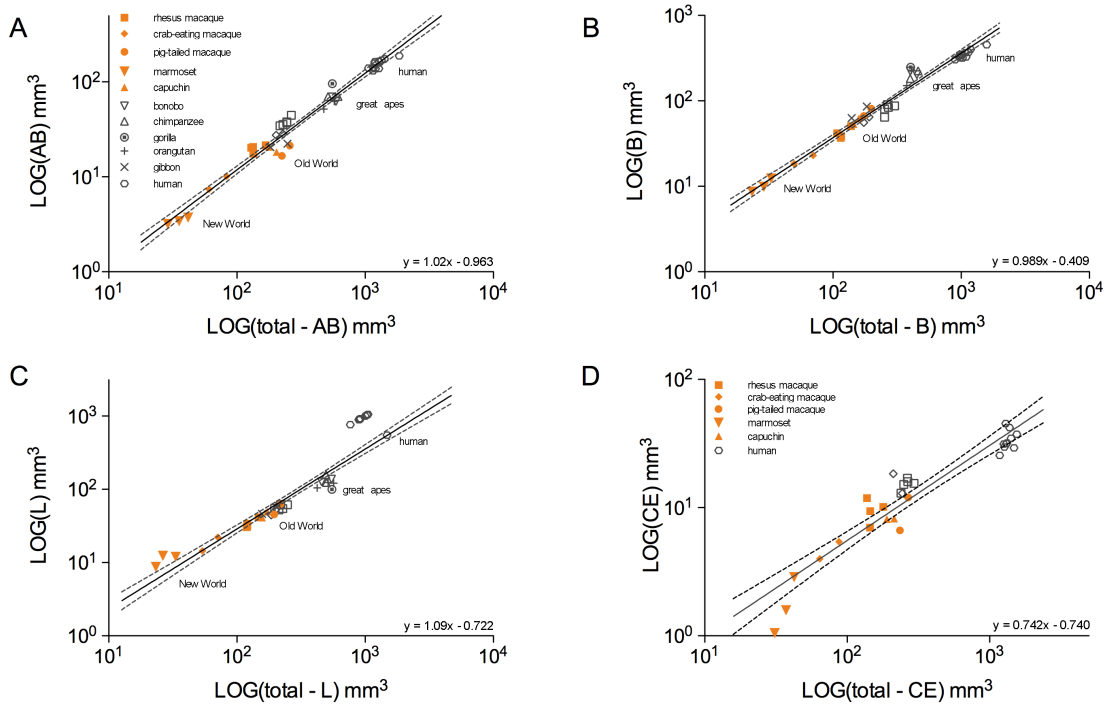


Figure 3.3 Volumes (mm^3) of accessory basal nucleus (A), basal nucleus (B), lateral nucleus (C), and central nucleus (D) across 4 primate taxa. Legend in (A) applies to (B) and (C). Orange, filled symbols indicate data collected in the present study. Gray, open symbols indicate data found in the existing literature.

The accessory basal, basal, and lateral nuclei were isometric, meaning the standard least-squares (SLS) regression slopes in Figure 3.3 were very close to 1 (AB slope = 1.02, SE = 0.086; B slope = 0.99, SE = 0.084; L, slope = 1.09, SE = 0.123). The marmoset and human data points fell above the 95% confidence intervals in the

lateral nucleus regression. In contrast, the great apes fell below the 95% confidence limits in this nucleus. Data points outside the 95% confidence limits were removed to evaluate their influence on the regression. When the humans were excluded from the analysis the SLS regression slope became negatively allometric (slope = 0.79, SE = 0.105, $R^2 = 0.899$, $p < 0.0001$). When the marmosets and the great apes, each in turn, were excluded from the SLS regression analysis the slope became positively allometric (marmosets: slope = 1.25, SE = 0.161, $R^2 = 0.906$, $p < 0.0001$; great apes: slope = 1.15, SE = 0.168, $R^2 = 0.914$, $p < 0.0001$). In contrast to the nuclei of the BLD, the central nucleus regression was negatively allometric with a slope of 0.74 (SE = 0.1382, Figure 3.3).

I conclude that the CE is growing at a slower rate than the BLD nuclei, which change at the same rate across primate taxa (New World, Old World, great apes). I have found suggestive evidence for a single change for the humans and the marmosets in the L that could reflect functional differences.

Comparison of neuron numbers in individual nuclei

Comparing volume and neuron numbers from each nucleus will help determine potential structural differences associated with our knowledge of functional differentiation between nuclei. Through the years there has been a longstanding debate about what contributes to cortical processing power. The question is whether it is the connections between neurons or the actual number of neurons that leads to higher cortical processing power.

Rockel (1980) sought to answer this question by evaluating the number of neurons within a cortical column 100 μm wide from the pial surface to the white matter (Rockel et al., 1974; Rockel et al., 1980). His findings indicate that there was no difference in the number of neurons per mm^3 across species with one exception; in the tupaia (intermediate primate between insectivores and prosimians) there were twice as many neurons per mm^3 in primary visual cortex than in six other areas of cortex. This finding was disputed by Haug (1987) who used an early form of stereology to evaluate cell numbers in a variety of cortical areas and found great variability in thickness, density, and cell numbers across species (Haug, 1987). To date the question of what contributes to increased cortical processing power remains. Using unbiased stereological techniques I am able to address this question for the amygdala, which is an area that is known to show some scaling changes across species and is critical within a system used for evaluating incoming emotionally salient information.

The following analyzes on neuron number and density include the 13 Old and New World specimens from the present study and the 10 human specimens from Schumann et al. (2005), which represent the only neuron number counts in the amygdaloid complex to date. SLS regressions for estimated total neuron number were run on the following nuclei: accessory basal, basal, central, and lateral nuclei (Figure 3.4 and Table 3.2). See Table 3.4 for neuron numbers from all 26 amygdaloid complex subdivisions that were evaluated.

The accessory basal and basal nuclei numbers were isometric, i.e. neuron numbers in each nucleus increase at the same rate as the neuron numbers in the entire amygdala (AB slope = 1.0, SE = 0.08, $R^2 = 0.939$, $p < 0.0001$; B slope = 1.04, SE = 0.084, $R^2 = 0.936$, $p < 0.0001$). Similar to the volume analysis, the central nucleus' neuron numbers increased at a slower rate than the AB, B, and L nuclei (CE slope = 0.59, SE = 0.15, $R^2 = 0.616$, $p < 0.0001$, Figure 3.4). The lateral nucleus SLS regression was negatively allometric (L slope = 0.94, SE = 0.118, $R^2 = 0.866$, $p < 0.0001$).

