ABSTRACT

Title of Dissertation: CHOLINERGIC CONTRIBUTIONS TO EMOTION REGULATION

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Theories based on clinical and neuroanatomical studies implicate the muscarinic cholinergic system in normal and pathological emotion regulation. Emotional and sensory experiences can be induced with intravenous administration of the local anesthetic procaine hydrochloride, which selectively activates limbic regions in humans and animals. Procaine has a high affinity for muscarinic cholinergic receptors *in vitro*. This research tests three hypotheses: (1) procaine binds to muscarinic receptors *in vivo*; (2) procaine alters functional connectivity among cholinergic brain regions and their targets; and (3) procaine-induced emotions are related to core cholinergic regions.

In Experiment I, anesthetized rhesus monkeys underwent positron emission tomography (PET) studies before and after administration of six doses of procaine on separate days using a radioligand with preferential binding to muscarinic M_2 receptors ([^{18}F]FP-TZTP). Procaine blocked [^{18}F]FP-TZTP in a dose-response fashion uniformly across the brain, while significantly increasing tracer flow in limbic compared with non-limbic regions.

In Experiment II, behavioral and physiological measures were assessed at baseline and following procaine in 32 healthy controls and 15 patients with bipolar disorder undergoing [^{15}O] PET yielding regional cerebral blood flow (rCBF). Procaine selectively increased rCBF in anterior paralimbic regions in healthy controls, but to a lesser degree in patients. Regions connected via cholinergic

pathways showed significantly different functional connectivity in both groups with procaine, however, prefrontal regions showed differential functional connectivity with cholinergic brain regions in patients compared with controls. Changes in activity of cholinergic regions explained the variance in anxiety ratings in an opposite manner in each group, and in euphoria ratings only in patients.

In conclusion, procaine binds directly with muscarinic receptors *in vivo* while selectively increasing limbic activity in anesthetized monkeys. Two key findings herein procaine-induced alterations in functional connectivity of core cholinergic regions in humans, and the association of core cholinergic regional activity with emotional experience support theories implicating cholinergic contributions to emotion regulation. Decreased anterior paralimbic activity and altered functional connectivity of cholinergic regions in patients with bipolar illness compared with controls revealed by procaine offers additional insight into the regional neurobiology of the disease, and may ultimately be targeted in therapeutic approaches to bipolar disorder.

CHOLINERGIC CONTRIBUTIONS TO EMOTION REGULATION

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Dedication

This work dedicated to my mother, Emmie C. Benson, and my father, Rush T. Benson, whose interests in sociology and engineering, respectively, unwittingly contributed to my curiosity in brain-behavior relationships.

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List of Abbreviations

A1 .....primary auditory cortex
AC .....anterior cingulate
ACh .....acetylcholine
AChE.....acetylcholinesterase
AChNet.....cholinergic neural network
ACTH .....adrenocorticotropic hormone
AINS.....anterior insula
AIR.....automated image registration
AM .....amygdala
AMPA .....alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
ARAS .....ascending reticular activating system
BA .....Brodmann area
BBB.....blood brain barrier
BD .....Bipolar Disorder
BDI.....Beck Depression Inventory
BF.....basal forebrain
BL.....baseline
BP.....binding potential
BP1 .....binding potential at baseline
BP2.....binding potential with procaine
BPI.....subtype I of bipolar disorder
BPII .....subtype II of bipolar disorder
C_0.....initial radioactivity level
Ca^{++} .....calcium
CA1-CA4.....Cornu Ammonis – hippocampus proper
cAMP .....cyclic adenosine monophosphate
CBF .....cerebral blood flow
CCK.....cholescystekinin
ChAT.....choline acetyl transferase
Ch2-VLDB .....cholinergic portion of the vertical limb of the diagonal band
Ch3-HLDB .....cholinergic portion of the horizontal limb of the diagonal band
Ch4-NMB .....cholinergic portion of the nucleus basalis of Meynert
CI-979.....milameline
CNS .....central nervous system
CRF .....corticotropin releasing factor
DA.....dopamine
DIA.....diastolic blood pressure
DLPFC.....dorsolateral prefrontal cortex
DMPP .....dimethylphenylpipereinium
DRE.....region of interest based on the work of Drevets et al., 1998
ΔBP .....change in binding potential

ΔBPmax.....maximum change in binding potential
EEG.....electroencephalogram
EKG.....electrocardiogram
fMRI.....functional magnetic resonance imaging
GABA.....gamma-aminobutyric acid
gCBF.....global cerebral blood flow
GE.....General Electric
GLU.....glutamate
GP.....globus pallidus
GSR.....galvanic skin response
HDRS.....Hamilton Depression Rating Scale
HIV.....human immunodeficiency virus
HLDB.....horizontal limb of the diagonal band
HR.....heart rate
HVA.....homovanillic acid
IC_{50}.....inhibitory concentration at 50% blockade
ICV.....intracerebroventricular
ITI.....inter-trial interval
K^+.....potassium
K_1.....radiotracer delivery
$K_{1\text{-base}}$.....radiotracer delivery at baseline
$K_{1\text{-proc}}$.....radiotracer delivery with procaine
k2.....clearance rate
Kd.....dissociation constant
Ki.....inhibitory concentration
[L].....ligand concentration
LAMP.....limbic associated modulatory protein
LOFC.....lateral orbitofrontal cortex
M1.....primary motor cortex
M2.....secondary motor cortex
M_1-M_4.....muscarinic receptor subtypes
mAChR.....muscarinic receptors
MAOI.....monoamine oxidase inhibitor
MAY.....region of interest based on the work of Mayberg et al., 2002
mCi.....milli-Curie
MOFC.....medial orbitofrontal cortex
MPFC.....medial prefrontal cortex
MRI.....magnetic resonance imaging
Na^+.....sodium
nAChR.....nicotinic receptors
NADPH-d.....nicotinamide adenine dinucleotide phosphate
NE.....norepinephrine
NGF.....nerve growth factor
NBM.....nucleus basalis of Meynert
NPY.....neuropeptide Y
PAG.....periaquaductal grey

pCO_2 .....partial concentration carbon dioxide
PET.....positron emission tomography
PGAC .....pregenual anterior cingulate
PI .....phosphotidylinositol
PR.....procaine
PTSD .....post-traumatic stress disorder
QNB .....quinuclidinyl benzilate
rCBF .....regional cerebral blood flow
$r_{controls}$ .....Pearson product-moment correlation for controls
$r_{patients}$ .....Pearson product-moment correlation for patients
r_{bd}.....Pearson product-moment correlation for bipolars
r_c .....Pearson product-moment correlation for controls
r_p .....Pearson product-moment correlation for procaine
r_{bl} .....Pearson product-moment correlation for baseline
ROI.....region of interest
RR .....respiration rate
S1 .....primary somatosensory cortex
SEP.....septal nuclei
SGAC .....subgenual anterior cingulate
SI .....substantia inominata
SPM99 .....statistical parametric mapping, version 99
SSAS .....Spielberger State Anxiety Scale
SSRI .....selective serotonin reuptake inhibitors
SYS .....systolic blood pressure
$t_{1/2}$ .....half-life
T1 .....MRI specification
TLC .....thin layer chromatography
TCA.....tricyclic antidepressant
TEG .....tegmentum
TP .....temporal pole
V.....volume of distribution
V1 .....primary visual cortex
V_{base} .....volume of distribution at baseline
V_{proc} .....volume of distribution with procaine
VApc .....ventroanterior pars compacta
VLDB .....vertical limb of the diagonal band
VLPFC.....ventrolateral prefrontal cortex
YMRS.....Young Mania Rating Scale
6-OHDA 6-hydroxydopamine
5,7-DHT.....5,7-dihydroxytryptamine
5-HT .....serotonin
3D SPGR .....3 dimensional - spoiled gradient echo pulse sequence
2-DG.....2-deoxyglucose
[^{18}F]FP-TZTP.....3-(3-(3[^{18}F]fluropropylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methypyridine
[^{11}C]-NMPB.....[^{11}C]N-methyl-4-piperidyl benzilate

Chapter 1: Introduction

> "There can be no knowledge without emotion. We may be aware of a truth, yet until we have felt its force, it is not ours. To the cognition of the brain must be added the experience of the soul."
>
> Arnold Bennett (1867-1937), British Novelist, 18 March1897

Relating emotion regulation to the neuromodulatory effects of the muscarinic cholinergic system may prove to be challenging, given the complex nature of emotions. Emotions involve cognitive, motivational, expressive, and physiological elements that can vary across time, according to environmental cues, and within and between individuals. Relying on any single entity to explain behavior may be viewed as reductionistic. However, the muscarinic cholinergic system has been tied to learning and memory (Bartus et al., 1982; Gold, 2003), attention (Wenk, 1997; Robbins and Everitt, 1995), consciousness (Delacour et al., 1997; Perry et al., 1999), and emotions (Mesulam, 1995). Mesulam describes the cholinergic projections as the "pathway likely to constitute the single most substantial regulatory afferent system of the cerebral cortex."

The muscarinic cholinergic system may serve as a bridge between emotional experience and underlying neurophysiological activity. In this regard, Sarter and colleagues (2001) suggests that cholinergic modulation may contribute to the top-

down optimization of task- or modality-specific information. This system is associated with fear-conditioning of the amygdala and loss of fear response with basal forebrain (BF) lesions (LeDoux, 1996), is implicated in arousal and attention (Robbins and Everitt, 1995), is localized in key regions known to mediate emotion (Mesulam, 1983; Flynn and Mash, 1993), and is hypothesized to be dysfunctional in affective disorders (Janowsky, 1972a).

The current impetus for the focus on the muscarinic cholinergic system is derived from a small body of literature linking the cholinergic system to limbic activity — where limbic is defined inclusively as relating directly to emotions and emotional behavior, as well as the autonomic and motivational connotations. The key points are summarized as follows. (1) David Janowsky and colleagues (1972a) theorized that there might exist an imbalance of cholinergic and adrenergic influences on limbic regions in patients with mood disorders based on his studies of cholinergic challenges in these patients (Janowsky et al., 1972b). (2) The identification of cholinergic receptors in the amygdala, a region theorized by many to be integral in emotional processing (Amaral et al., 1992). (3) The complex theory of emotion regulation developed by LeDoux (1996) suggests a role for the cholinergic system. (4) Animal studies showing increased limbic activity with procaine hydrochloride (Post et al., 1981), a drug considered to be a local anesthetic but also known to bind with muscarinic cholinergic receptors *in vitro* (Hisayama et al., 1989; Sharkey et al., 1988). (5) Anecdotal reports of emotional experiences occurring after local anesthesia with Novocain (procaine hydrochloride) during dental procedures and after routine antibiotic treatment with procaine-penicillin (Araskiewicz and Rybakowski,

1993). (6) Procaine induces selective cerebral blood flow hyperperfusion in the anterior paralimbic structures in the humans together with brief intense emotional and sensory experiences (Ketter et al., 1996). These effects suggest an avenue for examining the neuropsychopharmacology of emotional experiences, and possibly tying emotion to muscarinic cholinergic system more directly.

This dissertation examines the role of the muscarinic cholinergic system in emotion regulation and how the neuroanatomy, neurochemistry, and neurobiology of this system position it as a potential player in mediating emotion. Specifically, this work will explore how the drug procaine can induce these emotional and sensory experiences via a muscarinic mechanism. This will entail two experiments. A dose-response study with anesthetized rhesus monkeys undergoing positron emission tomography (PET) with a radioligand with preferential affinity for M_2 muscarinic cholinergic receptors will address the ability of procaine to bind to muscarinic receptors *in vivo*. As presented in Chapter 2, procaine does bind to these receptors at the same time the flow of the radioligand increases preferentially in limbic areas. In view of the robust emotional and sensory experiences induced by procaine in humans, an important question emerges regarding the relationship of brain regions known to have direct and indirect cholinergic innervation to these behavioral effects.

The study of emotion can be examined in several ways, including inducing emotions, either through self-induction or pharmacological induction paradigms, and by comparing normal versus pathological emotions. Both of these methods were employed in experiments presented in Chapters 3 and 4, where procaine administration in healthy controls is compared to patients with bipolar disorder during

measurement of regional cerebral blood flow (rCBF) with PET. The results suggest that the functional connectivity between cholinergic regions and their primary targets increases with procaine administration. In addition, procaine-induced changes in cerebral blood flow (CBF) in cholinergic regions are associated with changes in emotions.

Before beginning a detailed review and analysis of the problem, definitions of emotion and mood provide an understanding on the use of these related constructs throughout this work. The Oxford English Dictionary defines emotion as: "1. a moving out, migration, transference from one place to another; 2. a moving, stirring, agitation, perturbation (in a physical sense); 3. a political or social agitation, a tumult, popular disturbance; 4. a. any agitation or disturbance of mind, feeling, passion, any vehement or excited mental state; b. a mental 'feeling' or 'affection' (e.g., of pleasure, or pain, desire or aversion, surprise, hope, or fear, etc) as distinguished from cognitive or volitional states of consciousness."

The word emotion is derived from the Latin *exmovere*, meaning to move out or away, and is closely related to the English verb *to emote*. This suggests emotion can originate from an action viewpoint. There are important cognitive, physiological, and psychological components to emotion, as well. The over one hundred definitions of emotion (Kleinginna and Kleinginna, 1981) can be categorized by the experiential, behavioral, cognitive, motivational, or physiological nature of their roots. These various approaches to defining emotion illustrate the diversity in theories of emotions and its regulation.

For purposes of this work, definitions of emotion and mood will be as follows. Emotion can be defined as a patterned subjective experience of affective and bodily responses that vary according to valence, intensity, expression and arousal, and may include cognitive awareness of the process. Emotions are considered brief affective responses and their effects are thought to specific.

Mood is defined as a diffuse state of arousal that functions as a framework that can influence affective, cognitive and behavioral processes. It may or may not be the focus of attention. Generally, its effects are thought to be global and pervasive. The recognition of meaning and significance of events leading to arousal facilitates a self-regulatory process that reflects appraisal of life circumstances.

The example of anxiety suggests maybe semantics are also involved. Depending on the context, we understand anxiety to be an emotion or a mood. Thus, the label attached does not imply categorization into emotion, mood or temperament. The intensity of the emotion anxiety usually more severe and cannot be maintained as long as a mood of anxiety. On some, but not all, levels the quality of the experience is similar.

As compared to emotion, moods are thought to be typically less intense affective states and more time enduring. In mood disorders, the temporal domain is significantly altered where moods can extend for weeks or years. Moods are considered less variability and more restricted range of intensity and arousal level than emotions. Moods are thought to be involved in the instigation of self-regulatory processes. Mood can be comprised of set of emotions, and thus may be a more general state; for example, contrast an emotion of anger with the mood of irritability.

Some, but not all, researchers believe that emotion and moods also differ with respect to intensity, with moods are typically less intense than an emotion.

Ketter et al., (2003) has further differentiated mood and emotion by the presence or absence of precipitants, degree of autonomic arousal, the resulting products, and possible neural substrates. Thus, in general, emotions tend to be brief, distinct, and intense states that can occur in response to an acute precipitant, are accompanied by autonomic arousal, result in some form of action, and may be subserved by anterior paralimbic and brainstem structures. On the other hand, moods are more pervasive, composite and moderate conditions that may or may not have precipitants, can vary in the level of autonomic arousal, usually result in cognitions, and may be mediated by anterior cortical and limbic structures.

An examination of current emotion theory begins with a brief review of its historical roots in philosophical, psychological, and physiological perspectives (Section 1.1), and is followed by a detailed examination of important neuroanatomical (Section 1.2) and neurobiological (Section 1.3) substrates believed to be involved in emotional processing. As suggested above, emotion theories can be quite diverse. Discussion of current emotion theory (Section 1.4) will be limited to those more closely related to the theme of this dissertation. Finally, specifics relating the cholinergic system to emotional processing will lay the foundation for the research presented herein (Section 1.5).

1.1 Historical Perspectives[1]

A philosophical approach dominated very early discourses on emotion. The study of emotion began simply as identification of emotions as a field of interest. Philosophical discussions about the interface between emotions and the body followed the discourse on conscious thought, ranging from those who believed there was a physical basis for consciousness to dualist theories, a separation of the mind and body. Aristotle (367 BC) was not a dualist and believed that the body and psyche are one. In *Rhetoric*, he recognized the influence of emotion on "bonds of association," i.e., that emotion has a special strength on enhancing associations of the mind. Hippocrates (460 BC) connected conscious life, including emotions, to the brain. Herophilus (300 BC), based on dissections of human and animal brains, proposed the brain as the center of the nervous system and the *seat of intelligence*. Galen (170 AD) believed the soul was in the ventricles of the brain. He advocated the humoral theory of temperaments, or the four humors – blood/sanguine (warm-hearted and volatile), black bile/melancholic (sad), yellow bile/choleric (quick to anger and to action), and phlegm/congestion (sluggish). This model remained prominent for centuries and gave rise to terms describing temperament that are still commonly used.

Plato (427 BC), as a dualist, believed that emotions of desire and appetite were part of the irrational soul. Some early philosophers emphasized a distinction

[1] Information in this section is drawn from readings in *Nineteenth-Century Origins of Neuroscientific Concepts* (Clarke and Jacyna, University of California Press, 1987); *The Emotional Brain* (LeDoux J, Simon & Schuster, 1996); and *Great Psychologists*, (Watson RT, JB Lippincott Press, 1963).

between emotion and reason, believing that man was rational and knowing, with emotions to be part of our primitive animal spirits (nervous energy associated with physical sensation and movement) that must be minimized; reason was choice and emotion was instinct. Descartes (1596) set forth an extreme form of dualism, separating reason and emotion, stating that it was "the effect of body on the mind which produces passions." Nonetheless, he also presented a more advanced physiological explanation of the animal spirits basic in human behavior. This was achieved by sensing the environment via the sense organs and nerves thereby creating an impression on the pineal gland in the brain, where the soul apprehends this stimulus, and causes the animal sprits to activate the passions, the "bodily commotion" viscera and overt action or movement. These ideas predate James-Lange theory of emotion that "primacy is given to physiological processes prior to mental experience", but may be more in line with the thinking of Cannon because the lack of visceral input into the brain in his model.

Descartes considered the primary passions to be admiration, love, hatred, desire, joy, and sadness, and these could be combined to create introspective feelings or emotions. They served to cause 1) the flow of animal spirits, 2) the body to held ready for goal objects encountered in the environment 3) the soul to desire the these objects and 4) a persistent desire for these objects. Although, his beliefs that emotion debilitated the superior rational response by intervening between the environmental cues and the response eventually fell out of favor, he introduced a more sophisticated level of physiology into theories of emotion than his predecessors, and he set up a tradition of categorizing emotions as either basic or composite.

More recently, the physical basis of conscious thought was pursued in parallel with the development of psychological theories describing the constructs of thought and emotion and medical models of pathological emotional processing. Wundt (1874), who many consider as the father of modern psychology, promoted the view that combinations of feelings and ideation yield emotions. He thought emotions could be broken down into elements, with a direct analogy to chemical analysis of substances, or the way pure tones could be broken down into loudness, pitch, density and volume, and thus may have been influenced by Helmholtz. Through introspection and training subjects reported the "elements" of their emotions, and from these studies Wundt proposed a three-dimensional theory, pleasantness-unpleasantness, excitement-quiet, and tension-relaxation. Dimensional approaches to the study emotion are prevalent in many current theories, including Plutchik (1993) and Davidson (2002).

At this time, psychiatry was just becoming recognized as a specialty of medicine. Emil Kraeplin, a student of Wundt, is credited with the most astute differentiation and descriptions of what is now known as schizophrenia and bipolar disorder, which are still referred to today. In the early 1900's, Jung examined the association of affective determinants by studying the emotional preoccupation of his psychiatric patients through their associations of a list of 100 words with the ultimate goal to uncover the unconscious. Concurrently, Freud in his study of unconscious behavior developed his personality theory and the psychoanalytic approach, both of which were based on examining the pathological emotional experiences of his patients. These psychoanalytic approaches were important because they stressed the

experience as an integral component of emotion, but did little to advance neuroanatomy and neurochemistry of affect.

Franz Josef Gall popularized localization of function to specific areas of the brain, of which the holy-grail would be notion of the "grandmother cell", with phrenology. Based on the bumps on the skull, different emotions were to be controlled by various parts of the underlying brain. For example, love was localized in the occiput, lust the base of the occiput, and aggression (rage) the temporal region. Flourens criticized this point of view, believing the whole cerebrum was needed for conscious thought, including emotions. Thus, a controversy of distributed networks versus focal control emerged.

In the late 1800's Hughlings Jackson (1876/1931) described change of affect with right hemisphere damage resulting in negative (loss) and positive ("conservation of next most voluntary") changes in brain function, which he termed "dissolution." The term dissolution was used, acknowledging Spencer, as the opposite of evolution, a reduction of automatic movement. Broca showed the loss of speech could be localized in the "third frontal convolution of the left hemisphere." These contributions are reflected in current theories that specific functions, including emotional behavior, are attributable to specific areas of the brain, and in the broader context of whether the structure/function relationships can be defined in terms of localization versus distributed networks. Moreover, asymmetry in brain function is a central feature to the current work of Davidson (2002).

Theories of relationships between physiological and psychological aspects of emotional behavior became more intertwined to yield many current emotion theories.

Several investigators highlight this progress from a physiological point of view. Darwin (1872/1965) believed emotional expressions served practical functions. For example, dogs bare their teeth in preparation to bite. This contrasts with Charles Bell's idea that the behavior is for emotional expression. Darwin defined 3 principles of emotion expression: 1) the principle of serviceable habits – many expressive movements are vestiges of originally practical movements (ex: startle as a remainder of a larger flight reaction); 2) the principle of antithesis – opposite impulses tend to show opposed movement (cat's moves of affection are opposite of attack movements); and 3) direct actions of the nervous system (ex, trembling as an overflow into motor channels); these were originally voluntary movements that evolved into reflex actions through inherited habits.

Spencer, a contemporary and associate of Darwin, developed a theory of evolution based on philosophical, anthropological and geological arguments preceding Darwin's (1859); he may have even coined the phrase "survival of the fittest." He studied the association of the elements of "feelings" and "relations between feelings." He saw emotions as centrally initiated feelings that are related to psychological processes, to physiology, and to the adjustment of the psychological processes to environmental influences.

In 1884, James wrote the "bodily changes follow directly the PERCEPTION of the existing fact, and that our feeling of the same changes as they occur IS the emotion." His theory rested on the notion that visceral activity yields emotion and limited his studies to emotions with distinct bodily expressions. He and C.G. Lange thus helped establish the organic basis of emotion espoused in *The Emotions*. In

contrast, Cannon and Bard claimed the viscera were not sensitive enough to differentiate emotions, and react too slowly to be responsible for rapid emotions, and noted emotions still occur even after vagotomies and sympathectomies.

However, the James-Lange theory was a stimulus to move emphasis away from a philosophical approach towards psychology and physiology. Maybe more importantly, their work included a role of voluntary muscles in the expression of emotions that laid the groundwork for research examining facial expressions and posture in emotions. Cannon and Bard proposed the hypothalamic theory – emotion is produced by the hypothalamus that mediates input from the environment with cortical efferent activity to control the somatic and visceral motor systems – which served to initiate research the neurophysiology of emotion. The hypothalamus is still considered an important component of emotion theories.

In the early 1900's psychology emphasized the development of systematic methods of defining, measuring and describing psychological phenomena. As psychological events could not be measured overtly, the study of emotions was significantly disadvantaged, leading to the dominance of behaviorists. Watson (1930) stated emotions are bodily reactions to specific stimuli, not experienced states. Based on his studies of infants he proposed three basic emotions – fear, rage and love, and more complicated emotions were not inherited, but learned. Pavlovian and Skinnerian principles gained influence, linking environmental events to motivation through conditioning, what is now appreciated as a fundamental brain process. Although, behaviorism expressed an extremely limited view of emotional behavior,

these associative principles served as a foundation for more complex notions, like the fear-conditioning model of amygdalar function by Davis (1995) and LeDoux (1996).