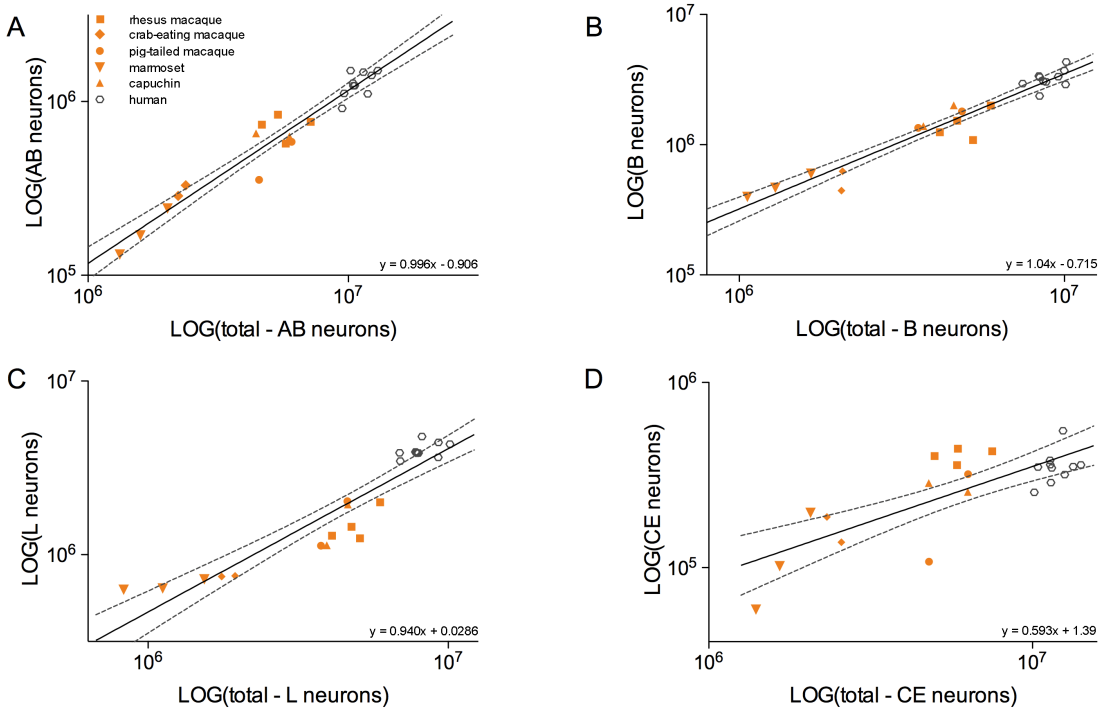


Figure 3.4 Estimated number of neurons in the accessory basal nucleus (A), basal nucleus (B), lateral nucleus (C), and central nucleus (D) across 3 taxa of primates. All plots contain human data from Schumann et al. 2005. Legend in (A) applies to (B - D).

I observed that CE is divergent from the BLD nuclei because both its volume and neuron number fail to increase at the same rate as the total amygdala. The BLD seems to sustain a growth rate that is similar to the increased amygdala volume found

as species become larger in body size, and potentially, as they increase their social networks.

Neuron density in individual nuclei

Measures of neuron density tell us how much neuronal processing power is contained in 1 mm^3. It is thought that if the density of neurons is the same within our unit measure, then differences in cortical processing power must then be determined from the connections, or neuropil, between those neurons. Neuron density, calculated as neuron number divided by volume, for each nucleus was compared with the whole amygdaloid complex neuron density. All nuclei had a regression slope of about -0.5 (AB slope = -0.54, SE = 0.099, $R^2 = 0.917$, $p < 0.0001$; B slope = -0.46, SE = 0.096, $R^2 = 0.892$, $p < 0.0001$; L slope = -0.58, SE = 0.094, $R^2 = 0.933$, $p < 0.001$; CE slope = -0.51, SE = 0.123, $R^2 = 0.864$, $p < 0.0001$, Figure 3.5). While all the nuclei were negatively allometric the slopes range from the highest, B nucleus at –0.46, to the lowest being the L nucleus at –0.58. I conclude from these data that the major nuclei of the amygdaloid complex have similar densities of neurons.

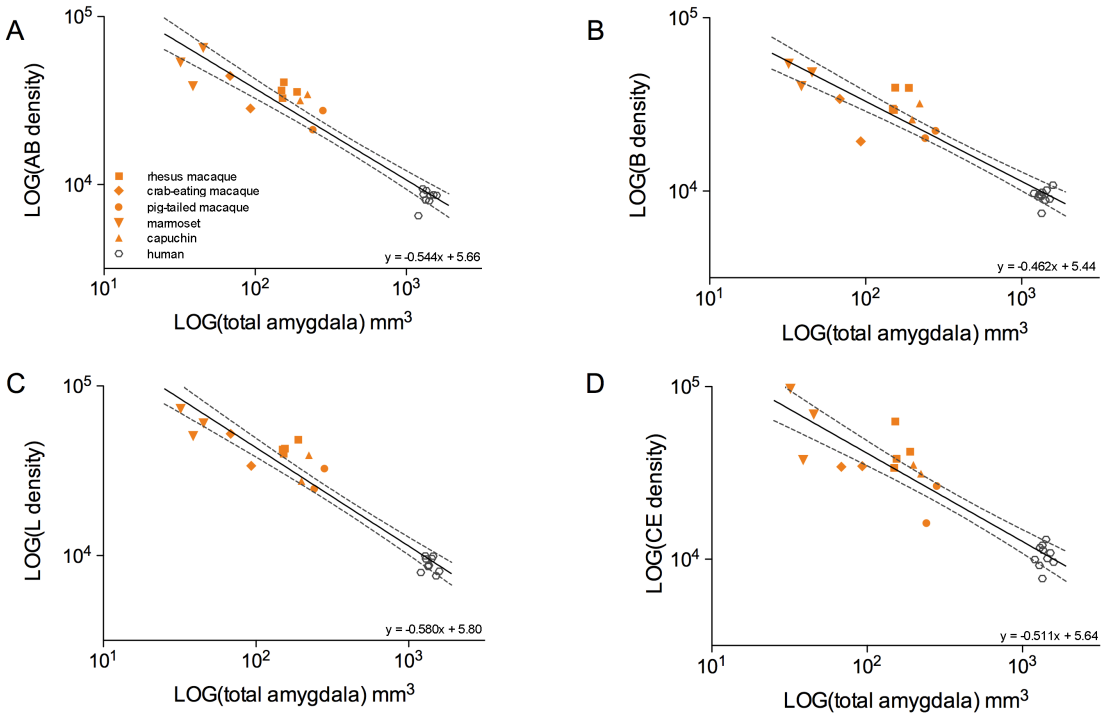


Figure 3.5 Neuron density (mm^3) in the accessory basal (A), basal (B), lateral (C), and central (D) nuclei across 3 taxa of primates. All plots contain human data from Schumann et al. 2005. Legend in (A) applies to (B - D).

Table 3.2 Amygdaloid complex and nuclei neuron numbers (10^5) for each specimen

TAXA		SPECIES	COMMON NAME	MONKEY	ACCESSORY BASAL	BASAL	CENTRAL	LATERAL	AMYGDALOID COMPLEX
Old World	CERCOPITHECIDAE	*M. fascicularis* (MF)	Crab-eating Macaque	MF1	3.29	6.25	1.37	7.54	27
				MF3	2.86	4.44	1.88	7.48	25.1
				Mean MF	**3.08**	**5.35**	**1.62**	**7.51**	**26.1**
		M. mulatta (MM)	Rhesus Macaque	MM1	8.39	15.3	3.58	14.5	62.1
				MM2	7.65	20	4.25	20.1	79.4
				MM3	5.72	10.8	4.39	12.4	63.2
				MM4	7.35	12.5	4.01	12.9	53.9
				Mean MM	**7.28**	**14.6**	**4.06**	**15**	**64.6**
		M. nemestrina (MN)	Pig-tailed Macaque	MN1	3.54	13.5	1.08	11.3	49
				MN2	5.87	18	3.19	20.3	66.5
				Mean MN	**4.71**	**15.8**	**2.14**	**15.8**	**57.7**
				Mean OW	**5.59**	**12.6**	**2.97**	**13.3**	**53.3**
New World	CEBIDAE	*C. jacchus* (CJ)	White-ear-tufted Marmoset	CJ1	1.32	3.98	0.594	6.27	14.6
				CJ2	2.42	5.98	1.98	7.21	22.6
				CJ5	1.7	4.67	1.02	6.39	17.6
				Mean CJ	**1.81**	**4.88**	**1.2**	**6.62**	**18.3**
		C. apella (CA)	Brown Capuchin	CA1	6.56	13.9	2.87	11.3	50.7
				CA2	6.29	20.1	2.57	19.5	65.7
				Mean CA	**6.43**	**17**	**2.72**	**15.4**	**58.2**
				Mean NW	**3.66**	**9.72**	**1.8**	**10.1**	**34.2**

Table 3.3 Amygdaloid complex nuclei and subdivision VOLUMES (mm^3) for 5 species of Old and New World primates

TAXA		SPECIES	COMMON NAME	MONKEY	DEEP NUCLEI									CORTICAL AND OTHER NUCLEI									
					Lateral nucleus				Basal nucleus			Accessory Basal		Central									
					Ld	Ldi	Lv	Lvi	Bi	Bmc	Bpc	ABmc	ABpc	AAA	AHA	CEL	CEM	COa	Cop	I	ME	PAC	PL
Old World	CERCOPITHECIDAE	*M. fascicularis* (MF)	Crab-eating Macaque	MF1	2.73	4.07	4.86	2.17	4.81	4.51	8.55	2.65	4.55	0.72	1.06	1.96	1.73	0.39	0.81	NA	2.18	3.03	3.01
				MF3	1.59	9.13	8.53	2.36	6.24	4.29	11.66	3.63	6.09	0.36	1.18	2.77	2.28	1.25	0.81	NA	2.85	5.99	1.94
				Mean MF	2.16	6.60	6.70	2.27	5.53	4.40	10.11	3.14	5.32	0.54	1.12	2.37	2.01	0.82	0.81	NA	2.52	4.51	2.48
				SD	0.81	3.58	2.60	0.13	1.01	0.16	2.20	0.69	1.09	0.25	0.08	0.57	0.39	0.61	0.00	NA	0.47	2.09	0.76
		M. mulatta (MM)	Rhesus Macaque	MM1	4.95	11.55	10.97	5.79	10.84	7.42	19.62	8.68	10.91	2.07	2.82	5.36	3.73	4.39	2.59	0.37	7.09	8.70	3.75
				MM2	7.06	12.68	12.70	8.27	18.30	6.20	24.67	7.76	12.05	0.99	2.28	5.00	3.97	5.49	2.55	0.69	5.93	15.68	7.05
				MM3	8.17	9.49	11.47	1.60	9.89	3.31	21.35	7.20	11.66	1.34	2.65	3.45	3.11	2.05	2.48	0.23	3.36	16.21	7.91
				MM4	3.83	10.53	11.05	4.56	9.71	7.51	22.99	8.70	11.13	1.00	0.81	5.14	4.84	2.26	2.92	0.24	4.58	8.52	6.28
				Mean MM	6.00	11.06	11.55	5.06	12.19	6.11	22.16	8.09	11.44	1.35	2.14	4.74	3.91	3.55	2.64	0.38	5.24	12.28	6.25
				SD	1.97	1.37	0.80	2.77	4.11	1.96	2.17	0.74	0.52	0.51	0.91	0.87	0.72	1.67	0.20	0.21	1.62	4.24	1.79
		M. nemestrina (MN)	Pig-tailed Macaque	MN1	8.11	12.40	15.09	7.59	16.96	11.87	34.52	7.15	9.12	3.75	3.32	3.20	2.57	4.76	4.55	0.57	6.42	17.62	10.61
				MN2	4.51	26.99	19.41	9.59	19.92	10.33	46.87	7.92	12.69	2.34	2.92	5.96	4.91	4.85	2.85	0.46	5.74	21.56	8.02
				Mean MN	6.31	19.70	17.25	8.59	18.44	11.10	40.70	7.54	10.91	3.05	3.12	4.58	3.74	4.81	3.70	0.52	6.08	19.59	9.32
				SD	2.55	10.32	3.05	1.41	2.09	1.09	8.73	0.54	2.52	1.00	0.28	1.95	1.65	0.06	1.20	0.08	0.48	2.79	1.83
				Mean OW	40.95	96.84	94.08	41.93	96.67	55.44	190.23	53.69	78.20	12.57	17.04	32.84	27.14	25.44	19.56	2.56	38.15	97.31	48.57
				SD	2.46	6.60	4.31	3.06	5.64	3.00	12.26	2.29	2.97	1.10	0.97	1.44	1.17	1.92	1.21	0.18	1.81	6.48	2.94
New World	CEBIDAE	*C. jacchus* (CJ)	White-ear-tufted Marmoset	CJ1	1.35	6.49	2.03	1.91	2.97	1.50	4.99	1.59	1.69	0.19	0.10	0.81	0.66	0.69	0.47	0.03	0.53	1.18	1.08
				CJ2	0.67	4.36	5.13	1.58	3.51	2.62	6.14	1.78	1.86	0.17	0.09	3.76	1.17	1.02	0.84	0.26	1.30	1.49	1.22
				CJ5	0.63	3.67	2.28	1.87	2.02	1.54	4.80	1.20	1.64	0.49	NA	0.83	0.66	0.49	0.49	0.03	0.69	1.60	0.88
				Mean CJ	0.88	4.84	3.15	1.79	2.83	1.89	5.31	1.52	1.73	0.28	0.10	1.80	0.83	0.73	0.60	0.11	0.84	1.42	1.06
				SD	0.40	1.47	1.72	0.18	0.75	0.64	0.73	0.30	0.12	0.18	0.01	1.70	0.29	0.27	0.21	0.13	0.41	0.22	0.17
		C. apella (CA)	Brown Capuchin	CA1	2.34	13.89	17.13	7.13	16.99	9.54	26.58	8.62	11.36	2.85	1.80	4.38	5.24	4.90	1.90	0.45	4.64	10.89	7.86
				CA2	5.27	16.76	16.51	9.73	14.74	8.64	37.91	7.35	9.76	2.30	3.96	3.38	3.89	6.57	2.34	1.24	3.58	7.26	6.09
				Mean CA	3.81	15.33	16.82	8.43	15.87	9.09	32.25	7.99	10.56	2.58	2.88	3.88	4.57	5.74	2.12	0.85	4.11	9.08	6.98
				SD	2.07	2.03	0.44	1.84	1.59	0.64	8.01	0.90	1.13	0.39	1.53	0.71	0.95	1.18	0.31	0.56	0.75	2.57	1.25
				Mean NW	10.26	45.17	43.08	22.22	40.23	23.84	80.42	20.54	26.31	6.00	5.95	13.16	11.62	13.67	6.04	2.01	10.74	22.42	17.13
				SD	1.93	5.92	7.59	3.76	7.20	3.98	15.30	3.57	4.87	1.28	1.83	1.69	2.11	2.81	0.86	0.50	1.85	4.39	3.30