Various portions of these early theories have contributed to the development of current theories of emotion. Subsequent researchers increasingly incorporated brain structure, and its physiology as integral components in theories emotion. In the following sections will review the neuroanatomy and neurobiology of emotion regulation before continuing with a discussion of current emotion theory.

1.2 Neuroanatomy of Emotion

The limbic system is considered a collection of neuroanatomical structures that contribute significantly to emotion regulation. In addition to mediating emotional behavior, the limbic system plays a key role in endocrine regulation (via the hypothalamus), as well as contributing to motivational and memory functions. The term *cerebri limbus*, which was first used by Willis (1664), describes the cortical border surrounding the brainstem that corresponds to the cingulate gyrus. Broca referred collectively to the cingulate gyrus, anterior olfactory region and hippocampus as the *grand lobe limbique*, however, he associated it with the closely related function of olfaction, rather than directly with emotion regulation. The cytoarchitecture of limbic cortex was initially described by Ramon y Cajal (1900, 1909) and Golgi (1883, 1903), when the field of neuroanatomy blossomed with their development of neuronal staining techniques.

Papez (1937) extended the Cannon-Bard theory of emotion regulation (Cannon, 1927, 1929, 1931; Bard, 1928, 1929, 1934) by combining known neuroanatomical connections of the limbic regions with the results of clinical studies (Penfield, 1934; Ranson, 1934) describing emotional disturbances after lesions of the cingulate and medial structures. His neuroanatomical circuit described emotional processing as part of his three-part theory of "man's volitional energies", which encompassed the affective, cognitive, and motoric components of experiencing emotion. He may have been one of the first investigators identifying the cerebral cortex as the locus of the cognitive component of feelings that arise from the various aspects of the affective experience. Previous work attributed cognitive aspects of emotion to subcortical regions, the hypothalamus (Bard 1928) and the thalamus (Masserman1943).

Yakovlev (1948) proposed a three-layer model of brain organization that integrated structure-function relationships with evolutionary considerations. Functions of emotion and affect occurred in the "intermediate layer" that also included functions of motor synergy, personality, arousal, and motivation. These functions were thought to be subserved by the basal ganglia, limbic thalamus, hippocampus and olfactory areas of orbitofrontal cortex (olfactory paleocortex).

Yakovlev's phylogenetic designation of this "intermediate" layer developing with the emergence of reptiles to early mammals preceded the triune brain theory of MacLean (1949, 1952). In the elaboration of three tiers of nervous system

development, MacLean[2] considered the middle paleomammalian stage, or "limbic system," the regions of the Papez circuit with the medial structures of Yakovlev. Integrating clinical and animal of both lesion and stimulation studies, MacLean's work attributed motivation and emotional expression to three cortical regions, the orbital frontal cortex, temporal lobe and the insula, and two subcortical structures, the amygdala and the dorsomedial thalamus.

Currently, the limbic system is generally considered to be a collection of cortical and subcortical structures, including the cingulate, parahippocampal gyri, hippocampus, amygdala, septal nuclei (SEP), and hypothalamus, and parts of the anterior nucleus of the dorsal thalamus and dorsal striatum. Nauta (1958) includes medial tegmental nuclei of the midbrain because of the reciprocal connections with the amygdala and hippocampus. Heimer argues that the ventral striatum should also be considered part of the limbic system (2003). While each of these regions may participate in other functions, together in processing internal and external information

[2] MacLean's indication as these regions as a middle stage of CNS development between reptiles and to mammals implied that mammals were direct descendents of reptiles and appears to be founded on the *scala naturae,* a hierarchical theory of evolution. However, comparative neuroanatomists do not accept this view. It is more accurate to say that mammals and reptiles share a common ancestor among the ancestral amniotes. While the association of evolutionary progress to more sophisticated function loosely works, the *scala naturae* is not accepted today because the rankings are based on homocentric value judgments not biology; discrepancies exist, for example, some so-called "lower vertebrates" that have acquired sophisticated sonar, radar, or electrosensory systems that are absent in so-called "higher vertebrate" class mammals.

they attach emotional valence to the organism's circumstance and generate the experience of emotions, cognitively and physically.

Several regions of the limbic system important to this thesis will be discussed in detail, including the cholinergic basal forebrain nuclei, the anterior cingulate and the amygdala. The cholinergic portion of the basal forebrain nuclei consist of the nucleus basalis of Meynert (NBM), and the nucleus of the horizontal limb of the diagonal band (HLDB); collectively, these regions are sometimes referred to as the substantia inominata (SI). In neuroimaging studies, the anterior cingulate (AC) and the amygdala (AM) are the structures most commonly activated in tasks where emotions are self-induced (Ketter, et al. 2003). The basal forebrain nuclei innervate the amygdala and cingulate cortex, the orbitofrontal cortex, insula, thalamus, and hypothalamus, as well as brainstem monoaminergic nuclei that are implicated in mood regulation. The discussion will end with two regions, the thalamus and the striatum. These regions have areas within well known to mediate limbic functions and considerable connections with core limbic areas, but they also have considerable cholinergic activity as well. While the thalamus has long been considered to have limbic functions, only recently has the striatum been appreciated in this regard (Alexander et al., 1990).

Because of the direct relevance of this subject matter to humans, the majority of the neuroanatomical findings reviewed are based on the species most closely-related to humans for which there is a significant database, old-world *Macaca* monkeys, either *Macaca mulatta* or *Macaca fascicularis*[3]; any departure will be

[3] These two *Macaca* species will have similar connections between brain regions, however, there will be differences with respect to cytoarchitecture. (Saunders R, personal communicaton)

noted. Utilization of non-human primates thought to be closer to humans, such as the chimpanzee or the gorilla, has not been customary, because they are considered an endangered species, the use of these animals is cost-prohibitive, or in the case of the gorilla, their size and strength makes them less manageable in a laboratory setting. The literature concerning the neuroanatomy of the rat is extensive, and in general, concurs with the monkey literature, however, there exists some meaningful differences in cytoarchitecture, pathways, and neurochemistry that make it a less optimal reference to human neuroanatomy. In particular, the connectivity of the amygdala differs somewhat between the two species (Aggleton and Saunders, 2000; Amaral et al., 1992). Nomenclature used throughout this dissertation as depicted in Figures 1 and 2 follow the work of Brodmann (1909) and Bonin and Bailey (1947) for human and monkey neuroanatomy, respectively. Identification of brain regions (Table 1) by using Brodmann areas (BA) is are common in brain imaging research. In rare cases the nomenclature of von Economo (1929) is used.

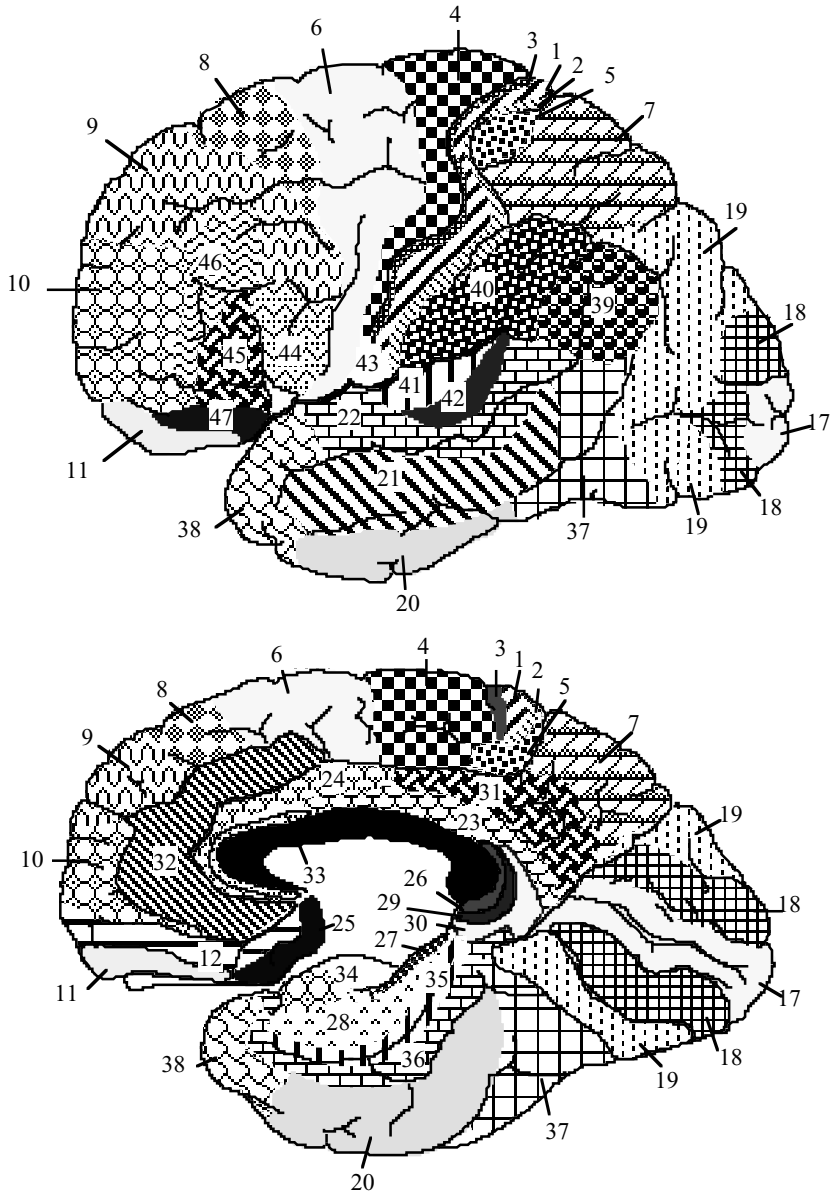


Figure 1. Cytoarchitectural Map of the Human Cortex.
Brodmann (1909) segmented the cortex using cytoarchitectonics. His nomenclature is used throughout clinical and preclinical work. Top: lateral surface. Bottom: medial surface. Black region represents the corpus callosum.

Table 1. Cortical Names of Brodmann Areas (BA)

BA	Cortical Region
1, 2, 3	Primary somatosensory cortex
4	Supplementary motor cortex
5	Parietal association cortex
6	Primary motor cortex
7	Parietal association cortex
8	Frontal eye fields
9, 10	Dorsolateral prefrontal cortex
11	Orbitofrontal cortex
12	Orbito- and ventrolateral prefrontal cortex
17	Primary visual cortex
18, 19	Secondary visual cortex
20, 21	Inferior and Medial temporal cortex
22	Auditory association cortex
23, 31, 33	Posterior cingulate
24, 25	Anterior cingulate
26, 29, 30	Retrosplenial cortex
32	Anterior cingulate
35, 36, 37	Parahippocampal cortex
28, 38	Uncus
39, 40	Parieto-occipito-temporal association cortex
44, 45	Broca's area
46	Dorsolateral prefrontal cortex
45, 47	Ventrolateral prefrontal cortex

Note: Some Brodmann areas span across cortex with more general names.

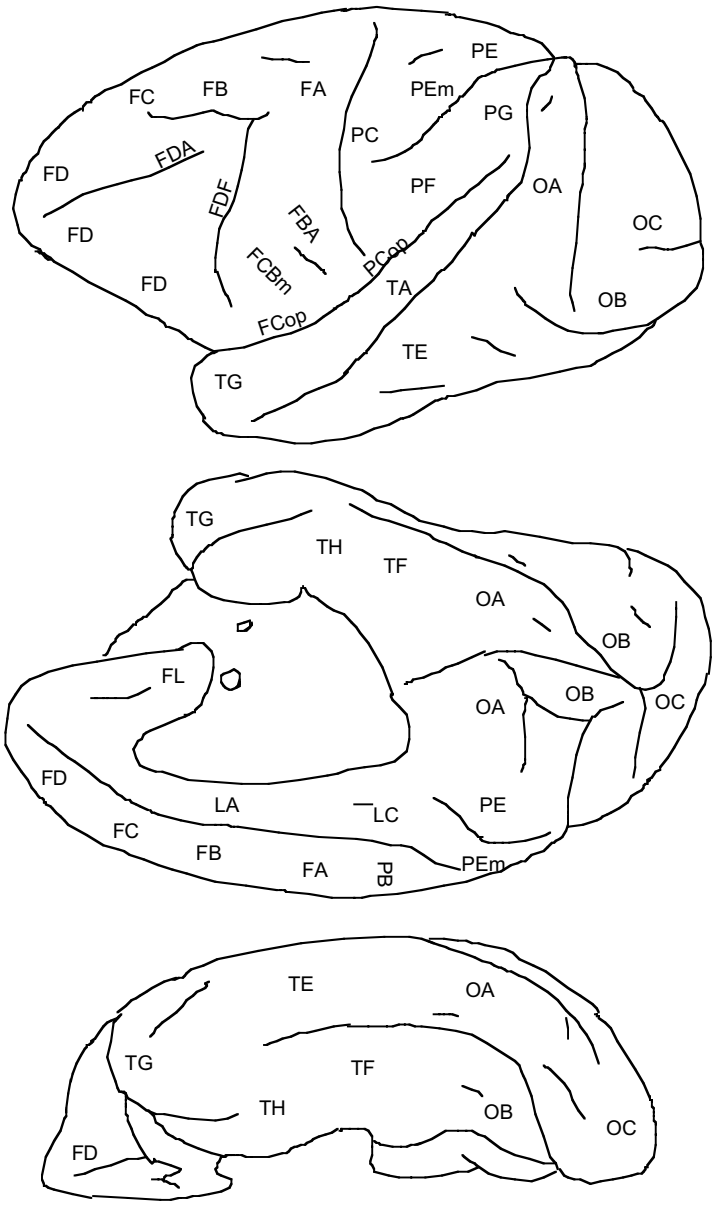


Figure 2. Cortical Neuroanatomy of the *Macaca mulatta.*
These diagrams display the locations and nomenclature of cortical brain regions developed by Bonin and Bailey (1947). Top: lateral surface. Middle: medial surface. Bottom: ventral surface. Abbreviations: FA-FL: frontal cortex; TA-H: temporal cortex; LA-LC: cingulate cortex; PB-PG: parietal cortex; OA-OC: occipital cortex.

1.2.1 Basal Forebrain Nuclei

The basal forebrain contains a group of nuclei, or rather somewhat undifferentiated cell clusters, dorsal to the anterior perforated substance at the ventral region of the brain where the telencephalon meets the diencephalon (Figure 3). It is variably referred to as the ventral striatum, substantia inominata, or septum, although these terms are not necessarily interchangeable. Many fibers of passage course through this region, e.g. fornix, stria terminalis, diagonal band of Broca, medial forebrain bundle, inferior thalamic peduncle, and ventral amygdalofugal pathway, which make it possible for terminal and en passant synapses. These regions contribute axons to these pathways for eventual termination rostrally in the frontal cortex and anterior cingulate, and caudally to the hypothalamus, midbrain and hindbrain.

Heimer and Alheid (1991) consider the basal forebrain the ventral regions under the dorsal striatum, pallidum and thalamus, thus containing both diencephalic (hypothalamus) and telencephalic (nucleus accumbens, septum, substantia inominata, the diagonal band nuclei, bed nucleus of the stria terminalis, hippocampus, amygdala, olfactory cortex and olfactory tubercle) components. However, considering the basal forebrain regions together, while adjacent to one another, as a unit as part of the limbic system does not account for the differences in the function, connectivity, and chemoarchitecture within this region. They have subdivided this area into three functionally related systems: the cholinergic cell groups, ventral striatopallidal system, and extended amygdala (Heimer and Alheid, 1991).

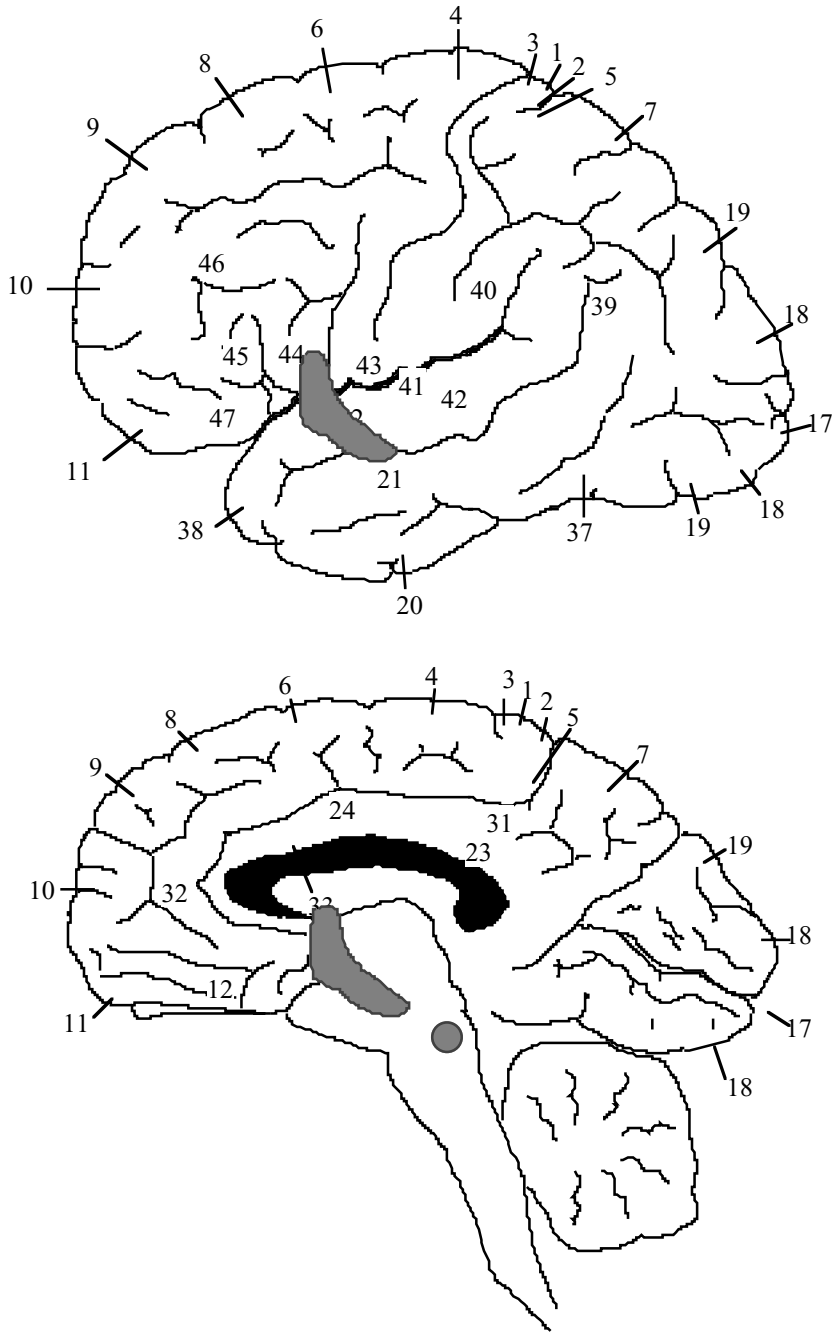


Figure 3. Location of Cholinergic Cell Bodies
Lateral (top) and medial (bottom) views of the human brain with approximate location of the cholinergic cell bodies, including the substantia inominata and septal nuclei, in the basal forebrain and the tegmentum (indicated in dark grey) superimposed. Although the cholinergic cell bodies do not extend to the cortex, the lateral view gives an idea of their location from this perspective. Numbers correspond to Brodmann areas (1909). Black region represents the corpus callosum.

The cholinergic cell groups are not totally distinct from the ventral striatopallidal and extended amygdala systems, but are better characterized as magnocellular corticopetal cell groups scattered across the forebrain from the septum to the diagonal band and extend through the ventral pallidum and extended amygdala, of which NBM is the major component. These cells are the origins of the cholinergic corticopetal projections in the rat and humans (Woolf, 1991; Mesulam, 1995) and release acetylcholine (ACh) synaptically. They also have diverse neuromodulatory substances affecting the cell bodies, such as galanin, gamma-aminobutyric acid (GABA), as well as other peptides (Záborszky, 1993). This region has similar connectivity as the extended amygdala, thus the function complements the extended amygdala as integral in the forebrain system evaluating the significance of external and internal events, and to effect appropriate behavioral responses through its widespread and highly organized projections to autonomic, neuroendocrine, somatic and visceral motor output. This system seems to be situated to perform a key role in motivational and adaptive behavior. Furthermore, the projection pathways and functions of the magnocellular cholinergic cell bodies, in many respects, suggests a mechanism whereby procaine via cholinergic mechanisms could yield the affective, hormonal, autonomic and blood flow activation pattern observed in humans (Ketter et al., 1996; Kling et al., 1994; Kellner et al., 1987).

Mesulam (1995) has studied the basal forebrain extensively in human brain tissue (note: any of the cholinergic studies by these authors cited herein are based on human neuroanatomy). Of the eight cholinergic cell groups delineated by Mesulam, four are these nuclei of the basal forebrain. His nomenclature corresponds to the

nuclei as follows: the medial septal nuclei - Ch1; the vertical limb of the diagonal band - Ch2 (Ch2-VLDB; also known as septum); the horizontal limb of the diagonal band - Ch3 (Ch3-HLDB); and the nucleus basalis of Meynert - Ch4 (Ch4-NBM). While these nuclei predominately express cholinergic markers throughout, there are areas within the nuclei that lack this neurochemistry. For example, within NBM, there exists large and small cells, both cholinergic and non-cholinergic, of which the 90% of NBM is associated with the cholinergic marker ChAT 90% of NBM; thus the use of the name Ch4-NBM when referring to the cholinergic portion.

The remaining four cholinergic clusters defined by Mesulam are in the brainstem, either midbrain nuclei near the junction with the pons, the pedunculopontine nucleus (Ch5), the lateral dorsal tegmental nucleus (Ch6), and parabigeminal nucleus (Ch8), or part of the diencephalon, the habenula (Ch7). For purposes of the work herein, Ch5, Ch6 and Ch8 together are considered the tegmental cholinergic cell body area (TEG) as represented in Figure 3. Each group can be further subdivided into smaller clusters that project to more selective targets; small letters following the group name denotes these divisions.

Ch4-NBM is a relatively small, but tightly packed region. It spans 13-14 mm from the level of the olfactory tubercle to the anterior hippocampus on the medial sagittal plane, and 18 mm within the substantia inominata in the transverse plane. In adult humans, this region contains ~200,000 neurons (Arendt et al., 1985), approximately ten times the amount of neurons as the locus coeruleus (Vijayashankar et al., 1979). The neurons are heteromorphic with isodendritic fields that overlap across divisions, and extend into passing fiber tracts (Ramon-Moliner and Nauta,

1966). All neurons contain acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) in the perikara, dendrites and axons (Mesulam et al., 1989), and 90% also express p75 nerve growth factor (NGF) receptors (Mufson et al., 1989).

Russchen and colleagues (1985a) have mapped the cholinergic projections of the NBM in non-human primates. They contend a continuum of cholinergic innervation exists from the ventral basal forebrain surface to rostral and lateral directions. The heaviest innervation is seen in the amygdala, subgenual (SGAC) and pregenual anterior cingulated (PGAC), medial orbitofrontal cortex (MOFC) and anterior insula (AINS).

The cortex is innervated by the forebrain cluster Ch4-NBM (Figure 4), as well as by midbrain clusters, Ch5 and Ch6 (Mesulam et al., 1983), and has no intrinsic cholinergic innervation (Mesulam, 1995). Ch4-NBM can be subdivided into six divisions based on the topography of the projections (Mesulam, 1995). For example, within Ch4-NBM, Ch4am projects to the medial wall of the cortex including the cingulate gyrus, while Ch4al projects to the amygdala, and to ventral orbitofrontal and prefrontal cortices. Ch4id and Ch4iv project to insular, dorsolateral prefrontal (DLPFC), parietal, peristriate occipital, and mid-temporal cortices, while Ch4p projects to the superior temporal gyrus and temporal pole.

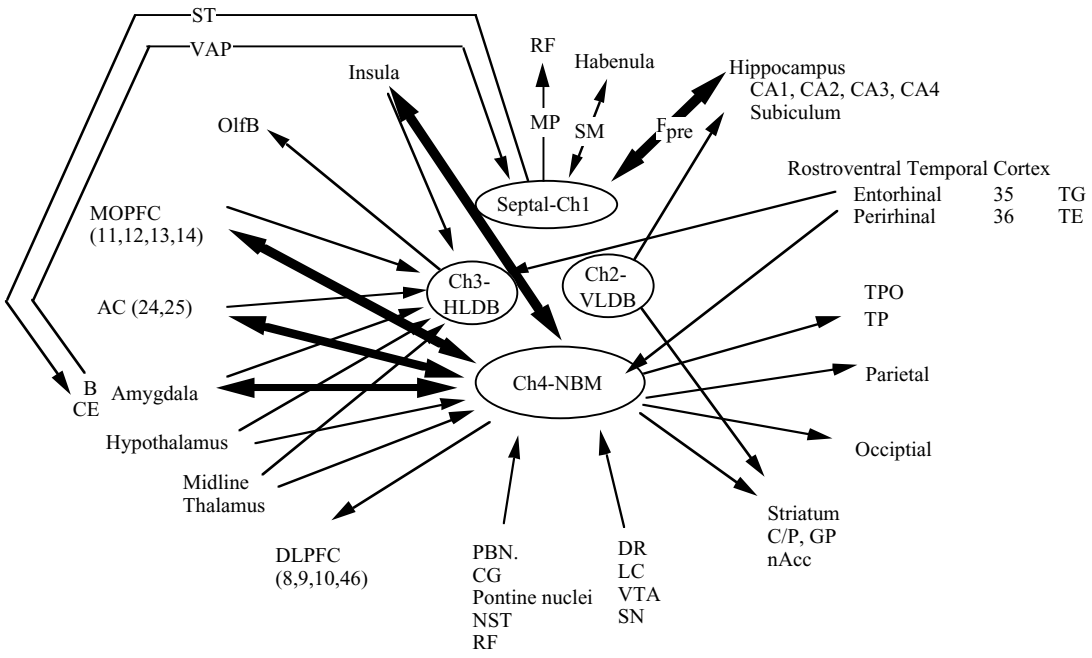


Figure 4. Cortical and Subcortical Connections with the Substantia Inominata. Schematic of the known afferents and efferents of the basal forebrain cholinergic nuclei. The densest cholinergic innervation (thick arrows) appears to be preferential to limbic structures. Of note, both the amygdala and the anterior cingulate have reciprocal connections with nucleus basalis. Numbers in parentheses are Brodmann's areas. Abbreviations: AC, anterior cingulate; B, basal nucleus amygdala; CE, central nucleus amygdala; CG, central grey; C/P, caudate-putamen; DLPFC, Dorsolateral prefrontal cortex; DR, dorsal raphe; Fpre, pre-commissural fornix; GP, globus pallidus; LC, locus coeruleus; MOPFC, medial orbitofrontal cortex; MP, mammillary peduncle; Ch2-VLDB, cholinergic portion of the vertical limb of the diagonal band; Ch3-HLDB, cholinergic portion of the horizontal limb of the diagonal band; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; NACC, n. accumbens; NST, n. solitary tract; OlfB, olfactory bulb; PBN, parabrachial n.; RF, reticular formation; septal-Ch1, septal nuclei; SN, substantia nigra; SM, stria meduallaris; ST, stria terminalis; TE, TG, TP, TPO: temporal regions; VAP, ventral amygdalofugal pathway; VTA, ventral tegmental area.

The cytoarchitectural distribution of these cholinergic projections has been studied with AChE, ChAT, and NGF, which co-localizes in many cholinergic neurons. All layers and regions of the cortex have dense cholinergic marker levels (Mesulam and Geula, 1992), but there is preferential density of markers in layers I, II, and upper III, and to limbic and paralimbic regions as well. Unimodal sensory areas have lighter levels of cholinergic markers.

Fields Ch4-NBM and Ch3-HLDB have major reciprocal projections with the amygdala, as mentioned below in the description of amygdala (Aggleton et al., 1987). These projections reciprocate back to the basal forebrain nuclei terminate almost exclusively in the Ch4-NBM region. The major amygdalar projections innervate the basal and central nuclei, with the lateral nucleus having a lesser degree of innervation. The medial nucleus is unique in that it has almost no cholinergic innervation. The highest density of cholinergic markers occurs in the amygdala and hippocampus.

Ch1 and Ch2 innervate the hippocampus primarily, with the densest projections to the hippocampus proper — CA1 (molecular layer), CA2, CA3, and CA4 — (Aggleton et al., 1987) via the pre-commissural fornix. The subiculum receives lighter projections from the septal area (Ch1). The septum receives hippocampal efferents, with the rostral hippocampus projecting more so the lateral septum and the caudal projections targeted the dorsal and medial aspects of the septum. The septal nuclei also innervate the habenula via the stria medullaris, the central amygdala nucleus via the stria terminalis, the basal amygdala nucleus via the

ventral amygdalofugal pathway, and the midbrain tegmentum via the medial forebrain bundle.

While the dorsal and ventral striatum are typically associated with the dopamine (DA) system, they also have considerable cholinergic innervation via interneurons (Graybiel, 1990), as well as receiving projections from all four basal forebrain nuclei. The nucleus accumbens, caudate, putamen, and globus pallidus (GP) receive projection from all four basal forebrain nuclei and Ch1 and Ch3 (HLDB) send 10 % or less.

Ch3 projects to the piriform and entorhinal cortex, but also to the olfactory bulb where it provides cholinergic tone to olfaction. Ch5 and Ch6 innervate the thalamus, while Ch7 projects to the interpeduncular nucleus and Ch8 projects to the superior colliculus.

Afferent projections to the substantia inominata magnocellular areas originate from cortical and brainstem regions. In the cortex, neurons from rostral and ventral areas, rather than dorsal or caudal regions, innervate the magnocellular nuclei. The cortical projections include projections from subgenual area 25, orbitofrontal areas (11 – 14), anterior insula, and rostroventral temporal areas 35, 36, TG, TE, entorhinal and piriform cortex (Russchen et al., 1985a; Záborszky, 1993). Sensory and motor cortex does not innervate cholinergic regions of the basal forebrain (Záborszky, 1993). The amygdala projections, as previously discussed, were primarily from basal nucleus to the Ch4-NBM. Brainstem projections originated from the midline thalamic nuclei, throughout the hypothalamic nuclei, and many midbrain nuclei, including the dorsal and median raphe, parabrachial nuclei, ventral tegmental area,

pars compacta of the substantia nigra, central grey, pedunculopontine, reticular formation, locus coeruleus, and nucleus of the solitary tract (Russchen et al., 1985a).

A good portion of the basal forebrain neurons are believed to be GABAergic interspersed with cholinergic neurons (Sarter and Bruno, 2002). The number of GABAergic cortical projections is comparable to cholinergic corticopetal neurons. The parvalbumin (PV - a marker predominately for GABA basal forebrain neurons) positive cells are predominately located in the ventral and lateral globus pallidus bordering the substantia inominata, while cholinergic regions are more medially and ventrally located in the substantia inominata. Záborszky (2002) suggests that specific neuronal clusters exist within which either ACh or GABA predominates, and each represents subdivisions of parallel basal ganglia circuits that mediate multilevel processing concurrently. In addition to the numerous GABAergic interneurons within the basal forebrain and cortex, GABAergic pathways include projections from the septal-diagonal band of Broca region to the cortex and hippocampus, striato-pallidal and striato-nigral connections. Jones (2004) believes ACh and GABA function to balance neuronal excitability.

In summary, the most dense locus of cholinergic cell bodies in Ch4-NBM area projects to anterior paralimbic regions, including the anterior cingulate, amygdala, medial orbitofrontal cortex, and the insula, that send reciprocal projections back to the this basal forebrain region as well. These reciprocal pathways represent a neuroanatomical basis of cholinergic influence on these essential limbic regions and are another example of recurrent loops commonly found in the limbic system.

1.2.1.1 Functional Attributes of the Basal Forebrain

Early investigations into the function of the basal forebrain suggested it was important in learning and memory. The finding of decreased number, size and function of the cholinergic cells in Alzheimer's disease encouraged this view (Bartus et al., 1982; numerous others – see review Wenk, 1997). Ch4-NBM lesions in animals were thought to impair their ability in learning and memory tasks (Bartus et al., 1985; Olton and Wenk, 1987) as suggested by deficits on radial arm maze task in rats (Wenk et al., 1986). However, the degree of destruction was not correlated with the degree of impairment, suggesting either an indirect relationship, or only partial reliance on the cholinergic innervation. Methods that produce more selective lesions, IgG-192 saporin (Wiley et al., 1991), that are injected directly into the region of the rat (rather than intracerebroventricular [ICV]) destroying more than 50% of the cells are beginning to show that the main deficit may be attention to brief salient sensory stimuli (Wenk, 1997; Robbins and Everitt, 1995), not learning and memory. Expectancy (Stoehr and Wenk, 1995), variable associativity (Chiba et al. 1995), and vigilance (McGaughy et al., 1996) are different aspects of attention that are affected by NBM lesions.

Wenk (1997) hypothesized that the corticopetal Ch4-NBM system is involved in the control of shifting attention to the potentially relevant stimuli that predict a biologically significant event or outcome. Thus, information of the emotional state, rewarding properties of a stimulus, arousal level originating from the amygdala may influence basal forebrain processing. This is supported by single unit recordings of

the NBM showing increased activity during attention to biologically relevant stimuli (Burton et al., 1976; Wilson and Rolls, 1990).

Basal forebrain cholinergic and GABAergic neurons can be distinguished by their physiological and pharmacological properties (Jones, 2004). Cholinergic neurons tend to be discharge at higher rates during cortical activation (compared to slow wave activity) and are excited by NE release from the locus coeruleus, glutamate from the reticular formation, and histamine from the hypothalamus. GABAergic neurons discharge at higher rates with slow-wave cortical activity and are inhibited by NE. Thus, they appear to have reciprocal roles in arousal systems.

The ventral striatopallidal system (Heimer and Alheid, 1991) consists of the nucleus accumbens (core; ventral striatum), olfactory tubercle (ventral striatum), and subcommissural substantia inominata (ventral pallidum). Projections of this ventral system follows the convention in the dorsal striatopallidal system, where the ventral striatum projects to the ventral pallidum and allocortical (hippocampal formation and olfactory cortex), periallocortical (entorhinal) and frontal and temporal proisocortical areas (insula, anterior cingulate, orbitofrontal, anterior temporal and perirhinal cortex) project to these ventral regions. Functionally, this system is analogous the dorsal system as well, where the dorsal is part of the extrapyramidal motor system, the ventral system is part of the limbic portions of the motor system, i.e., midline thalamic.

The extended amygdala (Heimer and Alheid, 1991) includes the bed nucleus of the stria terminalis, central and medial nuclei of the amygdala, the sublenticular substantia inominata, and medial accumbens (shell), not unlike the original concept

proposed by Johnston (1923). Cortical projections to the extended amygdala are from the same regions as the ventral striatopallidal system. The analogy to the dorsal striatopallidal system follows here also, although the output is to the hypothalamus and brainstem, thus is visceromotor. This pathway may be the basis of the hormonal and autonomic changes observed with procaine in humans (Kling et al., 1994; Kellner et al., 1987; Ketter et al., 1996).

In summary, the cholinergic cells of the basal forebrain appear to be ideally located to modulate the amygdala and nearby medial prefrontal (MPFC) and cingulate cortices to evaluate the incoming sensory stimuli for their level of significance with the goal of mediating effective behavioral strategies. This basal forebrain region has reciprocal connections to regions activated with acute procaine administration in humans that could contribute to the affective (amygdala), hormonal (hypothalamus) and autonomic (brainstem) phenomena observed with procaine challenge (Kellner et al., 1987; Kling et al., 1994; Ketter et al., 1996).

1.2.2 The Anterior Cingulate

The cingulate gyrus is a medial cortical structure that follows the inner aspect of the contour of the corpus callosum. Rostrally, it courses around the corpus callosum and tucks under the genu of the callosum, and caudally it gradually merges into the posterior parahippocampal gyrus. A distinct boundary of cytoarchitectural differences is observed between the anterior portion, which is agranular cortex (type 1) similar to motor cortex, and the posterior region, granular (type 2), although not like koniocortex (type 5) cortex as described by Von Economo (1929). The two

regions can be segregated functionally as well. Effector functions appear to be predominately in the anterior cingulate, while sensory–related inputs are thought to be associated with the posterior cingulate (Van Hoesen et al., 1993). Afferent and efferent connections with other brain regions often predominate, but not always.

While these distinctions exist, the anterior and posterior cingulate are coupled anatomically and functionally upon examination of the intra-cingulate circuitry. Functionally, the two regions interact via connections within the cingulate displaying two different patterns. First, the two extreme portions of anterior and posterior cingulate have reciprocal connections that may be inhibitory (Van Hoesen et al., 1993). Second, the intermediate regions of both anterior and posterior cingulate project to proximal cingulate cortex. The functional result of this intra-connectivity is bi-directional flow of information within the cingulate cortex (Van Hoesen et al., 1993). The rostral feed-forward network, or Van Hoesen's "outflow system", gathers signals via afferents from sensory-related cortex in the posterior cingulate and passes information rostrally to the anterior cingulate effector regions. The caudal feedback network provides anterior to posterior feedback to the posterior regions that project to the effector region.

1.2.2.1 Cingulate Pathways

The cingulate gyrus has considerable efferent and afferent extra-cingulate connections with multiple cortical and subcortical regions, allowing sensory-related information to be integrated with the affective processing, and in turn permitting modulation of somatic and visceral motor behavior. Cortical pathways, often

reciprocal, exist between the cingulate and the entire cortex. Subcortical connections are seen with the amygdala, hippocampus, striatum, thalamus, midbrain and hindbrain. A brief description of these connections follows and is summarized in Figure 5; for a more complete review, see Van Hoesen et al., 1993.

Prefrontal cortical regions, including dorsolateral prefrontal cortex (BA areas 8,9,10 and 46) and medial and orbital prefrontal cortex (BA areas 11,12,13, and 14), have reciprocal connections with the anterior cingulate regions 24 and 25, and posterior cingulate area 23 (Brodmann designation, 1909). The cingulate afferents terminate in all layers of the cingulate and are suggestive of a topographical organization, although topography has not thoroughly investigated (Van Hoesen et al., 1993). Orbitofrontal cortex also shows hints of topographical organization of cingulate projections that encompass the entire cingulate. Of note, caudal orbitofrontal cortex may be connected with rostral cingulate (BA area 24a, b) and more rostral orbitofrontal cortex connects with dorsal and posterior portions of the cingulate. Area 25, the closest in proximity to the cingulate, is reciprocally connected to medial portion of orbitofrontal cortex (Vogt and Pandya, 1987).

The cingulate cortex connects, often reciprocally, with many levels of the motor system, including motor cortex, the striatum and midbrain motor nuclei. Both primary motor cortex, M1 (BA 4) and supplementary motor cortex, M2 (BA 6) show a somatotopic organization, with the face area of M1 projects to anterior cingulate, hindlimbs to posterior cingulate, and the intermediate body areas represented by M1 projecting between the two extremes of the cingulate (Muakkassa and Strick, 1979).

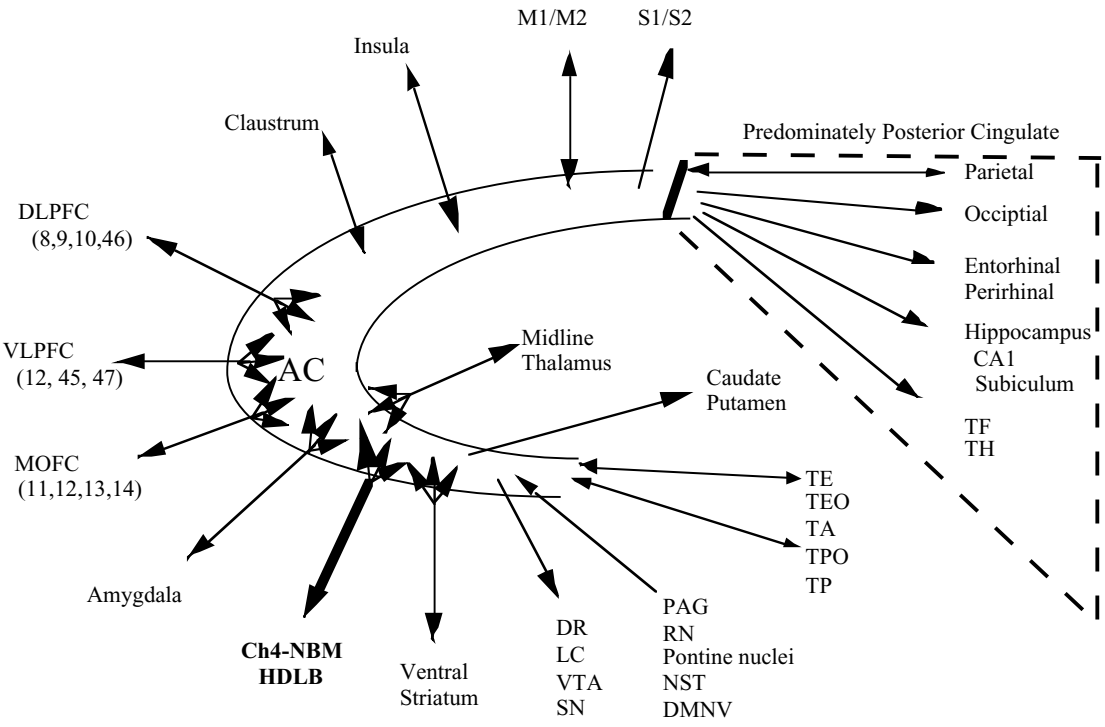


Figure 5. Pathways Involving the Anterior Cingulate
Schematic of the known afferents and efferents of the anterior cingulate in a sagittal view. Bold text and arrow reflects cholinergic innervation. Relative position of arrows on the cingulum is only generally specific; for example, the main targets of the amygdala and basal forebrain are the subgenual (25) and pregenual (24a) regions of the cingulate. The regions inside the dotted line project mainly from the posterior cingulate, however, there are minor anterior cingulate efferents to these regions. Numbers in parentheses are Brodmann's areas. Abbreviations: AC, anterior cingulate; DLPFC. Dorsolateral prefrontal cortex; DMNV, dorsal motor nucleus of the vagus; DR, dorsal raphe; LC, locus coeruleus; MOPFC, medial orbitofrontal cortex; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; NST, n. solitary tract; M1/M2, primary and secondary motor cortex; PAG, periaquaductal grey; PN, pontine nuclei; RN, red n.; S1/S2, primary and secondary sensory cortex; CA1, hippocampus; TF; TH; TE; TEO; TA; TPO; TP, temporal pole; VTA, ventral tegmental area. Data from Van Hoesen et al., 1993.

These connections are also reciprocal. Further studies showed that within each subdivision 24c and 23c there exist somatotopic projections to M1 and M2 (Morecraft and Van Hoesen, 1992), which also project to the cervical and lumbosacral segment of the spinal cord (Hutchins et al., 1988).

Regions typically associated with sensory-related functions, such as the parietal and occipital cortex, have the majority of their cingulate connections with the dorsal posterior areas (Van Hoesen et al., 1993). In the case of the parietal cortex, both lateral and medal surfaces, have extensive and often reciprocal connections (Baleydier and Mauguiere, 1980; Pandya and Kuypers, 1969) that terminate in layers cortical I-VI (Selemon and Goldman-Rakic, 1988) of dorsal posterior cingulate. These parietal connections are also intimately related to somatosensory cortex, S2 (BA 3,1, 2). In contrast, the anterior cingulate connections with parietal cortex are sparse, involving only the caudal portions of area 24 (Van Hoesen et al., 1993).

Temporal lobe regions involved in audition have reciprocal projections with both anterior and posterior cingulate. Anterior cingulate area 25 receives projections from superior temporal association areas Ts3 and Ts2 (von Economo nomenclature), while area 24 receives input from the Ts1 and the temporal pole. Neurons spanning the full length of TPO, on the dorsal bank of the superior temporal sulcus, project to posterior regions 23, 30 and 29. Rostral area 24 and 25 projects back to superior temporal gyrus and sulcus (Müller-Preuss et al., 1980). The temporal pole is the only temporal region that has reciprocal connections to the anterior cingulate regions 24a, b and c (Vogt and Pandya, 1987). These connections provide opportunity for

mediating neuronal activity related to auditory input and vocal expressions that may have emotional content.

Two regions located under the temporal lobe, the insula and the claustrum, appear to be involved in integrative functions of internal autonomic and external sensory information, respectively. Both regions have reciprocal connections with dorsal cingulate, primarily areas 24 and 23, which are arranged topographically, however, the claustrum also has projections to area 25.

The two major limbic regions in the medial temporal lobe, the amygdala and the hippocampus, exhibit predominately distinct connections with the anterior and posterior cingulate, respectively. The amygdala connections will be discussed in detail below, but briefly, the amygdaloid nuclei (basal, accessory, and medial) project to anterior cingulate, areas 25, and 24 a, b. The anterior cingulate has reciprocal connections to the basal nucleus, but also projects to the central nucleus of the amygdala.

While, the hippocampus and associated parahippocampal regions predominately have reciprocal projections with the posterior cingulate via the post-commissural fornix, the anterior region is not completely devoid of connections. Hippocampal CA1 and subicular regions project directly to most of the rostral and caudal cingulate (Rosene and Van Hoesen, 1977), and indirectly via connections to parahippocampal areas like the perirhinal and entorhinal cortices, which project areas 23, 24 and 25 of the anterior cingulate. Dorsal (24c) and posterior (23) cingulate cortices send projections to perirhinal, presubiculum and posterior parahippocampal regions TF and TH (nomenclature from Bonin and Bailey, 1947), but not to the

hippocampus. Thus, the majority of the hippocampal and parahippocampal connections, whether reciprocal or not, involve cingulate regions 23, 30 and 24c, while areas 24a, 24b and 25 appear to have fewer connections.

Efferent projections of the thalamus to anterior cingulate areas 25, 24a and 24b originate primarily in the midline parataenial, central densocellular, and reuniens, and mediodorsal (parvocellular division) nuclei, with slight variation with respect to anterior cingulate subdivisions (Vogt et al., 1987). Posterior cingulate regions receive input primarily from anteroventral, anterodorsal, and laterdorsal (to area 29), or anterior medial thalamic nuclei (to areas 23). These connections may also be reciprocal (Van Hoesen et al., 1993).

As in the case of all parts and types of cortex, the cingulate projects to the striatum, both dorsal and ventral regions (Baleydier and Mauguiere, 1980). The terminal projection pattern from the cingulate mimics the patchy matrix described by Graybiel (1990). The anterior cingulate projects to the caudate, putamen and the ventral striatum, while posterior cingulate has projects to only to the dorsal striatum (Baleydier and Mauguiere, 1980). Area 24 terminates more so in the putamen and 23 has stronger connections with the caudate.

Direct projections from the cingulate cortex to midbrain and hindbrain provide pathways out of the limbic system to control visceromotor and skeletomotor effector organs. The corticorubral pathway from cingulate areas 24c and some of 23c is distinctive, because this is the only limbic region projecting to the red nucleus, which receives most of its afferents from the cerebellum. Cingulopontine projections originate in areas 23, 24, and 25 (Vilensky and Van Hoesen, 1981).

1.2.2.2 Functional Attributes of the Anterior Cingulate

Early ablation and stimulation studies suggested that the anterior cingulate was involved in autonomic regulation, including cardiovascular, pupillary dilation, hormonal responses and motor inhibition (Smith 1945; Ward, 1948;Kaada, 1949, 1951; Frankel and Jenkins, 1975). Ablation of cingulate area 24 in macaque monkeys resulted in loss of "fear of man" and other affective behavioral changes (Smith, 1945; Ward, 1948). In addition, the avoidance reaction in the cats (McCleary, 1961) has been shown to be involved in noiceptive processing that has been associated with the anterior cingulate (dorsal and caudal portion) in man and animals, either in pain-avoidance paradigms or in studies examining pain-affect neural correlates (Koyama et al., 1998; Rainville et al., 1997).