Table 3.4 Amygdaloid complex nuclei and subdivision NEURON numbers (10^5) for 5 species of Old and New World primates

TAXA		SPECIES	COMMON NAME	MONKEY	DEEP NUCLEI									CORTICAL AND OTHER NUCLEI									
					Lateral nucleus				Basal nucleus			Accessory Basal		Central									
					Ld	Ldi	Lv	Lvi	Bi	Bmc	Bpc	ABmc	ABpc	AAA	AHA	CEl	CEm	COa	Cop	I	ME	PAC	PL
Old World	CERCOPITHECIDAE	*M. fascicularis* (MF)	Crab-eating Macaque	MF1	1.62	2.61	2.25	1.07	1.36	1.62	3.28	1.18	2.12	0.337	0.78	0.77	0.6	0.426	0.515	NA	1.41	2.61	2.5
				MF3	0.547	3.29	2.75	0.89	1.12	0.917	2.4	1.06	1.8	0.079	1.12	1.03	0.846	0.771	0.628	NA	1.81	3.09	0.903
				Mean MF	1.08	2.95	2.5	0.979	1.24	1.27	2.84	1.12	1.96	0.208	0.951	0.9	0.723	0.599	0.572	NA	1.61	2.85	1.7
				SD	0.757	0.482	0.357	0.126	0.17	0.495	0.618	0.0814	0.224	0.183	0.241	0.184	0.173	0.243	0.079	NA	0.281	0.339	1.13
		M. mulatta (MM)	Rhesus Macaque	MM1	1.94	5.23	4.68	2.61	3.75	2.45	9.08	3.1	5.29	0.896	2.11	2.24	1.34	2.86	2.28	0.155	4.15	5.87	2.03
				MM2	3.95	5.84	6.03	4.24	6.81	2.25	10.9	2.78	4.87	0.581	2.22	2.35	1.9	2.82	2.7	0.478	3.95	10.7	3.95
				MM3	3.54	3.68	4.57	0.624	3.01	0.77	7.07	2.83	2.89	0.46	6.27	2.27	2.12	1.03	5.07	0.14	3.08	9.53	4.28
				MM4	1.64	4.5	4.4	2.32	2.05	2.16	8.26	3.36	3.99	0.476	0.598	2.55	1.45	1.09	1.79	0.251	2.51	4.89	5.57
				Mean MM	2.77	4.81	4.92	2.45	3.9	1.91	8.83	3.02	4.26	0.603	2.8	2.35	1.7	1.95	2.96	0.256	3.42	7.75	3.96
				SD	1.15	0.932	0.749	1.48	2.06	0.768	1.61	0.269	1.06	0.202	2.43	0.14	0.368	1.03	1.45	0.156	0.766	2.8	1.46
		M. nemestrina (MN)	Pig-tailed Macaque	MN1	1.76	3.41	3.82	2.29	3.3	2.21	7.97	1.48	2.06	1.25	1.17	0.719	0.359	1.75	1.56	0.276	2.65	6.08	4.87
				MN2	1.38	9.54	6.47	2.93	3.67	1.55	12.8	2.19	3.68	0.759	1.44	1.77	1.43	1.7	1.65	0.201	2.09	7.7	3.53
				Mean MN	1.57	6.47	5.14	2.61	3.49	1.88	10.4	1.84	2.87	1	1.3	1.24	0.892	1.73	1.6	0.238	2.37	6.89	4.2
				SD	0.269	4.33	1.87	0.454	0.264	0.463	3.41	0.505	1.14	0.347	0.192	0.743	0.754	0.033	0.063	0.053	0.396	1.14	0.946
				Mean OW	16.4	38.1	35	17	25.1	13.9	61.8	18	26.7	4.84	15.7	13.7	10	12.5	16.2	1.5	21.6	50.5	27.6
				SD	1.13	2.2	1.45	1.21	1.8	0.635	3.52	0.904	1.33	0.361	1.83	0.76	0.614	0.908	1.44	0.124	0.976	2.87	1.55
New World	CEBIDAE	*C. jacchus* (CJ)	White-ear-tufted Marmoset	CJ1	0.616	3.84	0.653	1.17	1.09	0.645	2.25	0.654	0.661	0.054	0.067	0.315	0.278	0.401	0.314	0.019	0.245	0.646	0.661
				CJ2	0.469	2.9	2.95	0.893	1.79	1.37	2.83	1.3	1.12	0.15	0.047	1.29	0.681	0.781	0.699	0.259	0.956	1.44	0.688
				CJ5	0.48	2.95	1.44	1.51	0.965	0.916	2.79	0.866	0.831	0.285	NA	0.508	0.51	0.377	0.522	0.03	0.437	1.66	0.496
				Mean CJ	0.522	3.23	1.68	1.19	1.28	0.976	2.62	0.941	0.87	0.163	0.057	0.706	0.49	0.519	0.512	0.103	0.546	1.25	0.615
				SD	0.082	0.528	1.17	0.31	0.445	0.364	0.325	0.331	0.23	0.116	0.014	0.518	0.202	0.227	0.193	0.136	0.367	0.534	0.104
		C. apella (CA)	Brown Capuchin	CA1	0.877	3.88	4.28	2.3	3.48	2.15	8.24	2.76	3.74	1.53	0.663	1.2	1.67	2.03	0.865	0.252	1.75	4.81	4.19
				CA2	2.46	6.78	6.08	4.16	4.44	2.27	13.4	2.71	3.59	1.16	2.12	0.997	1.57	3	1.09	0.764	1.74	3.79	3.66
				Mean CA	1.67	5.33	5.18	3.23	3.96	2.21	10.8	2.73	3.66	1.35	1.39	1.1	1.62	2.51	0.979	0.508	1.75	4.3	3.93
				SD	1.12	2.05	1.27	1.31	0.677	0.083	3.63	0.0351	0.111	0.262	1.03	0.142	0.07	0.682	0.161	0.362	0.01	0.72	0.373
				Mean NW	4.9	20.4	15.4	10	11.8	7.35	29.5	8.29	9.94	3.18	2.9	4.31	4.71	6.59	3.49	1.32	5.13	12.3	9.7
				SD	0.843	1.59	2.18	1.31	1.54	0.726	4.85	1.01	1.54	0.666	0.974	0.431	0.635	1.16	0.3	0.302	0.706	1.75	1.82

CHAPTER IV

SCALING OF AMYGDALA NUCLEI AND THE DEVELOPMENT OF COMPLEX SOCIAL BEHAVIOR

Brain region volume has been taken as a proxy for a region's importance. For example, the human olfactory bulb is small for the size of the human brain because we rely on other senses, e.g. vision and hearing. Other animals, like the mole, depend on the olfactory system to find food underground where vision is not very helpful, and comparatively, their olfactory bulb takes up a larger portion of the brain. I seek to identify potential regions of evolutionary divergence in the amygdala. Comparison of volumes across species tells us about potential changes in behavior. In the present study, divergent growth could indicate increased use of the amygdala due to higher demands for social interaction in larger social networks that are formed by the Old World primates.