Three anterior cingulate regions are of particular interest because their projections to motor effector areas suggest their involvement in the expression of emotion. Rostral cingulate area 24(a, b) projects to the periaquaductal grey (PAG) region in the midbrain; stimulation of these portions of area 24 produces vocalizations associated with responses to noxious stimuli (Jürgens and Ploog, 1970; Müller-Preuss and Jürgens, 1976) that are emotional, not communicative, in nature. Ablation of the PAG results in a loss of these emotional vocalizations evoked from cingulate stimulation (Jürgens and Pratt, 1979).

Autonomic effector control appears to be exerted by the subgenual anterior cingulate region, ventral to the region associated with vocalizations, that has connections to the nucleus of the solitary tract and the dorsal motor nucleus of the

vagus in the cat (Room et al., 1985; Willet et al., 1986) and has been demonstrated to initiate gastric motility. A dorsal region of the anterior cingulate, which projects to the primary and supplementary motor cortex (Morecraft and Van Hoesen, 1991) and spinal cord (Van Hoesen et al., 1993), has been associated with the planning of movement, as evidenced by bursts of neuronal activity just prior to finger movement. Stimulation of this region generates forelimb, hindlimb, axial, and tail movements in monkeys (Mitz and Wise, 1987).

Anterior cingulate effector regions, areas 24 and 25, receive afferents from the basal nucleus of the amygdala (Porrino et al. 1981; Amaral and Price, 1984) and have some unique topographical features (Van Hoesen et al., 1993). This projection terminates most heavily in cortical layers II and I, and defines the boundary of the cingulate and supplementary M2 regions, thus the termination region may contain face representation (Muakkassa and Strick, 1979; Morecraft and Van Hoesen, 1992), suggesting a pathway to control facial affect. There is no overlap with parietal inputs to the region (Vogt and Pandya, 1987),

Vogt et al. (1992) and Devinsky et al. (1995) deduced from studies of lesions, electrical stimulation, microelectrode recording, and PET that the cingulate cortex can be separated into two functionally distinct regions, anterior and posterior cingulate. The anterior region, including areas 24 and 25, mediates what they terms “executive functioning”, or controlling the effector output, whether it be visceromotor, skeletomotor and endocrine outflow. Specifically, emotion, pain, maternal behavior, visceromotor, skeletomotor and attention have attributed to anterior cingulate function. Furthermore, they posit that this region evaluates the emotional valence of

an organism's situation and integrates emotional information to modulate these functions. This role of the anterior region of the cingulate is further supported by the neuroanatomical connections to the amygdala.

The anterior cingulate can be further subdivided in to affective and cognitive components (Devinsky et al. 1995). Affective regions include rostral areas 25, and rostral/ventral areas 24 and 33, while cognitive region is the dorsal/caudal areas 24 and 32. The affective region is involved in numerous emotional behaviors including conditioned emotional learning, vocalizations associated with expressing internal states, assessments of motivational content and assigning emotional valence to internal and external stimuli, and maternal-infant interactions. Area 25 projects to the parasympathetic nucleus of the solitary tract (Terreberry and Neafsy, 1983, Willett et al., 1986) and the dorsal motor nucleus of the vagus in the cat (Room et al., 1985, Hurley et al. , 1991). Area 24 projects to periaquaductal grey and intersects with area 25 (Vogt and Pandya, 1987) and orbitofrontal cortex (Morecraft et al. 1992). This region has prominent connections to the amygdala and visceromotor regions of the brainstem (Vogt and Pandya, 1987)

The posterior cingulate, including areas 29, 30, 23 and 31, serves an evaluative role in behavior, monitoring sensory information and the orientation of the organism with respect to its environment and its own memories. Neurons in this region are responsive to visual stimulation, and are involved in monitoring eye movements, complementing motor commands. Lesions disrupt tasks of spatial orientation and spatial working memory, like the ability to swim to a hidden platform. Anterograde and retrograde amnesia can occur from lesions of area 23a (Valenstein et

al. 1987). Disruptions of these memory functions are possibly related to the known neuroanatomical connections to the hippocampus and surrounding parahippocampal area (Squire et al., 2004). Posterior cingulate, in contrast to the anterior cingulate, did not activate with procaine-induced emotional and autonomic experiences (Ketter, et al., 1996).

In summary, the anterior cingulate appears to contribute significantly to the neural circuits mediating emotion, as well as autonomic regulation, pain processing and attention, with an overarching executive function that contributes to orchestration of situationally appropriate behavior. Procaine-induced activation of the cingulate cortex was quite striking in that a clear boundary of activation appears to segment the anterior and posterior portions (Ketter et al., 1996).

1.2.3 The Amygdala

The amygdala, a group of medial temporal lobe nuclei originally termed by Burdach (1819), became of particular interest to the study of emotional processing primarily because ablation studies show striking emotional changes. Klüver and Bucy´s (1937, 1939) rediscovery of the Brown and Schäfer´s (1888) remarkable effect of "psychic blindness" by temporal lobectomy provided rationale to include the amygdala in MacLean´s limbic system. Weiskrantz (1956) isolated this effect to the amygdala by showing that amygdalar lesions produced impairment of avoidance conditioning, and thus hypothesized that the function of the amygdala was to associate the affective and reinforcing properties of the stimulus with its sensory properties. Geschwind (1965) believed the animals behaved in a bizarre manner due

to the lack of the motivational significance of visual stimuli resulting via disconnection of the inferotemporal visual processing cortices from the medial limbic areas. However, Schreiner and Kling (1953, 1956) portray dysfunction in sensory processing, ingestion, and sexuality as well with amygdala lesions in cats.

The amygdala is a heterogeneous structure with respect to anatomy, function, and neurochemistry. This area has widespread cortical connections and has most known neurochemical substrates. Its main function appears to attach significance to external and internal stimuli. The following discussion integrates amygdalar neurochemistry and neuroanatomy, and serves as a basis for integrating its function and relationship to other limbic areas.

The amygdaloid complex is bounded ventrally by the uncus, caudally by the hippocampus, and medially and rostrally by the substantia inominata, and dorsally by the lentiform nucleus. Johnston (1923) originally mapped the amygdalar nuclei architectonically, and the refining of boundaries, cellular compositions, and pathways remains an active area of research. Terminology used herein will follow Amaral et al., 1992, who have taken much of the current nomenclature and refined it further for the monkey brain. As noted above, the neurochemistry and neuroanatomy of the amygdala in primates varies slightly from the rat (R. Saunders, personal communication), thus the following information may differ from studies based on the rat.

Currently, the core components of the amygdaloid complex are considered to be the lateral nucleus, basal nucleus, accessory basal nucleus, and central nucleus, while the superficial nuclei (or areas) include the paralaminar nucleus,

periamygdaloid cortex, medial nucleus, the nucleus of the lateral olfactory tract, the anterior and posterior cortical nuclei, intercalated nuclei and the periamygdalohippocampal area. Most of what is known about the amygdala is based on the core regions, thus these areas will be reviewed in more detail. Discussion of the remaining regions will be limited to selected features, as appropriate to the theme of this thesis.

1.2.3.1 Amydalar Nuclei

The lateral nucleus borders the external capsule rostrally and the putamen and ventral horn of the lateral ventricle caudally. Its cellular composition is heterogeneous with small to medium-sized cells that can be separated into two major subdivisions based on the staining differences in Nissl bodies and AChE. The ventrolateral compared to the dorsomedial portion is fairly densely packed and stains more darkly for AChE. Patches of tightly packed small neurons, which stain intensely for AChE on the lateral border, make up the heterogeneous lateral capsular nuclei (Amaral and Bassett 1989).

The neurochemistry of the lateral region with significant levels of cholinergic M_1, cholecystekinin (CCK), and galanin receptors, and the presence of $GABA_A$ benzodiazepine receptors is suggested by the high levels of immunoreactivity to antibodies of the $GABA_A$ receptor complex. In addition, high levels of NAPDH-d (a nitric oxide synthase), tyrosine hydroxylase (dopamine rate-limiting enzyme), somatostatin, and corticotropin releasing factor (CRF) have been found in cells or fibers in this region.

The basal nucleus is the largest of the amygdaloid complex, which originally encompassed the accessory basal nucleus. The terminology of its subdivisions used by different authors is variable, although boundaries remain relatively constant. The parvicellular division, also known as the medial basal or basomedial region, has the smallest cells of the basal nucleus. These densely packed cells stain darkly for Nissl bodies. Between this region and the magnocellular component of the basal nucleus lays the intermediate division. The cells in the magnocellular division are larger, more densely packed and more darkly stained than the parvocellular division. The magnocellular division, or the lateral basal or basolateral region, is the largest of the basal nucleus, and has the largest cells with many fibers coursing through it.

Moving dorsally from the parvicellular to magnocellular division yields three gradients: relative cell size increases from small to large; staining density for Nissl substance becomes darker; and cholinergic preparations with both markers increase in intensity. The chemoarchitecture is diverse, but as suggested above, there are high levels of cholinergic markers, ChAT and AChE, M_1 and M_2 receptors. Also reported are high levels of CRF, NADPH-d (nicotinamide adenine dinucleotide phosphate), somatostatin, and limbic system associated modulatory protein (LAMP), as well as benzodiazepine and CCK receptors.

The accessory basal nucleus is situated between the most medial amygdalar regions, the periamygdaloid cortex and the nucleus of the lateral olfactory tract, and the intermediate fiber bundle, a fiber tract between the accessory and basal nuclei, and extends the majority of rostral/caudal axis of the amygdaloid complex. This nucleus has parvicellular and magnocellular subdivisions, but also has a ventromedial

division, which contains the highest intensity of cholinergic markers of the accessory basal subdivisions. The entire nucleus is NADPH-d, somatostatin, LAMP, as well as M_1 and M_2, benzodiazepine, CCK and galanin receptors.

Similar to the basal nucleus, a gradient of cell size and intensity of cholinergic markers is observed spanning from the parvicellular with low cholinergic markers, to magnocellular with moderate to high levels of cholinergic markers. There is a "superficial division" that separates the amygdalohippocampal region from the accessory basal nucleus, which has architectonics more like the parvicellular than magnocellular.

The central nucleus is situated at the caudal third of the amygdaloid complex along with the posterior cortical nucleus, the amygdalohippocampal area and the caudal end of medial nucleus. The central nucleus has medial and lateral divisions and high levels of multiple neurotransmitter and neuromodulatory system markers, including tyrosine hydroxylase, serotonin, somatostatin, neuropeptide Y (NPY), vasopressin, CRF, neurotensin, and galanin receptors. However, this region has relatively lower levels of cholinergic markers.

The chemoarchitecture of the superficial nuclei is heterogeneous, but includes considerable cholinergic activity. The amygdalohippocampal area, the anterior cortical nucleus of the amygdala, the medial nucleus, periamygdaloid cortex, the nucleus of the olfactory tract and the anterior amygdaloid area have high levels of cholinergic markers, including ChAT, AChE, and M_1 receptors.

In summary, the lateral, basal, accessory basal and periamygdaloid cortex all express markers, including M_1 and M_2 receptors (Flynn and Mash, 1993), of the

cholinergic system. Of sixteen markers for neurotransmitter or neuromodulatory systems examined so far, only the cholinergic system, somatostatin and NADPD-d, nitric oxide synthase, express such widespread amygdalar involvement.

1.2.3.2 Intrinsic Amygdalar Pathways

Intrinsic connections across the lateral to medial axis tend to project predominately in a lateral to medial direction (Figure 6). For example, the lateral nucleus projects to all nuclei that are medial to it, and receives only minor afferents from the basal and accessory nuclei. This pattern is repeated across the lateral-medial axis of the amygdala and suggests predominately unidirectional information flow (Amaral et al. 1992). Further details of these connections will focus on the four core subdivisions; for a full examination of the connections, see review by Amaral et al., 1992. The basal nucleus sends efferents to the accessory basal and central nucleus, with only modest reciprocal projections. The accessory basal nucleus projects to the central nucleus, as well as other more medial nuclei. The central nucleus projects primarily to more medial nuclei, but also projects to the lateral and basal nuclei. The central nucleus receives afferents from all divisions of the amygdala.

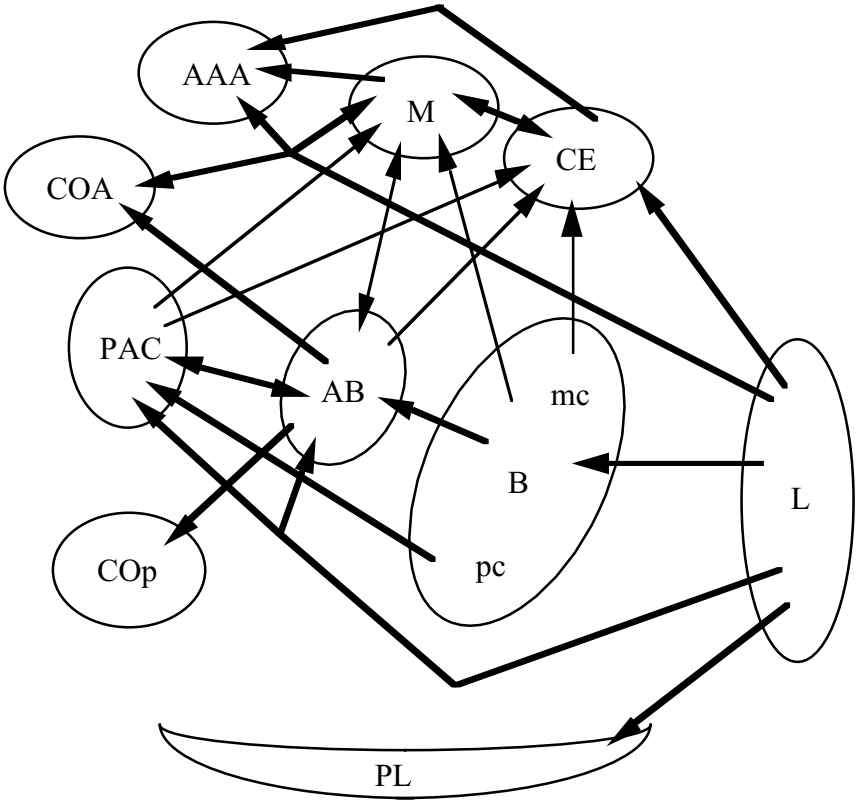


Figure 6. Intrinsic Connections of the Amygdala.
Schematic of the intrinsic connections of the amygdalar nuclei. Information processing generally flows in a lateral to medial direction. Thick arrows emphasize lateral to medial connections. Right side of figure is lateral, top is dorsal. Abbreviations: AAA, anterior amygdala area; AB, accessory basal nucleus; B mc, pc, basal nucleus, magnocellular and parvocellular; CE, central nucleus; Coa,p, cortical nucleus, anterior and posterior; L, lateral nucleus; M, medial nucleus; PAC, periamygdaloid nucleus; PL paralaminar nucleus. After Aggleton and Saunders, 2000.

1.2.3.3 Extrinsic Amygdalar Pathways

The extensive cortical and subcortical connections of the amygdala provide diverse pathways to affect behavior and modulate information processing. Subcortical pathways (Figure 7) exist between the basal forebrain, parahippocampal regions, striatum, thalamus, hypothalamus and the brain stem, while cortical connections (Figure 8) are seen with the prefrontal, cingulate, olfactory, insular, temporal, and occipital cortices. Of note, the parietal cortex is the only cortical area devoid of direct amygdala connections, however, polymodal information does make its way to the amygdala via sensory association areas in the temporal lobe.

The widespread amygdalar projections to rostral cortical regions are densest in anterior cingulate, lateral and medial orbitofrontal, and agranular insular cortices (Amaral and Price, 1984). This set of adjacent regions form a continuous density gradient emanating in two directions from the ventral subcallosum area, and subsequently will be referred as the core amygdalar efferents. In the medial to lateral dimension on the ventral surface, the core amygdalar efferents extend from medial orbitofrontal cortex (areas 13 and 14) laterally to anterior insula and lateral orbitofrontal cortex (LOFC; area 12). In a ventral to dorsal direction, in the medial plane, core amygdalar efferents include anterior cingulate areas 25, 24 and 32. Amygdalar connections diminish when moving beyond these regions, leaving some areas of patchy (8,9,45,46, premotor and 6) or no (10) amygdalar innervation.

The basal nucleus projects to all core amygdalar efferent regions topographically, and receives reciprocal projections from the anterior cingulate, insula, and caudal orbitofrontal cortex. The accessory basal nucleus, predominately

the magnocellular region, projects to anterior cingulate (area 25 and some of 32), insula, medial (14) orbital and lateral (12) orbital cortex, ventromedial wall of the medial wall of the prefrontal cortex, but only the caudal orbitofrontal and the medial wall of the prefrontal cortex reciprocate. The lateral nucleus projects to medial orbitofrontal cortex area 13 and insula with reciprocation only from the insula. The medial and periamygdaloid cortex projects to the insula.

Generally, reciprocation predominates, but several relationships stand out: the anterior cingulate innervates only amygdalar nuclei sending efferents to it; the basal nucleus reciprocates to all four of the dense innervations regions; and the insula appears to have the greatest number of connections, either afferent or efferent, with amygdalar nuclei. In humans undergoing procaine-induced emotional experiences, the anterior cingulate, insula and amygdala had robust concurrent activation (Ketter et al., 1996).

The lateral nucleus and some portions of the basal nucleus (Aggleton et al., 1980) receive projections from the anterior inferotemporal cortex (area TE), a unimodal visual processing region (Pandya and Yeterian, 1985). Primary visual and primary auditory cortices do not seem to directly innervate the amygdala (Aggleton et al., 1980), however, secondary auditory processing areas, superior temporal gyrus and association cortex do project to the lateral nucleus (Turner et al., 1980; Van Hoesen, 1981). Polysensory temporal areas, perirhinal cortex, parahippocampal cortex (regions TF and TH), project to the lateral, basal, and accessory basal nuclei, with the heaviest source of efferents coming from the temporal pole region (Turner et al., 1980) and regions more posterior contributing less so (Insausti et al., 1987).

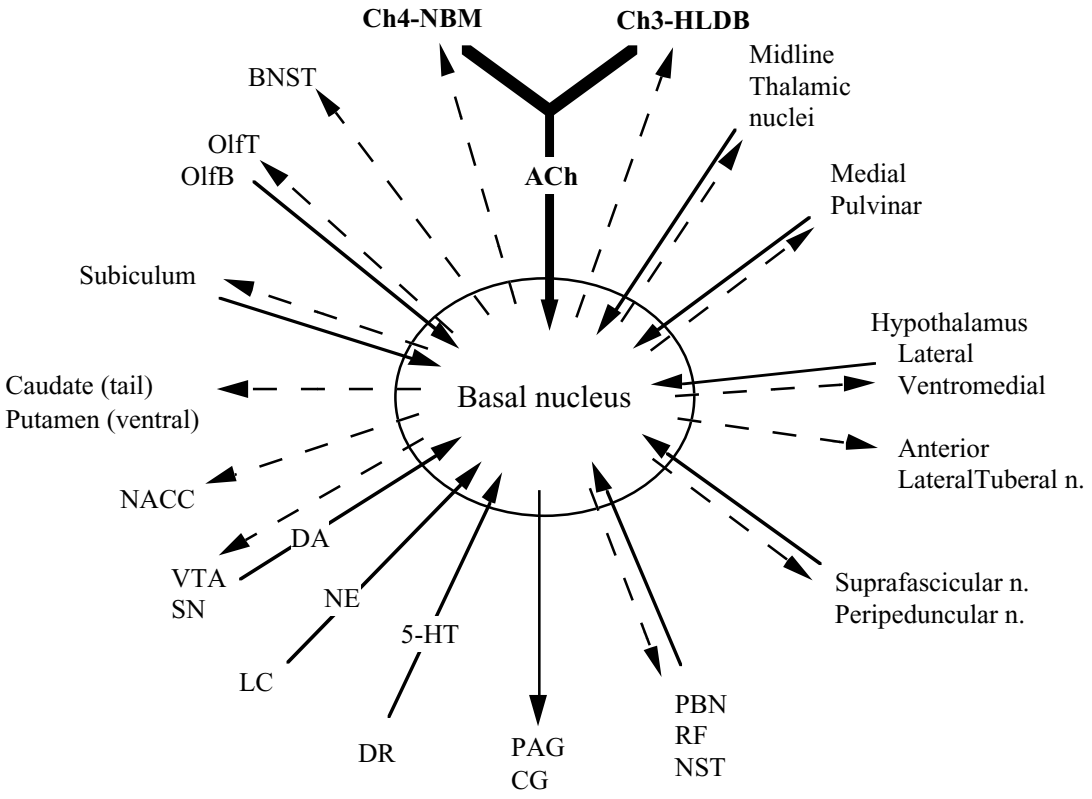


Figure 7. Reciprocal Subcortical Connections of the Amygdala.
A convergence of information concerning the internal milieu from widespread subcortical and brainstem nuclei. Also illustrated is the cholinergic (in bold) and monaminergic input to the amygdala. Again, this representation reflects mostly basal and lateral nuclei innervation, however, other amygdalar nuclei also receive a few projections. Abbreviations: ACh, acetylcholine; BNST, bed nucleus of the stria terminalis; CG, central, grey; DA, dopamine; DR, dorsal raphe; Ch3- HLDB, cholinergic portion of the horizontal limb of the diagonal band; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; LC, locus coeruleus; MT, MST: secondary visual processing areas; NACC, n. accumbens; NE, norepinephrine; NST, n. solitary tract; PAG, periaquaductal grey; PBN, parabrachial n.; OA, OB, OC: olfactory cortex; OlfB, olfactory bulb; OlfT, olfactory tract; RF, reticular formation; SN, substantia nigra; VTA, ventral tegmental area; 5-HT, serotonin. After Aggleton and Saunders, 2000.