Isometric Development of the Basolateral Complex Nuclei and Allometric Volume Changes in the Central Nucleus

The basolateral complex consists of the three largest nuclei in the amygdaloid complex. I found these nuclei to be isometric, having a SLS regression slope of 1. As the complex becomes larger the AB, B, and L nuclei increase in volume at the same rate as the rest of the amygdala. This finding suggests that these nuclei probably developed together. Barger et al. (2007) first asserted that the L in great apes was isometric. With the addition of my data and published data on humans from Schumann et al. (2005), the regression lines for the AB and B also show isometric convergence. In my data and the human data, only the CE develops at a slower rate than the rest of the amygdala when compared across six species. No CE data was available for the great apes.

The CE volume increases at a rate of 0.74, as the volume of the total amygdala increases in size. Thus CE is negatively allometric and could have developed divergently from the rest of the basolateral complex. CE has many intrinsic and extrinsic connections that make it central to both updating, indirectly a more executive frontal cortex system and directly the more primitive autonomic system. Changes in the CE suggest more global changes within theses systems that surely could change behaviors such as aggression, reconciliation, foraging, and decision-making. My finding that CE volume is not growing at the same rate as the rest of the amygdala could also be a result of functional changes in the basolateral division.

CE could be maintaining its survival functions by condensing the information it receives into a simple message to send to downstream autonomic areas. The human need for a strong connection to this flight or fight system, is probably reduced. Thus CE would not need to expand at the rate the rest of the amygdala is growing, i.e. increasing its functional output. It is also possible that cortical expansion from increased sensory input, and/or executive frontal cortex function, could increase the connections to the BLD. The basolateral complex would then be increasing in volume at a rate dictated by the increase in social, sensory, and decision-making functions required of the Old World monkeys, the great apes, and finally the humans. The changes I have found in the CE and the BLD are likely a combination of the need for greater social cognition based out of the BLD and a reduction in the need for a flight or fight system initiated out of CE. This theory is support by our knowledge of CE lesions and its functional connectivity to other cortical areas.

Lesions of the CE in the rhesus macaque produced marked behavioral changes and also a functional decrease in the levels of corticotrophin-releasing factor (CRF) (Kalin et al., 2004). Behaviorally, bilaterally CE-lesioned monkeys produced more coo-calls (interpreted as "help" calls), showed less fear when exposed to a toy snake, and exhibited less freezing when confronted by a human intruder. Despite the disruption in the fear circuitry, there existed no alterations in frontal cortex activity measured with electroencephalography (EEG). Other AC nuclei project to the nucleus basalis and these projections could be maintaining the amygdala's connectivity with the frontal cortex. This could explain why the frontal cortex activity was unaltered in the CE-lesioned monkeys.

CE is the common output of the amygdala, and it projects both to the nucleus basalis of Meynert (BNM), that has heavy cholinergic projections to the rest of the cortex, and to downstream autonomic areas, e.g. the medulla, pons, and hypothalamus (see Figure 4.1). The anterior cortical nucleus (COa), anterior hippocampal area (AHA), and anterior amygdaloid area (AAA) receive intrinsic projections from CE. The medial subdivision of CE receives heavy projections from the B and AB. Lighter projections come into CEm from L, PAC, and ME. In turn, CEm projects back to the parvocellular portions of B, and the lateral division of CE projects back to L (Amaral et al., 1992). CE is in a position to integrate the sensory information from L and the executive information from B and AB, before sending a cohesive message to the executive frontal cortex system. In this way CE is both updating the existing message and ensuring that the feedback loop, from frontal cortex, is complete. CE also projects

to the midline thalamic nuclei, midbrain, medulla, pons, and hypothalamus to influence the more autonomic functions driven by the AC (Amaral et al., 1992).

Perhaps the area CE projects to with the most widespread cortical influence is the Ch4 subregion (nucleus basalis of Meynert) of the basal forebrain (Mesulam et al., 1983). Three studies of the crab-eating macaque found that CE projects to the nucleus basalis and to the horizontal limb of the diagonal band (Aggleton et al., 1987; Price and Amaral, 1981; Russchen et al., 1985). The nucleus basalis consists of clusters of largish cells that stain heavily for AChE and a portion of these cells are found, along with the nuclei of the diagonal band and the medial septal nuclei, in the substantia innominata (Russchen et al., 1985). Other AC nuclei project to Ch3 (AB, ME) and Ch4 (AB, Bmc, Bpc, ME) with the heaviest projections terminating in Ch4 (Aggleton et al., 1987; Russchen et al., 1985). It is likely that neuroanatomical variations leading to changes in behavior would stem from this CE/basal forebrain pathway based on its extensive connectivity to most cortical areas.

There exist other more direct, and presumably quicker, pathways to update the amygdala. Direct projections from the substantia innominata (SI) and horizontal limb of the diagonal band (Ch3) back to the B nucleus were found with horseradish peroxidase injections into the AC of the rhesus macaque (Aggleton et al., 1980). Russchen et al (1985) and Aggleton et al. (1987) confirmed the projections from Ch4 to the magnocellular subdivision of the basal nucleus. Russchen et al. (1985) found that SI sends heavy projections to the rostrodorsal region of Bmc. The nucleus basalis plays a pivotal roll in updating the functions of the amygdala both via direct and

indirect-cortical projections. These basal forebrain cholinergic projections influence the entire cortex by modulating the actions of the areas to which they project.

There are two projections out of the nucleus basalis in rodent, monkey, and human (Saper and Chelimsky, 1984): the medial nucleus basalis has fibers that extend to the hippocampus and to the cortical areas on the medial wall of the hemisphere; the lateral nucleus basalis' fibers project through the external capsule to the lateral wall of the hemisphere (see Figure 4.1). In this way the entire cortex is influenced by the basal forebrain structures and the AC will receive feedback projections from frontal, occipital, temporal, insular, and cingulated cortices.