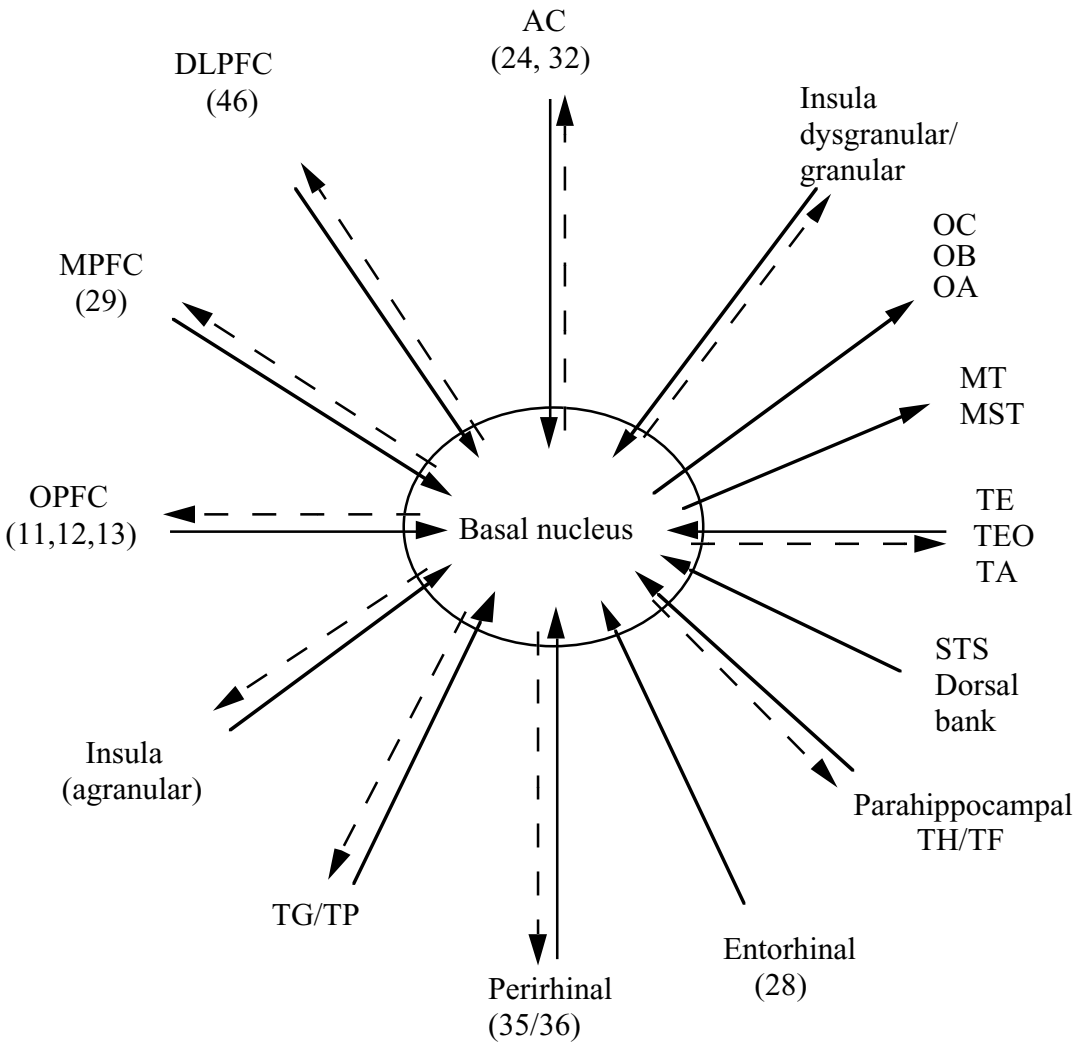


Figure 8. Reciprocal Cortical Connections of the Amygdala.
A convergence of external environmental information from widespread secondary and polymodal association areas descends onto the amygdala. The amygdala is unique in that in most cases it modulates its own afferents. This representation reflects mostly basal and lateral nuclei innervation, however, other amygdalar nuclei also receive a few projections. Abbreviations: AC, anterior cingulate; DLPFC, Dorsolateral prefrontal cortex; MPFC, medial prefrontal cortex; MT, MST: secondary visual processing areas; OA, OB, OC: olfactory cortex ; OPFC orbitofrontal cortex; TA, TE, TEO, TG, TH, TPO, TP: temporal regions. After Aggleton and Saunders, 2000.

In contrast, to the incomplete occipital and temporal efferents to the amygdala, the amygdala projects to directly all visual areas of temporal and occipital cortex in primates. The basal nucleus projects across the cortical mantle from area 17 to the temporal pole with progressively denser innervation in the rostral direction (Mizuno et al., 1981; Amaral and Price, 1984). This pattern is replicated in auditory cortex, although the specific amygdalar nuclei responsible for the projections remain unknown. The lateral and accessory basal nuclei projections to polysensory regions of the temporal lobe are also more dense in the temporal pole, but also increase as they progress from the lateral to medial perirhinal region (Amaral and Price, 1984). Projections to areas 35 and 36 are progressively denser along the caudal to rostral axis of the ventral surface of the temporal lobe; these originate in the basal and accessory basal nuclei area, but area 35 also receives afferents from the lateral nucleus (Stefanacci et al., 1996).

The olfactory bulb projects directly to the nucleus of the lateral olfactory tract, periamygdaloid cortex, and the anterior cortical nucleus, of which the first two nuclei reciprocate. In addition, amygdalar afferents from olfactory association cortices, such as the piriform cortex, terminate in the same amygdala nuclei that receive directly form the olfactory bulb in the rat (Carmichael and Price, 1996). Piriform cortex also projects to the medial and posterior cortical amygdalar nuclei in the rat (Krettek and Price, 1978; Ottersen, 1982). Olfaction is the only sense with direct connections to cortical areas, instead of first synapsing in sensory relay nuclei such as the thalamus.

Projections to the hippocampus and associated cortex for the most part follow other species, such as cat and rat, but there are significant exceptions; the accessory

basal nucleus sends efferents to the hippocampal formation and related cortices, entorhinal cortex and subiculum. There are considerably more amygdalo-hippocampal efferents than afferents, suggesting that the amygdala has more impact on the hippocampal processing than the reverse (Amaral et al., 1992).

The entorhinal cortex, as the major interface between the hippocampal formation and the amygdala (Witter and Amaral, 1991), and poly-modal sensory cortices (Insauti et al., 1987), receives amygdalar efferents from the lateral, accessory basal, anterior cortical and medial nuclei, and periamygdaloid cortex (Insauti et al., 1987; Saunders and Rosene, 1988). The basal nucleus also sends a minor projection to this area. Reciprocal projections to lateral nucleus have been questioned as to whether they consist of only fibers of passage piercing the lateral nucleus, or if they form *en passant* connections. The basal nucleus and periamygdaloid cortex are innervated by the subiculum and CA1 hippocampal formation (Saunders et al., 1988).

Since the basal forebrain nuclei have been discussed in detail already, a brief synopsis of the amygdalar connection follows. The nucleus basalis of Meynert, the nucleus of the horizontal limb of the diagonal band, and the amygdala have substantial reciprocal connections (Mesulam et al., 1983; Russchen et al., 1985b). This most exemplified by the reciprocal projection between the basal nucleus (parvicellular) of the amygdala and the nucleus basalis in the forebrain (Amaral and Bassett, 1989). In addition, the accessory basal and the central nuclei send a major projection to the magnocellular division of the basal forebrain. These regions of the basal forebrain are considered the origination of cholinergic cell bodies that project across the forebrain. The magnocellular division of the basal nucleus of the amygdala

has the highest levels of ChAT of any forebrain regions, suggesting this is the most substantial cholinergic projection of the basal forebrain (Hellendall et al., 1986).

The amygdalostriatal projection also has substantial efferents of the amygdala, projecting to both dorsal and ventral striatal structures. The nucleus accumbens receive efferents from the basal and accessory nuclei, while the caudate and putamen receives input from the magnocellular division of the basal nucleus (Parent et al., 1983; Russchen et al. 1985a). There also exists a lighter projection to the ventral pallidum from the (Russchen et al. 1985a).

The thalamus, usually considered a major sensory and motor relay, has significant innervation from limbic regions, thus midline and mediodorsal thalamic nuclei are considered limbic thalamus (Vogt et al., 1987). The central and medial nuclei of the amygdala project to midline thalamic nuclei, nucleus reunions and caudal centralis nucleus (Price and Amaral, 1981). The midline nuclei reciprocate back to the central, but also project to the basal nucleus of the amygdala (Mehler, 1980). The posterior thalamus, near the medial geniculate, sends projections to the lateral, accessory basal, medial and central nuclei (Mehler, 1980).

Mediodorsal thalamus receives projections from almost all amygdalar nuclei, but mostly from the lateral, basal, and accessory basal nuclei, and periamygdaloid cortex. The terminations are primarily in the magnocellular region that projects to the same orbital and medial prefrontal areas that receive amygdalar efferents (Porrino et al., 1981; Goldman-Rakic- and Porrino, 1985). Furthermore, there is a distinct amygdalo-thalamic topographical pattern (amygdala dorsal cells project to thalamic ventral cells) that is synaptically connected to the amygdalo-cortical and thalamo-

cortical topography. This direct and trans-thalamic amygdalar efferents projecting to the same orbital cortex region are "registered", as demonstrated by multiple axonal tracers that show overlap; i.e., the nuclei of the amygdala that projects to the orbitofrontal cortex, also projects to mediodorsal region that projects to the identical orbital cortex area. Although, the anterior cingulate projections do not have overlapping mediodorsal regions with the amygdala as seen with orbitofrontal cortex (Ray and Price, 1990), they may have indirect connections via interneurons to other thalamic nuclei.

The hypothalamus receives significant, primarily unidirectional, projections from the amygdala. The central nucleus has the most substantial efferents of the amygdaloid complex (Amaral et al., 1982; Price et al., 1987). The central nucleus also innervates the para-mammillary nuclei. The basal nucleus of the amygdala projects to the lateral tuberal nucleus. There are two projections from amygdalar nuclei to the medial hypothalamic nuclei. These include anterior cortical and medial nuclei to the anterior hypothalamus (Price et al., 1991), medial and accessory basal nuclei and the amygdalohippocampal area to the ventromedial and premammillary nuclei of the hypothalamus (Heimer and Nauta, 1969). Hypothalamic-amygdalar projections include the ventromedial and lateral hypothalamic nuclei send fibers back to the amygdalar central, medial, basal and accessory basal nuclei. The lateral hypothalamus has reciprocal projections back to the central and medial nuclei, via the ventral amygdalofugal pathway.

The central nucleus is the only amygdalar nucleus that projects to the midbrain, pons and medulla, via the caudal amygdalofugal pathway. Autonomic

control by the amygdala may be achieved by innervation of the periaquaductal grey, the parabrachial nucleus, the dorsal vagal nuclei, and the reticular formation (Price and Amaral, 1981). The amygdala receives various monaminergic fibers (Mehler, 1980; Norita and Kawamura, 1980) from the brain stem.

1.2.3.4 Functional Attributes of the Amygdala

The functional role of the amygdala has been the subject of numerous studies and theories since the discovery of amygdalar lesions in monkeys produce "tame" animals (Brown and Schäfer, 1888; Klüver and Bucy 1937; Weiskrantz, 1956; Schreiner and Kling, 1956). Visually agnosia, hyperorality, and hypersexuality are also associated with ablation of the amygdala in the cat (Schreiner and Kling, 1953). Geschwind (1965) hypothesized that sensory information from temporal lobe processing regions must impinge on the amygdaloid complex in order to be associated with the appropriate affective or motivational valence. The basis of this effect has been traced to the loss of fear conditioning by lesions of the basolateral complex (basal and lateral nuclei) and central nucleus (Brady et al., 1954; Davis, 1992; LeDoux, 1996), which occurs in conjunction with motoric, autonomic, hormonal and attentional alterations (Davis, 1995). Stimulation of the amygdala can produce complex responses of motor inhibition, autonomic arousal, and EEG changes that when taken together resemble fear responses (Davis, 1995).

The basolateral - centromedial functional division of the amygdala is continually being revised. Swanson and Petrovich (1999) segment the rat amygdala into four functional units, adding two olfactory systems to the traditional basolateral

and centromedial divisions: 1) frontotemporal system - basal and lateral nuclei have reciprocal projections with the frontal, parietal, cingulate, prefrontal, insular, hippocampal, and olfactory cortices, striatum, nucleus accumbens, and thalamus; 2) autonomic system - central nucleus projects to the SI, the bed nucleus of the stria terminalis, hypothalamus, and brainstem autonomic nuclei; 3) main olfactory system - cortical and basomedial nuclei projecting to SI, central and medial amygdalar nuclei, medial prefrontal, agranular insular, perirhinal, and hippocampal cortices, thalamus, striatum, nucleus accumbens, and parabrachial nucleus; 4) accessory olfactory system - medial, cortical and posterior nuclei project to the SI, central amygdalar nucleus, main olfactory system, ventral subiculum, medial prefrontal, agranular insula, and the nucleus accumbens. There exists discrepancies between this model based on the rat and what has been summarized in Amaral et al. (1992) with respect to cholinergic innervation in the monkey, as suggested by Saunders (personal communication).

In contrast to a segmented approach to amygdalar function, the presence of extensive intrinsic connections and 40 Hz oscillations (Gault and Coustan, 1965) of the amygdala suggest that the amygdala functions as relative unit (Aggleton and Saunders, 2000). In nonhuman primates and humans, amygdalar activity appears to have some selectivity to faces, and particularly for facial fear recognition. Bilateral amygdala damage in man produced deficits in recognizing facial affect of fear and anger, while sad, disgust and surprise affects were somewhat impaired, and happy was unaffected compared to brain-damaged and healthy controls (Adolphs et al., 1999). What was striking was the lack of consistency of errors the amygdala-

damaged subjects made; they did not confuse one emotion for another, but chose different emotions upon repetition of a trial. These results suggest a general function of the amygdala of emotion detection, rather than a specific fear-detector.

In a related study, amygdala-damaged subjects were less able to assess the trustworthiness and approachability from faces but not verbal description of these attributes, thus extending the impairment sustained into the social realm (Adolphs et al., 1998). These subjects performed as well as controls when assessing these attributes in the ten most negatively rated faces after slight alterations of facial features.

In summary, the anatomical connections of the amygdala have been extensively studied resulting in a large body of literature. As demonstrated in Figures 7 and 8 the intra-amygdala pathways as well as the projections to other brain regions are numerous (Aggleton and Saunders, 2000). Whether the amygdala operates as relative unit or with functional subdivisions remains to be elucidated. However, the functional response of procaine may very well be related to the amygdalar basolateral - centromedial division. For example, the anterior paralimbic rCBF activation (Ketter et al., 1996) may be related to the basolateral system projecting to prefrontal regions, while endocrine alterations (Kling et al., 1994) could be a function of corticomedial modulation of hypothalamic nuclei. It appears that the collective amygdalar nuclei communicate in such a manner that different aspects of emotional behavior can be compartmentalized to specific effector pathways and at the same time result in an orchestrated fashion. The broad rostral cortical region innervated by the basal nucleus suggests it may have a significant role in attention, i.e., it may be acting as a

general amygdalar switch that brings all the cortical regions "on-line" (activated together) when emotional processing is required to modulate executive functions.

1.2.4 Thalamic and Striatal Relationships with the Limbic System

Portions of the thalamus and striatum mediate limbic functions and have neuroanatomical connections with regions classically considered as limbic. The midline nuclei of the dorsal thalamus, because of its, often reciprocal, projections to limbic areas, as well as to sensory and motor areas, has long been hypothesized to be involved in emotional behavior as a route for sensory information to be accessed. The mediodorsal thalamus projects to the amygdala, and anterior cingulate areas 24a,b and 25. As mentioned in the discussion of the amygdala, the amygdala innervates the orbitofrontal cortex via direct and trans-thalamic connections and the anterior cingulate through direct and two-step thalamic (via thalamic interneurons) projections.

The striatum also has considerable connections with motor and limbic regions, that can be organized into dorsal and ventral striatopallidal pathways, respectively (Alheid and Heimer, 1996). Limbic pathways include ventral pallidal and striatal afferents from the prefrontal cortex, including, anterior cingulate and orbitofrontal cortex; limbic cortex receives from mediodorsal thalamus, which has reciprocal connections with the ventral pallidal and striatal structures. In addition, there are reciprocal connections of these ventral striatal and pallidal regions with the amygdala, septum, habenula, subthalamic nucleus, substantia nigra and ventral tegmental area.

The amygdalo-striatal projection is one of the most substantial efferents of the amygdala projecting to the nucleus accumbens.

As has been noted throughout this thesis, the involvement of the cholinergic system in the limbic system is widespread and substantial. The thalamus and striatum are no exception. All the thalamic nuclei express cholinergic activity, but especially in the mediodorsal, intralaminar nuclei, reuniens nucleus, anterodorsal nucleus, lateral geniculate and reticular nucleus, by pontine and tegmental cholinergic neurons (Mesulam, 1995). There are also cholinergic projections from the magnocellular basal forebrain nuclei to midline thalamic nuclei, including the nucleus centralis superior and dorsalis, ventral reunions nucleus, and paraventricular nucleus (Russchen et al., 1985a).

In the striatum there are high levels of cholinergic markers, most of which are a result of cholinergic interneurons (Graybiel, 1990), and also from Ch4-NBM (Mesulam, 1995). However, the co-existence of NGF receptor immunoreactivity suggests extrinsic innervation from basal forebrain nuclei. In addition, there are known cholinergic projections originating from the pontine and tegmental nuclei to striatal nuclei.

1.2.5 Neuroanatomical Circuits

Alexander and colleagues (1990) have brought renewed interest in the construction of neuronal circuits, which are more complex than the earlier work of Papez (1937), Yakovlev (1948), and McLean (1949, 1952). These distributed networks, rather than focalized locus of control, has sought to delineate parallel basal

ganglia-thalamocortical circuits mediating motor, oculomotor, prefrontal and limbic functions based on neuroanatomy and neurochemistry. Both the limbic and prefrontal loops are implicated in emotional behavior with distinctions being made as to the division of experiential and executive functioning with respect to these two circuits.

The limbic circuit connects the anterior cingulate and medial orbitofrontal cortex (MOFC) with ventral striatal and pallidal regions, the olfactory tubercle and nucleus accumbens and extended amygdala, that project on to the subthalamic nucleus that project onto limbic thalamic nuclei that returns projects up to the limbic cortex completing the loop. As outlined above, cholinergic neurons modulate the anterior cingulate, which has major outflow to effector regions of the brain. One might speculate that repeated passes through these loops could allow for amplification or reduction, and thus refinement of signals relaying putative emotionally significant information in an on-going basis. The functions of the limbic loop have not been studied as well, but they are hypothesized to be more attuned to the experience and expression of affect, and motivational processes.

The prefrontal circuit is more likely to incorporate the emotional valence of the situation at hand and "adjust" cognitive functions, such as memory and decision-making. For example, the dorsolateral prefrontal cortex (DLPFC) is activated during the Wisconsin Card Sort Test as evidenced by PET in healthy subjects, and less so in pathological states, such as schizophrenia (Weinberger et al., 1986).

Comparing the limbic and prefrontal circuits, differences between them mainly stem from the cortical regions involved, but also from the particular striatal and thalamic components are involved, as well. The limbic circuit involves the

anterior cingulate and medial orbitofrontal cortex, while the prefrontal incorporates dorsolateral prefrontal and lateral orbitofrontal cortices. The limbic circuit involves the ventral striatal structures and mediodorsal thalamus, while the prefrontal incorporates dorsal striatal region, caudate, and thalamic ventroanterior pars compacta (VApc). These differences may represent preferential neurochemistry of each circuit. For example, the limbic circuit includes anterior cingulate and MOFC, cortical regions of the limbic circuit that receive heavy cholinergic input from the basal forebrain. Moreover, the limbic and prefrontal circuits differ with respect to the involvement of the ventral pallidum and the substantia nigra, suggesting a dominance of acetylcholine or dopamine, respectively. The limbic circuit is involved in visceromotor and cholinergic areas, while the prefrontal circuit appears to be integrating areas more typically thought of as motor or dopaminergic.

1.2.6 Neuroanatomy Summary

Cholinergic cell bodies originating in the basal forebrain send afferents to numerous targets, but most heavily to the amygdala, the ventral and anterior portions of the anterior cingulate, the orbitofrontal cortex, and the insula. In addition, the majority of the cortex, dorsal striatum, globus pallidus, and hypothalamus receive significant cholinergic innervation, however, not as heavily as the aforementioned regions. The ventral and anterior portions of the anterior cingulate, the orbitofrontal cortex, and the insula also receive substantial afferents from the amygdala. Thus, there exists potential for these cortical regions to receive dual innervation from cholinergic cell bodies, direct and indirect. The neuroanatomical diagram in Figure 9

incorporates cholinergic pathways discovered from several neuroanatomists in a single model and provides a basis to test hypotheses regarding cholinergic function and its relationship to emotional behavior. This model will be referred to as the AChNet and will be tested in experiment II presented in Chapter 3 and 4.

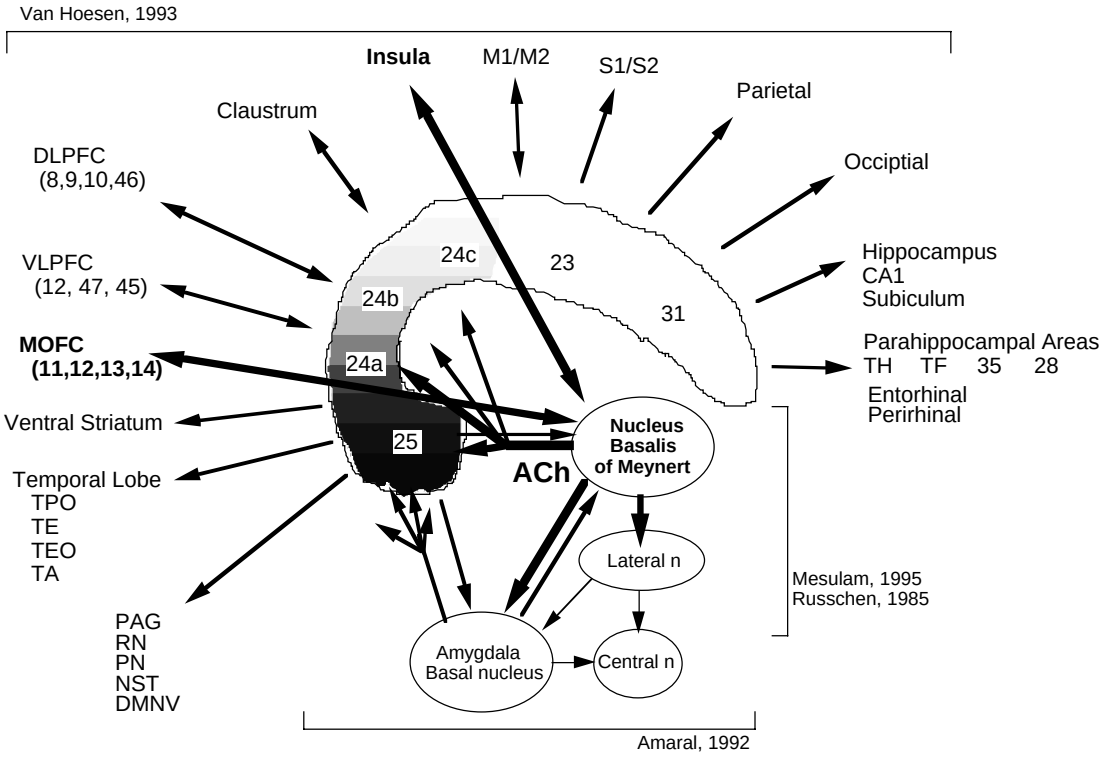


Figure 9. Schematic Summarizing the Cholinergic Influences on the Brain. Cholinergic innervation, direct and indirect, of the amygdala, anterior cingulate, and cortex suggests an important role in the limbic system, which implicates a pivotal function in emotion regulation. This is supported by pattern of cholinergic cell bodies and their most proximal and heavy targets that overlaps with the anterior paralimbic activation pattern of procaine. Shading represents density of dual innervation, direct cholinergic and indirect via the amygdala. Thick arrows indicate heavier innervation. Numbers in the anterior cingulate indicate Brodmann areas (1909). Abbreviations: AC, anterior cingulate; ACh, acetylcholine; DLPFC. Dorsolateral prefrontal cortex; DMNV, dorsal motor nucleus of the vagus; DR, dorsal raphe; LC, locus coeruleus; MOFC, medial orbitofrontal cortex; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; NST, n. solitary tract; M1/M2, primary and secondary motor cortex; PAG, periaquaductal grey; PN, pontine nuclei; RN, red n.; S1/S2, primary and secondary sensory cortex; CA1, hippocampus; TF, TH, TE, TEO, TA, TPO, temporal regions designated by Bonin and Bailey (1947); TP, temporal pole; VLPFC, ventrolateral prefrontal cortex; VTA, ventral tegmental area.

1.3 Neurobiology of Emotion

Understanding the neurobiology of emotion regulation can be aided by observing clinical and behavioral changes after lesions occurring in humans and animals, by examining effects of emotion induction by neuropsychological or pharmacological challenges, or by comparing normal to pathological emotion states in humans. Each method has its associated benefits and caveats. Therefore, the information gleaned from each approach can contribute uniquely to understanding the neurobiology of emotion when keeping their respective qualifications in mind. Any one neuromodulatory system is unlikely to be solely responsible for a single behavioral response, which are more likely related to the subtle interplay of the various systems. Excitatory and inhibitory neurotransmission occurs primarily via the amino acid neurotransmitters, glutamate (GLU) and gamma-amino-butyric acid (GABA), respectively, while neuromodulators, such as cholinergic, monaminergic, peptidergic, and chemical (e.g., nitric oxide), fine tune neurotransmission. A brief review of these mechanisms from several research perspectives follows.