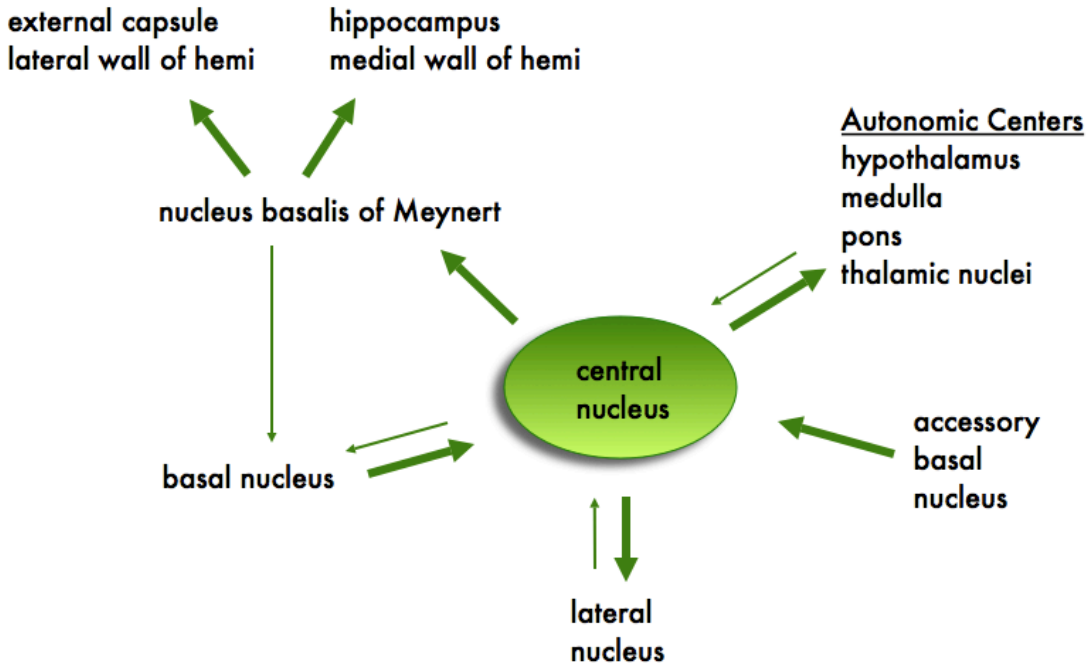


Figure 4.1 Intrinsic and extrinsic connections of the central nucleus. The central nucleus integrates information from the basolateral division. Then it sends projections to the autonomic centers that activate the fight-or-flight system and to the aceytlecholine-rich nucleus basalis of meynert in the basal forebrain, which is the main cholinergic input to the rest of the cortex (see text for details).

Volume comparisons with other studies

The first studies of amygdala volume, conducted by Stephan et al. (1977, 1981, and 1987), came to the following conclusions: 1) that the amygdala increases from smallest to largest in insectivores, prosimians, and simians (Stephan and Andy, 1977); 2) that the centromedial nuclei group exhibited no relative change when compared across species; 3) and that the cortico-basolateral amygdaloid group, in particular the small-celled components, had a greater increase than the total amygdala volume when changing from insectivore, to prosimian, and to simians. It is important to note here that the centromedial nuclei group contained the nucleus of the lateral olfactory tract (NLOT), which decreased in volume from 8% to 0% in simians, and that this nuclei group comparison masks the changes of the components even when they are as large as the change in the NLOT. Comparison of the cortico-basolateral amygdaloid group revealed that the simians' nuclei group is larger than that which is found in prosimians and insectivores. The conclusions from Stephan point to the corticobasolateral amygdaloid group as a regulator of the amount of environmental information capable of being processed by the neocortex since the lateral nucleus is the point of sensory and ultimately cognitive convergence (Stephan and Andy, 1977; Stephan et al., 1981; Stephan et al., 1987).

Unfortunately, I did not have an alternative structure to use for normalization; therefore I was unable to compare total amygdaloid complex volume ratios across species. Previous studies have used whole brain volume or medulla volume but neither of these structures was available for my specimens (Barton et al., 2003; Stephan et al., 1981). I did plot my data with medulla and neocortical volumes (Sherwood et al.,

2005; Sherwood et al., 2003; Stephan et al., 1981), and brain weight measurements (Bronson, 1981; Sherwood et al., 2003; Stephan et al., 1981) from previously published studies (see Figures 3.1 and 3.2). AC volume regressed against medulla volume was isometric (slope ~1) and neocortical volume was negatively allometric (slope = -0.67).

For comparative purposes Table 2.2 reviews all the published data on amygdala volumes for the species in the present study. Only two studies, Emery et al. 2001 and Stefanacci et al. 2003 (see control data), have determined volumes of four major amygdala nuclei in two difference species, *Macaca mulatta* and *Macaca fasicularis*. Comparison of the basolateral complex nuclei group from these two studies and the present study with Stephan's (1977, 1981, 1987) numbers show that all published numbers are consistent across studies (Emery et al., 2001; Stefanacci et al., 2003).

In addition to the studies mentioned above, there exists a small pool of data on the basolateral complex and AC volume in great apes and humans. In a study by Barger et al. (2007) six species of great apes and one human brain, the human had the largest AC volume and the largest basolateral complex (AB, B, and L). Most notably, they find an expansion of the L in the human, meaning that the L is larger than B. My findings, in accordance with others, show that the B is larger than the L in the Old World primates (Amaral et al., 1992; Emery et al., 2001; Stefanacci et al., 2003) and in great apes (Barger et al., 2007). In contrast, one of my two New World species, the marmoset, showed expansion of the L such that it was larger than the B. Clearly, no

conclusions can be drawn from this data set without evaluating additional species from the NW primate taxa but such a finding warrants further investigation.

Recently there have been four studies, which have determined amygdaloid complex volume using stereological techniques in humans. Schumann and Amaral evaluated, in a series of two papers, four AC nuclei (AB, B, L, and CE) volumes for 10 normal controls and 9 autistic individuals (Schumann and Amaral, 2005; Schumann and Amaral, 2006). They found no change in volume for the AC or nuclei between their cohort groups. In another study Berretta et al. (2007) evaluated four AC nuclei (AB, B, L, and CO) in 12 normal controls, 10 bipolar and 16 schizophrenic patients, finding the lateral nucleus to be decreased in volume in only their bipolar population (Berretta et al., 2007). They also noticed a trend (n.s.) toward a reduction in the volume of the L in their schizophrenic population. A separate study measured volumes of three AC nuclei (AB, B, and L) in a schizophrenic cohort and found reduced volume in the L and B compared to controls (Kreczmanski et al., 2007). These three studies confirm that the human L is larger than B. Their variable volume reductions in the L and B of patient cohorts and my finding that across species the basolateral complex should change at a rate dictated by the total amygdala volume, highlight the L as a nucleus which might be altered in affective disorders.

Neuron numbers of the basolateral nuclei increase at the same rate while the CE neuron numbers increase at a slower rate

I found that the basolateral nuclei cell numbers were isometric with the cell numbers in the amygdaloid complex. Once again the CE neuron numbers were

negatively allometric with the change in the total neuron numbers for the whole amygdala. The slower rate of increase in the CE is consistent with the similarly slow increase in volume. While there are limited data on amygdala volumes, there are no data containing cell numbers for the total amygdala in Old and New World primates (see Table 2.2).

Neuron number comparisons with other studies

Cell numbers have been reported for left neocortex 2.94 x 10^9 neurons (n = 4, rhesus macaque) (Lidow and Song, 2001) and for unilateral neocortex 1.35 x 10^9 neurons (Christensen et al., 2007). The former is twice as big but these numbers support a range between one and three million neurons in the rhesus cerebral hemispheres (see discussion in Christensen et al., 2007). Christensen et al. (2007) also found there to be 7.82 x 10^8 neurons (n = 4, rhesus macaque) in the parietal and temporal lobe combined (Christensen et al., 2007). Another study found there to be 2.02 x 10^8 neurons in the parietal lobe of the crab-eating macaque (n = 6) (Konopaske et al., 2007). I estimate there to be an average of 5.33 x 10^6 neurons (n = 8, Old World macaque species) in the amygdaloid complex. Since there are no published cell numbers for the amygdaloid complex, I can only abstractly compare my numbers to cell numbers from these other cortical areas. My finding of five billion neurons in the AC is smaller than the 600 billion neurons I would estimate to be in the temporal lobe (800 billion in the parietal and temporal lobes minus 200 billion in the parietal lobe).

Both changes in volume or neuron number are indicators of alterations in function and as such could play a role in disorders affecting the amygdala. Schumann

et al. (2006) found no change in volume between their cohort groups but a significant decrease in the number of neurons in the L of their autistic cohort. Berretta et al. (2007) identified a decrease in neuron number of the L (similar n.s. trend in AB) of bipolar patients, yet the volume of this region was the same as the control and schizophrenic cohorts (Berretta et al., 2007). In contrast, the study by Kreczmanski et al. (2007) found that schizophrenia patients had both a reduction in volume and neuron number in the L compared to controls (Kreczmanski et al., 2007).

Though these papers are not definitively conclusive regarding differences between patient and control groups, they do come to similar general conclusions: 1) volume alone is not enough to identify potential changes in microstructure of the AC nuclei; and 2) the lateral nucleus is probably altered morphologically in bipolar, autistic, and schizophrenic populations, and these differences likely cause functional deficits. Evolutionarily, in this cross-species study I have found that the L, along with AB and B, probably evolved together, so alterations in this "normal" development could foster develop of some psychiatric disorders.

Consistent density in four AC nuclei

In the present study, the density of neurons in the AB, B, CE, and L nuclei were found to decrease by –0.50 as the volume of the total amygdala became larger. This negatively allometric relationship means that neuron number is increasing at half the rate as the amygdala is expanding. It might seem strange that even though the CE showed different rates of change when comparing neuron numbers and volumes, it has a neuron density that is the same as the isometric basolateral complex nuclei. The rates

at which the neuron numbers and volume parameters changed in CE were similar, 0.7 for volume vs. 0.6 for neurons, resulting in a ratio that is equivalent to the basolateral complex nuclei. Here an increase in volume does not result in an equivalent increase in the number of neurons within that space.

One assumption I can make is that an increase in cortical processing requirements translates into a need for more synapses between neurons, thus volume increases to accommodate more neuropil. Investigation of synapse density in the AC nuclei would provide further insight to the components driving increases in cortical processing power. The question remains whether it is a subsequent increase in neuropil or an increase in number of cells that is needed to maintain optimum cortical processing power. My data suggest that it is the former.

Density comparisons to other studies

The closest brain region with neuron counts in Old World primates is the hippocampus. In the hippocampus the density of neurons is 0.07 x $10^6/mm^3$ in the CA1 region and 0.11 x $10^6/mm^3$ in the CA2-3 regions (Christensen et al., 2007). CA1 subregion was found to be 23.45 mm^3 and CA2-3 regions were 7.40 mm^3, n = 8 rhesus macaques. I found that AC nuclei of comparable size, CE and AB (9.59 and 19.97 mm^3, n = 4 rhesus macaques) have densities lower than reported in the hippocampus, 0.042 and 0.036 x $10^6/mm^3$ respectively, and in the entire neocortex 0.16 x $10^6/mm^3$. If I had only compared densities, I would have come to the erroneous conclusion that there is no difference, evolutionarily or otherwise, between the AB, B, L, and CE nuclei. My findings regarding CE volume and neuron number indicate that this

nucleus has developed divergently from the nuclei of the basolateral complex, and that this difference could indicate a difference in main function of this nucleus across species.

While there are no amygdala neuron density data for Old or New World primates, data does exist for humans. Both Schumann and Amaral (2006) and Berretta et al. (2007) found no change in volume but a decrease in neuron number, for L and AB nuclei respectively, that consequently resulted in a decrease in neuron density in their patient cohorts. Similar to my CE density findings, Kreczmanksi et al. (2007) report decreases in volume and neuron number that do not result in density changes in their patient cohort. This is due to the fact that the percent reduction in volume must be the same as the reduction in neuron number, which makes my CE density the same as the other nuclei AB, B, and L. This finding supports the fact that parameters such as volume, neuron number, or density, cannot be used singularly to draw conclusions about a species or a particular patient group.

Social Behavior in Old and New World primates

I was unable to compare differences in social behavior across species due to lack of species breadth. Such comparisons are important to make and should be considered in future work. I will briefly review what we know about social behavior in Old and New World primates and introduce a few a few additional species (patas monkey, squirrel monkey, and owl monkey) that would make interesting comparisons to my existing dataset.

Old World Primates

Extensive research has been done on the social organization of the Old World primates and a graded social categorization has been proposed for the macaque species (Thierry, 1990; Thierry, 2000). The four grades of social organization in the macaque species are delineated by a number of characteristics including: degree of egalitarianism, degree of respected hierarchy, level of tolerance among group members, role of kinship relations, and number of affiliative interactions (see Figure 4.1). Macaque species in grades 1 and 2, demonstrate rigid hierarchical conformity, decreased conciliatory contacts, and increased aggression. All of the species that I have evaluated are in grades 1 and 2 (rhesus macaque, crab-eating macaque, pig-tailed macaque). Macaque species that fall in grades 3 and 4 are characterized as being more tolerant, having relaxed dominance hierarchies, and increased rates of affiliative contacts. Some examples of less aggressive grade 3 and 4 macaques include the following species: *M. sylvanus* (barbary macaque), *M. arctoides* (stump-tailed macaque), and *M. tonkeana* (tonkean macaque).

A good non-macaque species for future evaluation is the *Erythrocebus patas* monkey. Patas monkeys live in groups similar in size as the other Old World primates. Unlike the macaque species, the pastas monkeys' hierarchy is stable only 25% of the time, yet this stability has no affect on rates of aggression or affiliation (Goldman and Loy, 1997; Isbell and Pruetz, 1998). The degree of hierarchy and dominance is of debate but regardless, based on the literature, I can definitively conclude that they are much less aggressive than the grade 1 and 2 macaque species. Thus, the patas monkey will be a great comparison to the highly aggressive macaques already analyzed.