1.3.1 Lesion Studies

Lesion studies have suggested neural substrates of emotion, e.g., Klüver-Bucy syndrome (1937, 1939). Examples of linking different emotions to specific brain areas include rage induced by septal nuclei lesions and loss of fear conditioning associated with amygdalar lesions. Of these, septal rage may be closely associated to a single neuromodulatory system because it is has a concentration of cholinergic cell

bodies (Mesulam, 1995), despite the fact that it contains many neuromodulatory substances (Záborszky et al., 1993). In contrast, the amygdala also has diverse neurochemistry, but most likely lacks neuromodulatory cell bodies.

While the ablation technique has been beneficial in isolating potential regions involved in a behavior, it is unclear whether the lesion effects are direct or indirect effects, i.e., the resulting behavior may be related to the integral function of the region, a disruption of its relationships with other regions, or both. Another problem is the interpretation of emotional behavior in animals, as this must be, in some cases, inferred from some measurable parameter, and leads to difficulties in the extrapolation from animals to humans.

With these caveats in mind, this portion will focus on the relative effects of lesioning the cholinergic and monaminergic systems to compare how these systems can interact in arousal and attentional. Studies of arousal and attentional mechanisms may serve as a window into in emotional processing of animals. Indeed, these general brain functions are not in their own right unrelated to emotional processing, but are hypothesized to be an essential part of it (LeDoux, 1996).

At first glance, the broad diffuse innervation patterns of the cholinergic and monaminergic systems appear to lack anatomical specificity. See Figure 10. This does not necessarily mean that the neuroactive substances lack functional specificity. Intuitively, this is most likely not the case. With regard to arousal and attentional mechanisms, Robbins and colleagues (1995, 2002) has done a series of elegant lesion studies delineating the functions of the cholinergic and monaminergic systems.

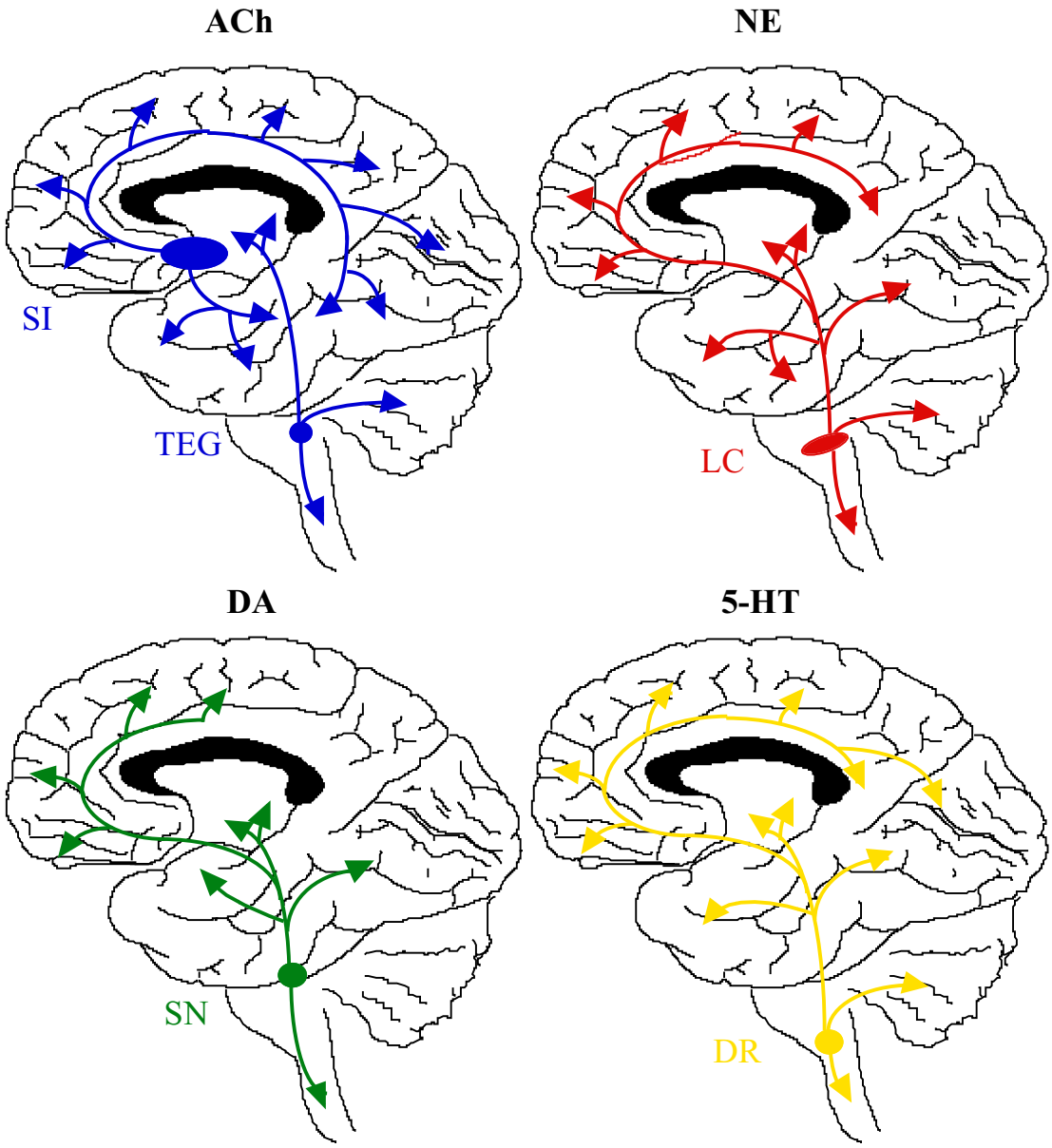


Figure 10. Distributions of the Acetylcholine and Monoaminergic Projections Schematic of putative cholinergic and monoaminergic projections of the human brain. These drawings are primarily based on the neuroanatomical pathways in the rat. The cholinergic system is the only classic neurotransmitter with cell bodies in the forebrain, as well as the brainstem. Abbreviations: ACh= acetylcholine; NE=norepinephrine; DA=dopamine; DR= dorsal raphe; 5-HT=serotonin; SI=substantia inominata; LC=locus coeruleus; SN=substantia nigra TEG=tegmentum at the junction of the midbrain and pons.

He compared the behavioral responses of rats tested in a five-choice task after cytotoxic lesions of the respective nuclei containing the cholinergic and monaminergic cell bodies. The five choice test is an adaptation of continuous performance test of attention; rats are trained to detect brief visual stimuli that are presented randomly at one of five locations in session of 100 trials separated by a 5s inter-trial interval (ITI). The animals are tested in four conditions: baseline, distraction condition (where random bursts of white noise occur just prior to stimulus presentation), an unpredictable condition (when stimuli are presented randomly), and drug condition (d-amphetamine injected into n. accumbens which produces increased locomotor activity, and impulsive and premature responding).

Cholinergic lesions with alpha-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) or quisqualate microinjected into the nucleus basalis results in reduced accuracy performance of 70% from the baseline condition. This effect can be mimicked with ICV hemicholium, nucleus basalis (NBM) injections of muscimol (this GABA agonist reduces cortical cholinergic function). All three of these effects reversed by physostigmine (Muir et al., 1992a; Muir et al., 1992b). In this paradigm, the cholinergic system seems to function to ensure the information acted upon is accurate and implies the relative saliency of the stimulus with respect to the environment is mediated by cholinergic mechanisms (Robbins and Everritt, 1995). This may well have deep roots in adaptive behavior, as it absolutely necessary for survival to distinguish friend from foe or safety from danger.

These results agree with the long-standing notions that cholinergic system is involved in arousal, attention, and memory, as well as aggression, sexuality and thirst

(Goldberg and Hanin, 1976). Impairment of visual or auditory signal detection can occur after scopolamine injections (Warburton, 1977). However, a general role may be to facilitate focus on the environment and orchestrate an appropriate behavioral response (Panksepp, 1986) and utilize these functions as part and parcel of emotional processing (LeDoux, 1996). Caution is required in interpreting excitotoxic lesions as these types of lesions also destroy other neurochemicals (Dunnett et al., 1991), thus may be related more generally to the functions or connections of NBM. This is supported by the fact that damage to VLDB does not produce the same effect (Marston et al., 1994).

Lesions with 6-hydoxydopamine (6-OHDA) into dorsal noradrenergic bundle produces no gross behavioral deficits and animals can do simple associative tasks, such as conditioned taste aversion (Robbins and Everitt, 1995). Performance on 5-choice test is impaired only in distraction, unpredictable, or drug conditions, but not at baseline. This suggests that normal functioning of the norepinephrine (NE) system acts to preserve attentional mechanisms in stressful situations, and may be increasing the signal to noise ratio to offset these effects (Foote, 1983). NE appears to fulfill many of the properties of the cortical arousing system envisaged by Hebb (1955) and research supports enhancement of processing especially in the cortex and hippocampus.

With lesions produced with 6-OHDA injected into the ventral striatum, performance on the 5-choice task is not impaired in accuracy, but response latency is increased and overall probability of responding is decreased. The implications of reduced motivation with DA lesions concur with the long-standing putative notion of

reward-punishment functions associated with the dopaminergic system (Wise, 1984). The DA system's main functions may be to achieve of activation of behavior in response to cues that signal availability of incentives or reinforcers.

After dorsal raphe lesions with 5,7-dihydroxytryptamine (5,7-DHT), performance on the 5-choice task showed there was no impairment of accuracy, but increased impulsive responding in all conditions was observed (Robbins and Everitt, 1995). Impulsivity resulting from removal of serotonergic tone agrees with the overall function of behavioral inhibition that has also long been associated with the serotonin system (Panksepp, 1986; Robbins and Everitt, 1995). This can be in the context of a variety of behaviors that are inhibited by serotonin, such as feeding, aggression, and play (Vogt, 1982), or induced by serotonin, sleep (Jouvet, 1972). The function of the 5-HT system has long been associated with regulation of appetite, sleep, locomotor activity, and pain response (Panksepp, 1986; Jouvet, 1972; Vogt, 1982).

1.3.2 Normal versus Pathological Psychopharmacology

The neurobiology of emotional processing has relied heavily on psychopharmacology of affective disorders, where several monaminergic theories have been generated based on the alleviation of symptoms with antidepressant medications, such as Prozac. Pharmacology studies on these mood-altering drugs have implicated the serotonin (5-HT), norepinephrine (NE) systems, and dopamine (DA), although more recent studies have shown some efficacy of cholinergic drugs in the treatment of depression (Burt, 1999).

Initially, tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (MAOIs) were identified as successful treatments in depression. Imipramine, the first TCA to be identified, blocked the reuptake of NE into presynaptic neurons (Glowinski and Axelrod, 1966; Glowinski and Iverson, 1966). These experiments led to the generation of the catecholamine theory in the pathogenesis of depression (Prange, 1964; Bunney and Davis, 1965; Schildkraut, 1965). Indolamines were added as a potential mediator of mood dysfunction, as these were thought to be modulating serotonergic neurons (Brogden et al., 1981; Bryant et al., 1982). Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline, paroxetine, citalopram, and fluvoxamine, soon followed supplanting NE as the major neuromodulatory system implicated in depression. The fact that both NE and 5-HT systems have consistently showed abnormalities suggests that these systems play a central role in affective disorders (Garth and Nemeroff, 2002). The strongest support for serotonin system dysfunction in mood disorders comes from the alleviation of depressive symptoms with SSRIs. The "serotonin hypothesis" (Meltzer and Lowy, 1987; Maes and Meltzer, 1995) was generated in from evidence of pre- and post-synaptic dysregulation in depression.

Typically the indolamine dopamine is associated with schizophrenia. Despite this, there is evidence of DA dysregulation and that some antidepressants may act through a dopaminergic mechanism in mesolimbocortical system (Willner, 1995). This system projects from the substantia nigra and the ventral tegmental area in the midbrain to the septum, amygdala, hippocampus, and prefrontal cortex. Low cerebrospinal fluid (CSF) homovanillic acid (HVA), the major DA metabolite has

been reported in patients with depression (Roy et al., 1985 and many others), Parkinson's and Alzheimer's disease (Van Praag et al., 1975; Wolfe et al., 1990), suggesting either a common etiology of depressed mood or psychomotor symptoms. Dopaminergic psychostimulants such as amphetamines, cocaine, and methylphenidate cause transient mood elevation in healthy volunteers and patients with depressed mood, as well as individuals abusing substances. This suggests a role of the DA system in the induction of normal emotions and moods. Of interest, scopolamine, a non-specific muscarinic antagonist, diminished self-administration of cocaine suggesting that cholinergic agents may regulate the release of DA (Ranaldi and Woolverton, 2002), thus participating emotional responses.

While most tricyclic antidepressants have anti-cholinergic actions that yield adverse effects (such as dry mouth and difficulty in urination), it is also possible that relative cholinergic blockade could contribute to their efficacy and increased proclivity to cause switches in mania compared with less anticholinergic antidepressants (Peet, 1994; Guille et al., 1999). On the other hand, donepezil, a cholinesterase inhibitor, has been reported to be efficacious in treating bipolar disorder (Burt et al. 1999) and relieves some of the anti-cholinergic adverse effects with some risk of mania induction (Jacobsen, 1999), indicating that maintaining a relative cholinergic balance may be instrumental to mood disorders.

1.3.3 Emotion Induction Studies

Pharmacological emotion induction studies have implicated the cholinergic system in affective processing. This has originated from the finding that individuals

with depression are more susceptible to the effects of muscarinic agonists than healthy volunteers. For example, physostigmine, a cholinesterase inhibitor, can reduce manic symptoms and exacerbate depressive symptoms in patients with bipolar disorder (Janowsky, 1972b). Furthermore, physostigmine administration results in relapse of depressive symptoms in bipolar patients successfully treated with lithium, while healthy controls do not develop depressed mood. The patients with bipolar disorder also have concomitant hormonal disturbances and emotional arousal expressed as dysphoria (a mixed anxiety depression state, with more anxiety than depression) with physostigmine administration. Procaine also produces similar emotional and endocrine responses (Kellner et al., 1987; Kling et al., 1994; Ketter et al., 1996).

Several theories regarding the relative balance of neuromodulators with acetylcholine, including cholinergic-noradrenergic (Janowsky, 1972a) and cholinergic-serotonergic (Overstreet et al., 1998), and their interactions have been suggested. Arecoline also has been reported to cause dysphoria (Nurnberger et al., 1989). Sitaram and colleagues (1987) found enhanced REM latency induced by arecoline, a general muscarinic agonist, in mood disorder patients whether or not currently depressed, and in individuals with a family history of depression compared to those without such history.

1.3.4 Interactions Between the Cholinergic and Other Neurotransmitter Systems

Opportunity for complementary actions between the cholinergic system and monaminergic systems is supported by the existence of many cortical and subcortical

brain regions innervated by cholinergic, serotonergic and noradrenergic projection neurons, however, the dopaminergic system has predominately a subcortical afferent pattern and limited cortical projections. With regard to the anterior cingulate, the cortex serotonergic projections terminate in all layers, but most heavily in layers I, II, V and VI in area 24 of the anterior cingulate (Crino et al., 1993); thus there is potential for interaction at layers I and II as cholinergic terminate for the most part in these layers as well as layer III (Van Hoesen et al., 1993). Noradrenergic innervation of the anterior cingulate occurs in all layers of area 24 thus allowing for cholinergic interaction in layers I, II and III (Crino et al., 1993), and there is also dopaminergic innervation of the anterior cingulate, layers I, II, V and VI (Crino et al., 1993).

Other potential regions of complementary actions can occur in the ventral and dorsal striatal and pallidal regions. Amygdalar (lateral, basal, and central nuclei) co-localization of ACh with NE may be more restricted than that of 5-HT, with maybe the major nuclei where interaction can occur is in the central nucleus (Amaral et al., 1992). Interaction of the cholinergic system with the DA systems is well documented in the both the dorsal (Graybiel, 1990, 2000) and ventral striatum (Holt et al., 1997; Záborszky and Cullinan, 1992), where ACh stimulates dopamine release. Peptidergic actions may potentiate this dopamine release.

The monoaminergic systems all project to the basal forebrain nuclei. Documented interactions between ACh and NE can occur in the basal forebrain (Záborszky et al., 1991) and 5-HT (Nilsson et al., 1988). The frontal cortex is only area with feedback connections to brainstem/midbrain monoaminergic and

cholinergic cell groups; these connections allows for regulation of its own input (Goldman-Rakic, 1987).

The basal forebrain area has numerous GABAergic cell bodies that are projection neurons as well as interneurons. GABAergic projections from the cortex synapse on cholinergic cell bodies in the basal forebrain (Sarter and Bruno, 2002). Within the striatum, cholinergic markers co-localize with GABA and other neurotransmitters (Graybiel, 1990). These are just a few examples of sources of most likely numerous interactions between the cholinergic and GABAergic systems.

This brief assessment touches upon but a few of the multiple potential sources of complementary actions between the cholinergic and monaminergic systems. To summarize the relevant neuropharmacological findings, a balance between acetylcholine and both serotonin and norepinephrine in the anterior cingulate, amygdala, and basal forebrain has been documented. There is considerable evidence indicating the dopamine and cholinergic systems act in complementary ways in the dorsal (Graybiel, 1990, 2000) and ventral striatum (Holt et al., 1997; Záborszky and Cullinan, 1992). In addition, amino acid, peptidergic and unknown neuromodulatory systems may inhibit or facilitate the actions of ACh and other neurotransmitters. As multiple neuromodulatory systems function together, the relative role of any single system with respect to the emotional processing may be limited.

1.4 Theories of Emotion Regulation

Strongman (1987) loosely categorizes the numerous current theories from their origins in by physiological constructs, such as motivation and/or arousal

(Lindsley, 1951, 1970), by behavioral (Watson, 1930; Pavlov, 1938; Skinner, 1953; Millenson, 1967; Gray, 1995), by psychoanalytic or experiential, or by cognitive approaches. There are composite approaches (Plutchik, 1980; Izard, 1993) incorporating to varying degrees both psychological and physiological aspects. Some models have delved into neurophysiology and psychopharmacology, while incorporating constructs from behaviorists, cognitive scientists and psychopathology (Panksepp, 1986; LeDoux, 1996). In addition, examination of neuropsychiatric conditions has generated medical models (Mayberg, 2002; Davidson, 2002; Damasio, 2001).

Motivational theories consider dimensions of arousal, activation and pleasantness/unpleasantness as models of emotion. Lindsley (1970) took into account attention, sleep, vigilance, and motivation in his model that involved the descending and ascending reticular systems (ARAS), based on electroencephalogram (EEG) studies. Lindsley proposed that the reticular system must be activated in order for emotional behavior to occur, as opposed to the limbic system, which functions to organize input into particular expressions.

Arousal has been suggested to be modulated by brainstem the monaminergic nuclei, ACh, NE, DA, and 5-HT (LeDoux, 1996; Robbins and Everitt, 1995; Mesulam, 1995). The relationship of arousal to the cholinergic system may be unique in that there is the additional major group of cholinergic cell bodies in the basal forebrain that can influence attention, motivation and arousal more so than the monaminergic systems.

1.4.1 Composite Theories

There are many investigators that have created composite theories, which take into account the various perspectives of emotional behavior. Izard (1993), Panksepp (1986), and LeDoux (1996) stand out due to their efforts to integrate neurobiological, cognitive, and psychological concepts that resulted in a comprehensive model of emotion. Emotions can be associated to the underlying neural processes, with the eventual goal of ascribing individual emotions to a particular neural circuit. For example, the amygdala is the most likely the pivotal component of the neural system subserving fear (Davis, 1995; LeDoux, 1996). Emotion also includes an expressive component, where affect can be assessed. Motoric aspects, such as facial and vocal expression, visceral activity, and eye, head and postural movement, help to define emotions. This perspective also creates the possibility to identify emotions, and assess with precise and objective methods, such as research utilizing faces expressing specific emotions (Ekman and Friesen, 1978). Emotions are part of and register in consciousness. The words emotion, feeling and emotional experience are often used interchangeably. Experiential factors, such as motivation, perception, tendency to action and feeling state, are not always in the forefront of consciousness, but add to the gestalt/qualia of emotion.

1.4.2 Izard

Izard recognized the value of examining emotions from various perspectives and created one of the more comprehensive model of emotion to date (1993). Izard proposed that because of the significant role of emotion in evolution and adaptation

(Darwin, 1859, 1872/1965) several avenues for generating them must have developed. Furthermore, redundancy in the nervous system creates the possibility for different pathways in the genesis of emotion. Izard (1993) proposed a multi-system model of emotion activation, where four inter-related systems can generate and modulate emotion. The four systems are neural, sensorimotor, motivational, and cognitive processes. He suggested that these four systems related to each other, dependently or independently, and had different underlying mechanisms and processes. Ultimately, however, generation of emotions relies on neural processing subserving each system, making neural processing the only system necessary and sufficient for emotion generation. Finally, at any time, each system may exert dominance in terms of driving emotion supplanting the one currently active. For example, a sudden sharp pain to the foot may initially induce anger (sensorimotor induction), but quickly be replaced by sympathy upon appraisal of the accidental nature of the situation – a child dropping something (cognitive induction).

Izard (1993) argued that evidence from evolution and biology suggested a hierarchical organization, with neural processing at the basic level, serving sensorimotor, motivational and cognitive levels to generate emotion, with cognition at the highest level. In a developmental sense, neural and sensorimotor systems engage before motivational (e.g., reproduction) and cognitive (e.g., higher order reasoning) systems. Information processing can also be arranged hierarchically. In general, the more complex the situation, the more likely higher levels of the activation system will be engaged.

1.4.3 Panksepp

Panksepp (1986) developed a complex multilevel theory that ultimately rests on neurophysiology, and was based on integration of introspection (or anthropomorphism) and animal brain research. At the same time, his theory is traditional, with the incorporation of evolutionary perspectives that mammals share emotional circuits in the limbic system, the "obligatory internal dynamics." He made five assumptions: 1) distinct emotion processes are reflected in specific hard-wired circuits; 2) primitive emotion processes are shared between humans and other animals; 3) the number of basic emotional circuits are limited, but much can be contrived through mixtures and social learning; 4) neurotaxonomy may possibly be considered through introspection; and 5) a scientific understanding of emotion processes may be gained through the study of brain organization. He proposed: 1) genetically hard-wired unconditioned responses are made to life-challenging circumstances; 2) an adaptive activation or inhibition of classes of related actions exist; 3) with recurrent feedback, emotion circuits change their sensitivities; 4) neural activity can go on longer than circumstances that give rise to it; 5) reinforcement can condition activity in the emotion circuits to environmental stimuli; and 6) interaction occurs between the emotion circuits and the brain mechanisms of consciousness.

He described four emotion-mediating circuits that connect the midbrain, with the limbic system and the basal ganglia, subserving expectancy, fear, rage and panic. Dopamine and ACh mediate the expectancy and rage circuits, while the panic and fear circuits are mediated by benzodiazepine receptors and endorphins. Each has distinct neuroanatomical connections in the common midbrain and limbic system

regions, of which brain amines, such as serotonin and norepinephrine, may be involved differentially.

At a psychological level, he draws into his theory ideas of how learning and reinforcement play a role in shaping emotional responses and their neural substrates. For example, in the expectancy circuit, learning has been shown to be an intrinsic property. "With an emotive state-dependence on memory retrieval, and thus, emotional experience, higher brain circuits might access lower brain circuits leading to the cognitive appraisal as an influence on the development of emotions." Panksepp, thus, created a model where the brain emotion system forms unique limbic circuits specialized for each of the four basic emotional/experiential states, which are initially hard-wired genetically, but through subjective experience can be modulated and/or combine to result in more complex emotions.