Figure 4.2 Scaling of macaque social organizations

1st GRADE			2ND GRADE			3RD GRADE			4TH GRADE		
Taxa	Common Name	Phyletic Lineages	Taxa	Common Name	Phyletic Lineages	Taxa	Common Name	Phyletic Lineages	Taxa	Common Name	Phyletic Lineages
M. mulatta	Rhesus	Fascicularis	*M. fascicularis*	Longtail	Fascicularis	*M. arctoides*	Stumptail	Sinica-Arctoides	*M. tonkeana*	Tonkean	Silenus-Sylvanus
M. fascata	Japanese	Fascicularis	*M. nemestrina*	Pigtail	Silenus-Sylvanus*	*M. sylvanus*	Barbary	Silenus-Sylvanus	*M. maurus*	Moor	Silenus-Sylvanus
						M. silenus	Liontail	Silenus-Sylvanus	*M. nigra*	Crested	Silenus-Sylvanus
						M. radiata	Bonnet	Sinica-Arctoides	*M. nigrescens*	(Gorontalo)	Silenus-Sylvanus
						M. sinica	(Toque)[1]	Sinica-Arctoides	*M. hecki*	(Heck's)	Silenus-Sylvanus
						M. thibetana	(Tibetan)	Sinica-Arctoides	*M. ochreata*	(Booted)	Silenus-Sylvanus
						M. assamensis	(Assamese)	Sinica-Arctoides	*M. brunnescens*	(Muna-Butung)	Silenus-Sylvanus

BEHAVIORAL CHARACTERISTICS:			
↑	Aggression	↓	Aggression
↓	Rates of Affiliative Contacts	↑	Rates of Affiliative Contacts
↑	Dominance Gradient	↓	Dominance Gradient
↑	Female Preference for Relatives	↓	Female Preference for Relatives
↑	Female Protectiveness of Infants	↓	Female Protectiveness of Infants
↓	Shared Care of Infants	↑	Shared Care of Infants

*Only exception to phylogeny explaination of the scale.

[1](indicates evidence is based only on a few behavioral traits)

Adapted from B. Thierry in Natural Conflict Resolution (2000)

New World Primates

Studies on social characteristics in New World primates are sparse. In particular when considering aggression and subsequent post-conflict affiliation not much is known about these interactions in New World primates. Reconciliation is defined as an increase of affiliative contacts between 2 former opponents shortly after a conflict. This type of post-conflict affiliation has been found in the great apes, Old World matrilineal groups (including baboons, mangabeys, mandrills, guenons, patas monkeys, and macaques), Prosimians *Lemur catta* and *Eulemur fulvus rufus*, and the New World *Cebus apella* (Westlund et al., 2000). Westlund et al. (2000), contrary to their hypothesis, found that the *Callithrix jacchus*, despite its low levels of aggression, did not reconcile more often than other species (New World Monkey: *C. jacchus* 31%; Old World Monkeys: *M. arctoides* 41%, *M. mulatta* 9%, *M. maurus* 40%, *Colobus guereza* 50%, and *Theropithecus gelada* 30%). The marmoset, *C. jacchus*, lives in small groups that contain a monogamous pair plus their offspring. The "family" forages together, shares food, travels, and works together to protect the group against predators. Aggression in this species focuses on either a struggle over an object or an aggressive vocalization, which generally also involve biting, slapping, wrestling, or chasing.

The dominance style of the marmoset is more relaxed than in the macaque species. Fellow New World primates, *Saimiri sciureus* (squirrel monkey) and *Brachyteles arachnoids* (woolly spider monkey) also display a less aggressive stature (Baldwin, 1992; Strier, 1992). The squirrel monkey forms large troops that live in

expansive home ranges, which are 2 characteristics that are very similar in the Old World macaque species. In contrast, the squirrel monkey is small, average 900 g, in body size and they perform no social grooming that is a common social activity in Old world primates and the New World capuchin. Within captive squirrel monkey groups, the greater the intensity of the conflict the greater chance for affiliative contact (78% for high intensity levels), or reconciliation, after said confrontation (Pereira et al., 2000). This evidence would support the hypothesis, put forth by Westlund (2000) that less aggressive species have higher rates of reconciliation when conflict does arise. There still remains the question of why there is a difference between the New World marmoset and squirrel monkeys—social group size might contribute to these differences in reconciliation behavior since the marmoset has a much smaller social group size than the squirrel monkey.

The owl monkey has a group size similar to that of the marmoset and this is a good reason to compare the 2 New World species. Yet, this comparison is not ideal because the owl monkey, *Aotus trivirgatus*, is the only nocturnal monkey and the difference in visual processing could also be reflected in the amygdala's lateral nucleus since it receives sensory input from V1. Owl monkeys live in monogamous family groups, similar to the marmoset, with the male as the primary caregiver of the young, and they are thought to be highly aggressive both in captivity and in their natural environment (Hunter and Dixson, 1983; Wright, 1978). The only quantitative study in the literature found that wild owl monkeys spend 53% of their time feeding, 22% of its time resting, 21% of its time traveling, and 4% of its time in agonistic

activity (Wright, 1978). Agonistic activity ranged from alarm calls and direct encounters with conspecifics.

The New World capuchin monkey spend the majority of their time, when not foraging, in social grooming situations. Because the capuchin has a social system that closely resembles that of the macaque species, they are an ideal group to use as a comparison between the Old and New World primate orders. One area in which they differ from Old World monkeys is social grooming, also called allogrooming and is defined as a form of cooperative behavior. Capuchin dominant members groom subordinate members, which is the opposite of grooming patterns in the macaque species, and more often grooming occurs across within the same rank, e.g. not as often between low-ranking and high-ranking individuals (Parr et al., 1997). Another difference between capuchin and the Old World primates is the temporal reconciliation patterns; Old World primates often reconcile within the first 5 minutes following conflict while the New World capuchins wait between 5-20 minutes before reconciliation (Verbeek, 1997).

All the species discussed above represent a range of social behaviors within the New and Old World primate orders and would allow for adequate control comparisons between species and across taxa. Across species I would predict that individuals with larger amygala's would have larger social group sizes. Within species, e.g. the four grades of macaques, I would predict that greater aggression levels would correlate with increases in volume of the BLD.

Summary: All in a Nut[1]

I have evaluated neuron numbers and volumes of amygdala nuclei in 13 Old and New World primates. In short, I found that the central nucleus volume is negatively allometric, while the accessory basal, basal, and lateral nuclei are isometric or changing at the same rate as the entire amygdaloid complex. In conjunction with these volume changes the central nucleus neuron numbers increase at a slower rate than the neurons in the three nuclei of the basolateral complex. Finally, the neuron density remains consistent across all four nuclei evaluated.

My data indicate that the central nucleus may have evolved differently from the three other amygdala nuclei. The isometric relationship between the neuron numbers and volume of the basolateral complex nuclei and the AC lend support to the idea that these nuclei evolved together. When viewed in a larger context that includes existing data from great apes and humans my findings suggest that neuroanatomical changes in the central nucleus could underlie social behavior and consequently psychopathologies known to encompass functional changes in the amygdala.

[1] Signature song on album Heavy Mental by the rock band The Amygdaloids. Amygdaloids T. 2007. Heavy Mental: All in a Nut. New York City.

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