1.4.4 LeDoux

Integrating previously separate concepts from behaviorism, neuroanatomy and neurochemistry, LeDoux suggested limbic circuits subserving emotion are malleable by experience. LeDoux (1996) incorporated current theories of working memory, consciousness, arousal systems and subjective experience. Based on his work on fear-conditioning of the amygdala, he created a model of the general emotion system in effort to build up core knowledge on one emotion that may eventually be expanded to other emotions. He chose to focus on fear as it is pervasive within human experience, is an important feature of psychopathology, and is expressed similarly across species.

According to LeDoux (1996), there are three general principles of emotion. First, subjective emotional experiences result from becoming consciously aware that an emotion system is active. However, the roots are in the unconscious processes. Emotions are created by the establishment of a conscious representation of the underlying processing systems, much like other cognitive processes such as working memory. Second, working memory may be the cognitive interface between bottom-up and top-down processing systems. This requires brain systems to have various short-term buffers of information readily available for different aspects of cognition, like sensory, spatial, linguistic or emotional aspects of behavior. For example, the lateral prefrontal cortex performs general purpose working memory tasks, while anterior cingulate and orbitofrontal cortex may be specialized as emotion-related working memory tasks.

Third, by combining the first two principles, emotions come about when the specialized emotion systems are represented in consciousness through utilizing the principles of working memory. The emotion system is dependent on the neural network subserving it, which heavily relies on the amygdala for species-specific and experience-specific contextual information, and the anterior cingulate and orbitofrontal cortex for evaluative processing.

LeDoux's hypothesized a series of events occurs with activation of the amygdala. First, there is direct influence on the cortex via direct connections from the amygdala to the cortex. These pathways allow the defense system network of the amygdala to influence attention, perception and memory in situations where we are facing danger. The vast, often reciprocal, connections of the amygdala with sensory

processing areas, visual and auditory, at multiple levels of the processing allows for modulation of the same processes generating input into the amygdala. Amygdalar connections with hippocampal systems mediate the long-term memory processing. The most important connections include those with the anterior cingulate modulating executive functioning and orbitofrontal cortex mediating working memory of reward and punishment.

A second focus is the nature of the amygdala-triggered arousal – attentional mechanisms that are dependent on level of arousal. Cortical and thalamic cells become unsynchronized upon arousal with selectively driven cells activating more so. Four arousal systems are each dominated by different forebrain cholinergic or brainstem nuclei neurotransmitters (ACh, NE, DA and 5-HT). Together, these systems mediate arousal level, but each with subtle differences. However, the basal forebrain cholinergic system appears to be particularly important. Damage to nucleus basalis prevents stimuli that "warn of danger" from eliciting arousal and stimulation of the amygdala or nucleus basalis elicits cortical arousal. Moreover, drugs that block ACh actions on the cortex prevent the effects on arousal of conditioned stimuli, amygdala stimulation or nucleus basalis stimulation from occurring. Thus, detection of a dangerous situation initiates the amygdala to release ACh from the basal forebrain across the cortex.

Arousal can occur from non-threatening or novel events, but they are not prolonged as in the case of events with emotional significance and do not involve the amygdala. Cortical arousal occurs directly from sensory input and indirectly from brainstem activation from cortical efferents (Lindsley, 1951, 1970); LeDoux suggests

that these kinds of arousal quickly habituate. If there is emotional significance to the stimulus, the amygdala acts to perpetuate the arousal. The neurophysiology underlying sustained activity in a circuit may be achieved by recurrent loops or bursting activity. Thus, one might speculate that the anatomical connections of the anterior cingulate with dual innervation from the NBM and amygdala may subserve extended arousal and attention in emotional circumstances.

LeDoux hypothesized the core of emotional processing to be the nonspecific emotional system, which can be triggered by any emotional event, and interaction of this general emotion system with appraisal and evaluative processing systems that generate species-specific actions. Attentional mechanisms and long-term memory of emotionally significant events are posited to be a result of the anterior cingulate and orbitofrontal cortex processing. Additional information from short- and long-term working memory by lateral prefrontal cortex converges into a representation that has a precise meaning and consequently a specified course of action. Attentional mechanisms also come into play by blocking other ongoing cortical input.

Species-specific visceral and motoric expression is hypothesized to occur via amygdala connections with hypothalamic and brainstem nuclei, which feedback to brain. This allows for a host of neuromodulatory mechanisms to ensue to produce visceral and motoric aspects of emotional expression, feedback to the brain and result in modulating the expression. Cannon's idea that the viscera do not respond with a specific pattern has been shown not to be the case; galvanic skin response (GSR), heart rate, skin temperature all show differential expression with the different emotions. The visceral reaction is too slow to account for James' feedback theory,

however, the striated muscle response is quick enough and has been theorized as a potential source of feedback (Izard, 1993).

To recapitulate LeDoux's model, the whole picture is the combined functioning of the specialized emotion system (amygdala and its connections), cortical sensory buffers, cortical working memory, cortical arousal and bodily feedback. "When all of these systems function together a conscious emotional experience is inevitable" (LeDoux, 1996).

1) Emotional feelings (of fear) are represented in working memory.
2) Complete feelings (of fear) involve activation of the amygdala.
3) Feelings are sustained by the arousal system keeping attention on the emotional situation.
4) Emotional experiences are sustained by feedback from the body providing distinct sensations that make emotions feel different from one another.
5) Emotional feelings can happen without direct cortical projections from the amygdala, i.e., the working memory of a feeling can be deduced from indirect sources, however, the emotion will be different.
6) Emotional feelings can happen without being conscious of the eliciting stimulus – "The emotional responses and the conscious content are both products of specialized emotions systems that operate unconsciously."

1.4.5 Damasio

Damasio (2001) proposed that the combination of both visceral and motoric feedback underlie the "gut feelings" associated with emotion. When subjects who

performed certain facial expressions characteristics of emotions were queried about how they felt, there was a significant influence by whether the expressions were associated with positive or negative emotions (Ekman, 1992, 1993; Adelmann and Zajonc, 1989).

Damasio hypothesized from the work of Valins (1966) that the "as if" feedback becomes cognitively represented in working memory to thereby influence feelings and decisions. In this study, subjects were given false feedback about their heart rate when viewing pictures (Valins, 1966); the subjects believed that their heart rate was changing enough to make them feel as though they were emotionally aroused, and that they like certain pictures more than others. Damasio suggested this supported the existence feedback could act to influence subjective experience.

1.4.6 Mayberg

The last two models to be discussed in detail have originations in the pathophysiology of emotion. Mayberg's model of emotion regulation originated from the observation that there is a common affective response to structural deficits and resulting brain dysfunction in disease states. Parkinson's, Huntington's, and Alzheimer's disease (Mayberg, 2002) can cause secondary depression, a depressed mood in conjunction with the primary motor or memory disorder. She developed a model of emotion regulation drawn from examining common functional neuroanatomical substrates in depressed states observed in primary and secondary mood disorders, the functional changes associated with antidepressant or

psychotherapeutic interventions in mood disorders, the remission of symptoms whether or not on active treatment, and induction of emotions.

She observed that remission of symptoms, whether from antidepressants, placebo, or psychotherapy, often (but not always) yielded changes in cortical structures mediating attention and cognition, such as dorsal anterior and posterior cingulate (24c, 23), dorsolateral prefrontal cortex (9, 10, 46), premotor, and parietal cortices. In contrast, antidepressants often (again, but not always) yielded changes in limbic regions associated with vegetative-circadian functions, such as subgenual anterior cingulate, insula (anterior and posterior), orbitofrontal cortex, amygdala, hippocampus and hypothalamus. The limbic and cortical components are modulated by the subcortex component including the rostral anterior cingulate (24a; not subcortex, but activity matches these other subcortical areas), basal ganglia, thalamus, and brainstem, of which all have been reported to have increased metabolic of blood flow activity in mood disorders (Ketter et al., 1999; Kimbrell et al., 2002). Her belief is that the failure of this regional network to respond to homeostatically in times of increased stress results in the various symptoms observed in depressed patients, and may explain the heterogeneity of the disorder depending on the nature of the network's dysfunction (Mayberg, 2002).

1.4.7 Davidson

Based on models like Plutchik (1980) and Gray (1995), Davidson (2002) suggested two essential emotion systems, approach and withdrawal. Each is associated with positive or negative affect in the general form of moving towards a

desired goal or way from aversive stimulation, respectively. These emotion systems are subserved by specific neural networks, which often can be distinguished by the laterality of the regions involved. For example, the left prefrontal cortex is considered part of the positive affect system, because focal damage of the dorsolateral prefrontal cortex results in depressive symptoms, in line with the reasoning that depression is associated with deficits of positive affect. Although the method lacks spatial resolution, electrophysiological (EEG) asymmetries have been shown, with positive affect to be associated with the left prefrontal cortex and negative affect with the right prefrontal cortex (Davidson, 1998). PET studies of emotion induction have also shown this laterality (George et al., 1995).

1.4.8 Summary

According to Strongman (1987) current emotion theory has evolved such that several principles have come out of the work on emotion: (1) Emotion can be viewed as a system that both affects and can be affected by other systems; (2) Emotions can influence arousal and motivation; they can be energizing or motivating, or they can be draining and demoralizing; (3) Intricate links exist between cognition and emotion; (4) Emotional expression is an important component of the emotional experience; (5) Some form of hierarchy in emotions may exist, some are primary and some are secondary, indicative of a nature versus nurture division; (6) Similarities and differences exist between the various emotions, and they can range in intensity, in which the quality may change upon reaching some vague threshold; and (7) Emotion

is multi-faceted; this has promoted many avenues for exploration. These principles have in some cases continued to develop in a similar vein, however, most theories have incorporated several of these ideas.

Clearly, the study of emotion is complicated. However, with techniques to analyze brain and behavior relationships becoming more sophisticated, the information gleaned utilizing these newer methods has resulted in the development of these complex theories. Of the theories reviewed here, LeDoux has developed an intriguing theory spanning scientific fields from consciousness to neurochemistry. The purported involvement of the cholinergic system speculated in his theory provides strong rationale for this thesis and theoretical background to discuss the results of the proposed experiments.

1.5 The Muscarinic Cholinergic System Involvement in Emotion Regulation

There appears to be substantial evidence that the cholinergic system plays a role in emotion and its regulation. For example, lesions of the NBM interferes with arousal elicited by stimuli that may "warn of danger" and the fear-conditioning process (LeDoux, 1996), and the reduced accuracy in tests of arousal and attention, functions closely related to emotional processing (Robbins and Everritt, 1995). Older studies have shown that lesions of the septal nuclei, which have high concentrations of cholinergic cell bodies, induce septal rage.

Connections between cholinergic cell groups and the amygdala and anterior cingulate regions may subserve novel and sustained attentional mechanisms necessary for assigning emotional valence to particular situations, and initiate

appropriate action (LeDoux, 1996). This is consistent with Panksepp's theory (1986) of the rage and expectancy circuits being influenced via ACh and DA neuromodulatory effects, and agrees with the general arousal system of ARAS (Mesulam, 1995). The importance of the cholinergic system in the neurochemistry of the basal forebrain region, including the cholinergic cell groups, ventral striatal region, amygdala, and extending to the anterior cingulate, but also projecting across the cortical mantle, supports Mesulam's notion that the cholinergic system may be the most substantial regulatory system.

Challenge studies with known cholinergic activity in man have also supported a role of this system in emotional processing. Physostigmine, an ant cholinesterase, administration in humans yields dysphoria (Janowsky, 1986). Similar emotional and endocrine responses occur with procaine hydrochloride (Novocain) administration, although the mechanism of the latter remains to be established and is the focus of this thesis. Since 1950s, case reports have described emotional and sensory responses to the antibiotic procaine-penicillin (Araskiewicz and Rybakowski, 1993), where procaine is tagged onto penicillin to enhance central nervous system (CNS) penetration. Anxiety and perceptual disturbances, such as auditory, visual, gustatory and somatosensory hallucinations, along with autonomic arousal emerge within seconds following injection of procaine-penicillin, with these responses lasting from 5 to 30 minutes. Procaine-penicillin has a longer half-life than procaine hydrochloride due to a different metabolic pathway, and thus yields more prolonged effects than procaine.

Similarly profound, but briefer, emotional and sensory experiences occur upon intravenous administration of procaine hydrochloride, a local anesthetic that has been used as an investigational tool in humans for probing the limbic system (Ketter et al., 1996; Parekh et al., 1995; Kling et al., 1994; Kellner et al., 1987;). A dose of 1.84 mg/kg in healthy individuals produces emotional responses including dysphoria, euphoria (elation), visual and auditory sensory experiences, hormonal changes such as increased adrenocorticotropic hormone (ACTH), cortisol, and prolactin (Kellner, 1987) and EEG fast activity increases in the temporal lobes (Parekh, 1995). The experiences described by the subjects in these experiments are reminiscent of those described by Penfield, Gloor and Halgren (Penfield and Jasper, 1954; Halgren et al., 1978; Gloor et al., 1982) after direct stimulation of limbic cortex during epilepsy surgery.

With the development of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), the neural substrates involved in emotional processing can be determined at the neuroanatomical level at the same moment that emotions are experienced. Measurement of cerebral blood flow (CBF) with $H_2{}^{15}O$ PET during procaine administration yielded a 23% increase of global blood flow and even greater regional increases in anterior paralimbic areas (Ketter et al., 1996). Furthermore, both euphoria and fear responses correlated with changes in left amygdala CBF; euphoria had an inverse relationship with changes in left amygdala CBF, while fear had a positive correlation. Also, increases in mesial occipital and global CBF were associated with visual hallucinations.

Preliminary analysis of the CBF response to procaine in affectively ill patients compared to healthy volunteers blunted (diminished increases) global CBF and regional absolute paralimbic CBF activation. Patients with mood disorders have blunted rCBF in the "resting" state (when they are asked to monitor their internal emotional state), suggesting that some of the procaine's effects on blood flow may be related to baseline differences. However, even after subtracting out the baseline differences blunted anterior paralimbic activation is still seen compared to healthy controls. PET studies during self-induced emotional states showed overlapping rCBF increase in anterior paralimbic regions (George, et al., 1995). Thus, emotional states either self-induced or pharmacologically-induced, resulted in activation of overlapping anterior paralimbic areas. Taken together, the above data suggests that procaine may be a useful tool for investigating the neural substrates of emotional and sensory experiences.

1.5.1 Procaine as a Muscarinic Cholinergic Ligand

Animal studies have linked emotional responses to the actions of procaine and have implicated the muscarinic cholinergic system. Procaine administered to animals selectively activated electrophysiological activity in limbic structures (Wagman et al., 1967; Racine et al., 1975; Racine et al., 1979; Munson et al., 1970). Utilizing deoxyglucose methodology (2-DG), lidocaine, an amide local anesthetic, was shown to selectively activate limbic metabolism in rats (Post et al., 1984). Local anesthetics, including cocaine, lidocaine and procaine, can induce kindled seizures and aggressive behavior in rodents (Post, 1981). Cholinomimetics induce kindled

seizures similar to electrical amygdaloid kindling, which can be blocked by muscarinic antagonists (Cain, 1981). Furthermore, atropine inhibits and physostigmine weakly facilitates procaine induced kindling in rats (Heynen, 1995).

The mechanism of procaine' s sensory and emotional effects is not known. Evidence from neuroanatomy, neuropharmacology and neurochemistry suggests muscarinic M_2 receptors might play a role in action of procaine (Mesulam, 1995; Nieuwenhuys, 1985; Vogt, 1993; Adamec et al., 1985). The following sections will detail each of those perspectives.

1.5.2 Neuroanatomy

Since the cholinergic neuroanatomy has been extensively reviewed already, a brief synopsis of the relevant brain regions and their connections follows. The reciprocal connections between the amygdala and substantia inominata and further reciprocation between limbic cortex and thalamus provide an interface between limbic cortex and visceral hypothalamic areas whereby sensory and cognitive phenomena influence emotional aspects and autonomic responses. Of particular interest are the bi-directional cholinergic pathways between the CH4-NMB and the amygdala and the anterior cingulate, and projections to medial orbitofrontal cortex and regions ventral to both of these areas. Thus, the anterior cingulate appears to receive direct and indirect via the amygdala cholinergic input (Figure 9). This dual innervation may subserve the maintenance of attentional mechanisms described by LeDoux (1996) required in emotional processing that is adaptively oriented.

Moreover, it is precisely these regions that are selectively activated with acute procaine administration in humans (Ketter et al., 1996).

Mesulam (1995) emphasized the importance of the amygdala and limbic basal ganglia as mediators of emotional behavior. The cholinergic telencephalic projections (paralimbic areas) can be classified into two major groups: 1) the anterior projection group including all regions of the cortex, basolateral amygdala, reticular nucleus in the thalamus and basal ganglia nuclei or the amygdalocentric portion of the limbic system suggesting a role for ACh in emotional processing; and 2) The septal nuclei and the vertical limb of the diagonal band (VLDB) within the substantia inominata cholinergic projections innervate the hippocampal complex. This population of cholinergic neurons corresponds to the posterior or hippocampocentric portion of the limbic system that may mediate memory and learning. Heimer and Alheid (1991) suggested this septohippocampal system appears to be a separate functional unit from other basal forebrain systems. Vogt defined reciprocal cingulate connections with amygdala, insula, anterior temporal lobe, and basal forebrain in similar manner (Vogt et al., 1992). He described the anterior cingulate as ”executive” mediating affective, attentional, semantic processing and vocal functions, and the posterior cingulate as “evaluative” being involved in motor and sensory processing.

The pedunculopontine and lateral dorsal tegmental nucleus are the major groups of cholinergic cell bodies in the brainstem; these areas project to the intralaminar thalamus and onto the cortex as part of the ascending reticular pathways. Brainstem cholinergic pathways also project to the lateral hypothalamus and basal forebrain. Thus, phylogenetically more recent telencephalic structures mediating

more sophisticated functions and older brainstem areas contributing to consciousness and arousal constitute the neuroanatomy of the cholinergic system.

1.5.3 Neuropsychopharmacology

The neuropsychopharmacology of the cholinergic system is divided into two major receptor systems, nicotinic and muscarinic, originally isolated by their affinity to nicotine and muscarine, respectively. Nicotinic receptors are ligand-gated ion channels with a pentameric structure of subunits that allows passage of potassium (K^+) and sodium (Na^+) (Changeux, 1993). Four muscarinic receptor subtypes(M_1-M_4) have been discovered through pharmacological studies, while cloning studies identified five subtypes (m1-m5), with concordance between the two methods, i.e., the M_1 corresponds to the m1, etc., although m5 remains unpaired. The five subtypes are believed to be comprehensive based on total nonselective agonist binding as compared with partitioning of selective antagonist binding.

Muscarinic receptors can be divided into two groups, based on coupling to specific G proteins. M_1, M_3 and M_5 receptors act via Gq stimulating PI turnover, while the M_2 and M_4 couple with Gi inhibiting cyclic adenosine monophosphate (cAMP) production. Increase phosphotidylinositol (PI) turnover results in release of calcium (Ca^{++}) stores from the endoplasmic reticulum and increased phosphorylation of membrane receptor proteins, K^+ and Ca^{++} channels. This results in increased likelihood of channel opening and further increases in Ca^{++} levels. Thus, direct and indirect increases in Ca^{++} increase neuronal excitability. cAMP activation also phosphorylates membrane proteins, increasing the likelihood of K^+ or Ca^{++} channels.

By inhibiting cAMP production, M_2 activation results in reduced phosphorylation of K^+ or Ca^{++} channels, thereby decreases neuronal excitability.

$M_1/M_3/M_5$ compared to M_2/M_4 receptors have more complex transduction pathways yielding additional opportunities for signal amplification. Therefore, $M_1/M_3/M_5$ class of receptors is believed to have less need for sensitivity to ligands or of signal transduction within the receptor/ G protein complex. In contrast, the M_2/M_4 receptors are thought to need to be more sensitive to signal activation having less steps in the transduction pathway and less opportunity for amplification. Besides the active site, the M_2 receptor contains an allosteric site creating the possibility of potentiation of agonist activity (Lena and Changeux, 1993).

Nicotinic receptors are localized predominately in the nucleus basalis of Meynert, cortex, thalamus, striatum, hippocampus and cerebellum in humans (Ripoli et al., 2004). Four methods for muscarinic receptor localization (competitive binding curves, direct binding studies with same selective antagonist, immunoprecipitation and identification of clones through in situ hybridization) yield similar findings. M_1 receptor density increases caudal to rostral with highest densities in hippocampus, cortex and striatum and lowest densities in the brainstem nuclei. M_2 receptors have more uniform and low-level distribution with the highest density in brainstem nuclei and lower levels rostrally. However, there are some significant exceptions; intermediate to high M_2 receptor density is found in the olfactory bulb, cortical layers I and II, lateral and medial septum, striatum, hippocampus, and amygdala. Flynn and Mash (1993) found the highest levels of M_2 receptors in hypothalamic nuclei, including the lateral hypothalamic area, and the anterior nucleus and other thalamic

areas. The non-M_1/M_2 distributions generally show increases caudal to rostral with the highest densities in the striatum and cortex and lowest densities in brainstem nuclei.

1.5.4 Neurochemistry

Acetylcholine has two active moieties, an acetate and a choline, each binding with one of the cholinergic receptor systems. The choline portion binds muscarinic receptor's active site and the acetate portion binds the nicotinic receptor's active site. The binding of a positively charged choline moiety to the active site in the muscarinic receptors suggests that anionic amino acid residue(s) are involved (Hulme et al., 1990). Aspartate (ASP) 105 and 111 provide carboxylic residues with a usual pK around 3.9. Depending on the pH and adjacent residues' effect on the active site, the aspartates may have a negative charge drawing in ACh. Adjacent to the aspartates are threonine 234 and tyrosine 506 with hydroxyl residues that may encourage agonist, but not antagonist binding. The OH residues may create an oxyanion hole stabilizing the double bond oxygen in the ester linkage of acetate during transition states. Site-directed mutagenesis studies show that the aspartates, threonine and tyrosine are essential for ACh binding (Brann et al. 1993; Fraser et al., 1989).

Procaine (mw=236) has a similar structure to ACh with respect to the choline-like ester linkage of a hydrophilic tertiary amine (Figure 11). It differs with respect to the acetate end; a lipophilic aromatic replaces the methyl group. When protonated, procaine can be viewed as an ACh molecule with an aromatic ring substituted for the methyl end group. The positive charge of the choline-like end may bind to the same

active site as ACh, however, the bulky ring may keep procaine from fitting completely into the receptor. The tyrosine and threonine residues cannot confer their stabilizing effect resulting in partial binding to the active site. The amine portion of the procaine is thought to bind to residues inside the pore.

The acetate portion of ACh is known to bind to the nicotinic receptor; procaine's lipophilic ring may be too bulky to allow significant binding to the nicotinic receptor's active site. However, it is also well known that procaine non-competitively binds to a high affinity site in the nicotinic receptor pore, blocking ion conductance (Klobin et al., 1979; Lena and Changeux, 1993; Yost and Dodson, 1993), not blocking the active site.

Procaine's chemical properties make it very easy to pass the blood brain barrier (BBB; Table 2). It has low lipid solubility constant and is protonated at normal body pH to facilitate membrane passage with the acid/base transporter. Elimination occurs mostly via butyrylcholinesterases in the plasma and the synapse. At high concentrations enzyme inhibition may occur (Butterworth and Strichartz, 1990; De Jong, 1994).

Animal studies show that procaine displays a variety of neurochemical actions. It blocks voltage-gated sodium, but not potassium channels, in the *Torpedo californica* and the *Electrophorus electricus* by binding in the channel pore and altering the gating mechanism keeping the channel in an inactive state (Butterworth and Strichartz, 1990). The affinity constant for Na^+ channel blockade is in the millimolar range.

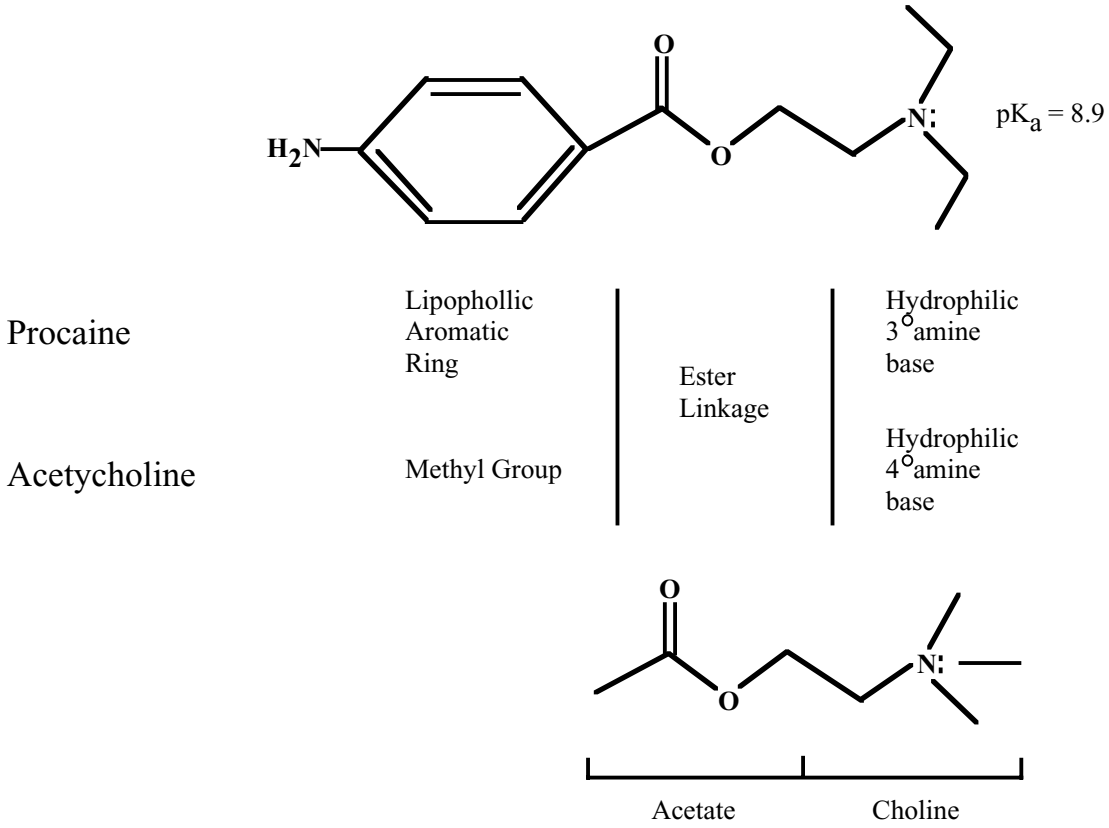


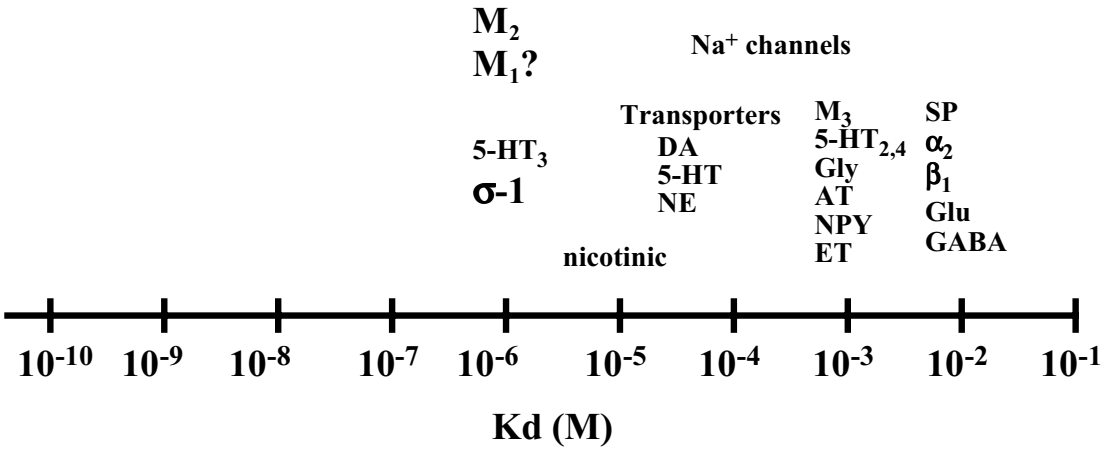
Figure 11. Structural Comparison Between Procaine and Acetylcholine. Procaine has an additional moiety of an aromatic ring that may aid its passage through the blood brain barrier and produce differential binding properties. The tertiary, rather than quaternary, amine base of procaine may a more limited differential effect on passage or binding from acetylcholine.

Table 2. Physio-Chemical Properties of Procaine Hydrochloride.

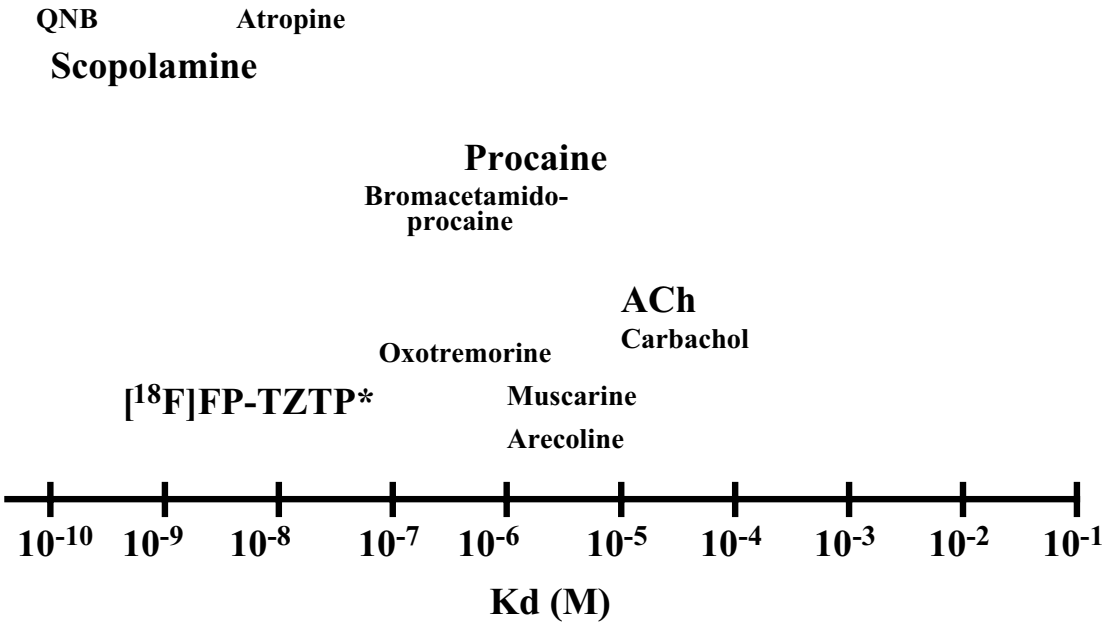
2% base at pH 7.4
Low protein binding (5.8%)
Low lipid solubility constant
Half-life estimations: $t_{1/2}$ = 7.7 minutes in humans $t_{1/2}$ = 45 minutes in rhesus monkeys (1st estimation)
Metabolized predominantly by butyrylcholinesterase [plasma] > [brain] Only 10-20% of cholinesterases are butyrylcholinesterase Majority of enzyme concentrations found in synapse
Enzyme inhibition occurs at higher concentrations
CSF concentrations 50% of plasma levels 5 minutes after 1 mg/kg injection may be as high as 90% on 1st pass rate of entry estimated to be faster than quinine and slower than pentobarbital
May be passing BBB with acid/base transporter

Procaine competitively displaces quinuclidinyl benzilate (QNB) at M_2 receptors in the guinea pig ileum (Hisayama et al., 1989) with a Kd (dissociation constant) of 4 μM and inhibits QNB binding in the rat brain also at low μM concentrations (Saraswati et al., 1992).The affinity constants for the M_1 receptor cited are virtually identical, however, the tissue used for the assay was rat hippocampal membranes. Since the approximate levels of muscarinic receptors two thirds M_1 and one third M_2. This is a considerable proportion of M_2 receptors, thus M_1 binding is suspect. The affinity constant of procaine for the nicotinic receptor on rat brain membranes is 50 - 100 μM depending on the agonist used (methylcarbachol or dimethylphenylpipereinium [DMPP]) (Saraswati et al., 1992). With the exception of sigma, and 5-HT_3 receptors, the affinity constants for virtually every other system is millimolar or higher (Sharkey et al., 1988; Fan and Weight, 1994). Although the influence of these receptors with similar affinities to procaine cannot be disregarded, the distributions of these receptors do not complement the procaine activation pattern as well as the muscarinic receptor. Specifically, sigma receptors are mostly found in hippocampus, striatum, thalamus, cerebellar hemisphere and vermis, and across the cortex (Elsinga et al., 2004) and 5-HT_3 receptors are localized in the layers II-III of the cortex, hippocampus, and amygdala, caudate putamen, nucleus accumbens, and within the brainstem, motor nuclei and area postrema in rats (Morales et al., 1998). Procaine has relatively similar affinity to muscarinic receptors as acetylcholine (Figure 12).

A.



B.



*** 3-(3-(fluoropropylthio)-1,2,5-thidiazol-4-yl)-1,2,5,6-tetrahyro-1-methylpyridine**

Figure 12. Relative Affinities of Procaine
Relative affinities of procaine for (A.) various neurotransmitter and neuromodulators receptors and (B.) muscarinic compounds for the mAChR (muscarinic receptor). The receptors with the highest affinity for procaine are the muscarinic M_2 and possibly the M_1 , and the sigma receptors. Procaine has approximately a ten-fold higher affinity for the acetylcholine receptor than ACh. Abbreviations: ACh (acetylcholine), AT (angiotensin), DA (dopamine), ET (endothelin), GABA (gamma-aminobutyric acid), Gly (glycine), Glu (glutamate), NE (norepinephrine), NPY (neuropeptide Y), QNB (quinuclidinyl benzilate), SP (substance P), α_2 (alpha2 NE), β_1 (beta1 NE), σ-1 (sigma 1), 5-HT (serotonin).

1.5.5 Summary

There are several ways the cholinergic system and its neuroanatomical connections could participate in procaine's emotional and sensory experiences, as well as the selective anterior paralimbic activation. The widespread cholinergic innervation across the cortex, basal forebrain and brainstem is consistent with arousal theories of cholinergic stimulation producing increased global brain activity and may also explain, in part, the global increase in cerebral blood flow seen with procaine. However, given the widespread distribution of Na^+ channels (Worley and Baraban, 1987) and known procaine bind to them, other mechanisms could contribute to these global effects. Cholinergic activation could modulate more regional specific effects, such as the anterior paralimbic increases in rCBF (Ketter et al., 1996) and temporal lobe fast EEG activity (Parekh et al., 1995). Cholinergic mechanisms in arousal-related low voltage fast EEG activity has been reported in the rat (Steriade et al., 1990; Stewart et al., 1984), and may be balanced with GABAergic transmission (Jones, 2004). The regional concentration of cholinergic cell bodies in the basal forebrain and projections to anterior paralimbic regions is consistent with muscarinic mechanisms contributing to such regional effects. The regions most activated by procaine appear to have heavy cholinergic and amygdalar innervation (Figure 9).

Core limbic areas, amygdala and hippocampus, have the highest density of cholinergic innervation, both M_1 and M_2 (Flynn and Mash, 1993; Mesulam, 1995). Differential distribution of the cholinergic system in sensory - limbic pathways could play a pivotal role in emotion. Moreover, cholinergic drugs are known to influence limbic functions such as emotion, reward and aggression. Taken together, the

anterior paralimbic activation pattern with procaine and its effects on M_2 muscarinic cholinergic receptors suggest a role of cholinergic mediation.

In summary, cholinergic modulation could contribute significantly to procaine-induced emotional and sensory experiences. This is suggested by procaine's: 1) preferential affinity to M_2 muscarinic cholinergic receptors *in vitro,* which are localized in key limbic areas; 2) ability to selectively activate limbic structures associated with emotion regulation while producing concomitant clinical electrophysiological effects; and 3) similarities in action to muscarinic agonists. Taken together, the anterior paralimbic activation pattern with procaine and its effects on M_2 muscarinic cholinergic receptors suggest a role of cholinergic mediation. This dissertation explores whether the blood flow and affective changes observed with procaine are related to binding to muscarinic receptors.

The question at hand has two parts and will be addressed by two experiments addressed in the next three chapters. First, does procaine bind to M_2 muscarinic receptors *in vivo*? This will be evaluated by examining the binding of [^{18}F]FP-TZTP, a specific radiotracer for the M_2 receptor with agonist activity, in the presence and absence of procaine in rhesus monkeys (Chapter 2). Second, what is the relationship between procaine's functional response of affective changes and blood flow effects to the muscarinic system? Regional cerebral blood flow measured with [^{15}O] PET and behavioral data collected during procaine administration in humans, healthy controls and patients with bipolar disorder, will be examined testing the cholinergic model described above with two-fold methodology. A preliminary analysis describing any potential differences in response to procaine administration between the two groups

will be used to compare and contrast the findings of the second method (Chapter 3). The two-fold methodology includes: 1) assessing functional connectivity, as defined as the correlative relationships between brain regions, to confirm modulation of cholinergic pathways with procaine administration; and 2) multivariate multiple regression, based on the neuroanatomical model (AChNet) in Figure 9, will assess the relationships between core cholinergic brain regions and the robust cerebral blood flow responses that occurs with procaine administration (Chapter 4).

Chapter 2: A Potential Cholinergic Mechanism of Procaine's Limbic Activation

The following is the article as published in full in *Neuropsychopharmacology* **29**, 1239-1250. An afterword discusses the links between this preclinical work and the clinical work presented in subsequent chapters, and addresses any outstanding concerns arising since publication.

A Potential Cholinergic Mechanism of Procaine's Limbic Activation

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2.1 Abstract

The local anesthetic procaine, when administered to humans intravenously (i.v.), yields brief intense emotional and sensory experiences, and concomitant increases in anterior paralimbic cerebral blood flow, as measured by positron emission tomography (PET). Procaine's high muscarinic affinity, together with the distribution of muscarinic receptors that overlaps with brain regions activated by procaine, suggests a muscarinic contribution to procaine's emotional and sensory effects. This study evaluates the effects of procaine on cerebral muscarinic cholinergic receptors in the anesthetized rhesus monkey. Whole brain and regional muscarinic receptor binding was measured before and after procaine administration on the same day in three anesthetized rhesus monkeys with PET and the radiotracer 3-(3-(3[^{18}F]fluoropropylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([^{18}F]FP-TZTP), a cholinergic ligand that has preferential binding to muscarinic (M_2) receptors. On separate days each animal received six different doses of i.v. procaine in a randomized fashion. Procaine blocked up to ~90% of [^{18}F]FP-TZTP specific binding globally in a dose-related manner. There were no regional differences in procaine's inhibitory concentration for 50% blockade (IC_{50}) for [^{18}F]FP-TZTP. Tracer delivery, which was highly correlated to cerebral blood flow in previous monkey studies, was significantly increased at all doses of procaine with the greatest increases occurring near procaine's IC_{50} for average cortex. Furthermore, anterior limbic regions showed greater increases in tracer delivery than nonlimbic regions. Procaine has high affinity to muscarinic M_2 receptors in vivo in the rhesus monkey. This, as well as a preferential increase of tracer delivery to paralimbic

regions, suggests that action at these receptors could contribute to i.v. procaine's emotional and sensory effects in man. These findings are consistent with other evidence of cholinergic modulation of mood and emotion.

2.2 Introduction

The local anesthetic procaine has unique neuropsychopharmacological properties that make it useful as a discrete probe of the limbic system and associated studies of emotion. In this regard, it has been used as an affective challenge in healthy volunteers (Ketter et al., 1996; Servan-Schreiber et al., 1998), and patients with mood disorders (Ketter et al., 1993) and panic disorder (George et al., 1993), as well as in individuals abusing alcohol (George et al., 1990) and cocaine (Adinoff et al., 2001).

Procaine (1.84 mg/kg), when administered intravenously (i.v.) in healthy volunteers (Kellner et al., 1987), results in brief intense emotional (ranging from euphoria to dysphoria) and sensory experiences (visual, auditory, and olfactory illusions/hallucinations) in association with increased relative anterior paralimbic cerebral blood flow (CBF) as measured with [^{15}O] water positron emission tomography (PET) (Ketter et al., 1996). In healthy individuals, procaine also induces the following: hormonal changes such as increased adrenocorticotropic hormone (ACTH), cortisol, and prolactin (Kling et al., 1994); and increased temporal lobe fast activity electroencephalogram (EEG) (Parekh et al., 1995). Furthermore, the right amygdala blood flow correlated with the degree of affective arousal, while the left amygdala CBF correlated positively with the degree of dysphoria and negatively with the degree of euphoria (Ketter et al., 1996). The experiences described by the subjects in these procaine studies are reminiscent of those reported after direct stimulation of

limbic cortex during epilepsy surgery (Gloor et al., 1982; Halgren et al., 1978; Penfield and Jasper, 1954). The emotional and endocrine changes also resemble the effects of acute challenge with the anticholinesterase drug physostigmine (Janowsky et al., 1986).

The neurochemical mechanisms of procaine's emotional, sensory, and endocrine effects could provide valuable insights into the neurobiology of normal and pathological emotion regulation, but have not been clearly elucidated. Procaine is classically associated with the blockade of voltage-gated sodium channels producing the local anesthetic effect for which it was designed and synthesized by Einhorn in 1905. Procaine produces 50% inhibition of the action potential at 1.1 mM in frog and rat sciatic nerve (Butterworth and Strichartz, 1990), by binding in the channel pore and altering the gating mechanism to increase the probability of the inactive state, thereby reducing the likelihood of an action potential (Butterworth and Strichartz, 1990). Site-directed mutations in rat brain sodium channels show reduced use-dependent blockade, confirming this hypothesis (Scholz, 2002). Procaine is also hypothesized to have direct effects on the lipid bilayer and hence indirectly influence components in the neuronal membrane (De Jong, 1994).

Procaine also interacts with many neurochemical systems, but is most potent at the muscarinic cholinergic receptor. Procaine competitively displaces quinuclidinyl benzilate (QNB), a nonspecific muscarinic antagonist at putative M_2 receptors in the guinea-pig ileum with a delivery rate constant (Ki) of 4 μM (Hisayama et al., 1989) and inhibits QNB binding (Ki=4 μM) in rat hippocampal membranes known to have M_1 and M_2 receptors (Sharkey et al., 1988). In contrast, the affinity of procaine for

M_3 receptor was 5 mM in the guinea-pig mesenteric artery (Itoh et al., 1981). Procaine inhibits methylcarbachol or dimethylphenylpipereinium (DMPP) binding presumably at nicotinic cholinergic receptors on rat brain membranes at higher concentrations (Ki=50-100 μM depending on the agonist used) than observed in the muscarinic system (Saraswati et al., 1992). Sigma receptors are the only other sites at which procaine binds in the low micromolar range (3.6 μM; Sharkey et al., 1988).

The affinity of procaine for other systems include transporters (dopamine (DA): 104 mM, norepinephrine (NE): 217 mM, and serotonin (5-HT): 276 mM) and receptors (serotonin (0.1-10 mM, depending on subtype), neuropeptide Y (5 mM), angiotensin (5 mM), endothelin (5 mM), adrenergic (α_2: 5 mM, β_1: 100 mM), glycine (1 mM), glutamate (1 mM), and gamma-aminobutyric acid (GABA) (1.5-5.4 mM per subunit); Aoshima et al., 1992; Cunningham and Lakoski, 1988; Fishlock and Parks, 1966; Itoh et al., 1981; Napier, 1992; Ritz et al., 1987; Sharkey et al., 1988; Sugimoto et al., 2000; Sato et al., 2000). It should be noted that for some ligands affinity constants were not determined, but stated doses had no biological effects, such as D_1 and D_2 receptors (Napier, 1992). Thus, based on affinities, procaine would be expected to have primary actions on the muscarinic cholinergic and sigma receptor systems that could be important contributors to its clinical effects.

Cholinergic M_1 and M_2 receptors are present in core limbic areas, such as amygdala and hippocampus, as well as primary sensory regions. While M_1 receptors have primarily a cortical distribution, M_2 receptors have a more uniform distribution across the brain, cortically and subcortically (Mesulam, 1995; Mesulam et al., 1983; Flynn and Mash, 1993). Telencephalic cholinergic projections originating in the basal

forebrain nuclei, such as the nucleus basalis, send efferents to most of the cortex, including the anterior cingulate, as well as to subcortical regions, the basolateral amygdala, and hippocampus (Mesulam, 1995; Mesulam et al., 1983). Of particular interest are the efferents of the basolateral amygdala that heavily innervate the anterior cingulate and nearby medial orbitofrontal cortex (Russchen et al., 1985a, b). These anterior paralimbic regions with direct and indirect cholinergic connections are also the areas that exhibit the most robust activation by procaine in humans (Ketter et al., 1996). Thus, the distribution and the potential functional consequences of the cholinergic receptors in limbic pathways could allow cholinergic mechanisms to play an important role in emotion (Heimer, 2003).

Cholinergic drugs have been shown to influence limbic regions known to mediate mood, reward, and aggression. For example, neuronal activity in the dorsolateral prefrontal and orbitofrontal cortex, and the amygdala of monkeys has been shown to be altered by iontophoretic administration of cholinergic agents (Aou et al., 1983; Inoue et al., 1983; Lenard et al., 1989). Similar to GABA, procaine microinjection (6-20 mg) into rat nucleus basalis inhibits frontal cortical neuronal firing in response to conditioned stimuli (pairing with medial forebrain stimulation) (Rigdon and Pirch, 1984). Moreover, procaine selectively activates limbic structures electrophysiologically in rats (Munson et al., 1970; Racine et al., 1975, 1979; Wagman et al., 1967). Utilizing 2-deoxyglucose methodology, lidocaine, a closely related amide local anesthetic, selectively activated limbic structures in rats (Post et al., 1984). Local anesthetics, including cocaine, lidocaine and procaine, can produce kindled seizures and aggressive behavior in rodents (Post, 1981). Intra-amygdalar

injection of cholinomimetics also induces kindled seizures (similar in appearance to those evoked by electrical amygdaloid kindling), which can be blocked by muscarinic antagonists (Cain, 1981). Furthermore, atropine, a muscarinic antagonist, inhibits and physostigmine weakly facilitates procaine-induced kindling in rats (Heynen et al., 1995).

In summary, cholinergic modulation could contribute significantly to procaine-induced emotional and sensory experiences. This is suggested by procaine's: (1) preferential affinity to M_2 muscarinic cholinergic receptors that are localized in key limbic areas; (2) ability to selectively activate limbic structures associated with emotion regulation while producing concomitant clinical electrophysiological effects; and (3) similarities in action to muscarinic agonists. In this study, we specifically explore the potential role of the muscarinic system in procaine's effects by measuring muscarinic receptor binding in anesthetized rhesus monkeys with the PET radioligand [^{18}F]FP-TZTP, 3-(3-(3[^{18}F]fluoropropylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine (Kiesewetter et al., 1995) before and after procaine administration.

We hypothesized that the binding of [^{18}F]FP-TZTP would be reduced by procaine as a function of the dose administered. In addition, we hypothesized that the K_1 of the ligand, which correlates strongly with CBF (Carson et al., 1998), would have a pattern of specific limbic increases similar to those observed with CBF in human studies (Ketter et al., 1996), although this effect could be attenuated by anesthesia.

2.3 Methods

2.3.1 Subjects

Four adult male rhesus monkeys (*Macaca mulatta*) weighing 11.7, 8.8, 8.0, and 7.1 kg were studied with brain imaging techniques while receiving general anesthesia during the entire procedure. One animal was unable to complete the whole series of studies due to arterial port failure, and thus was not included in the analysis. All studies were performed under a protocol approved by the NIH Clinical Center Animal Care and Use Committee. The monkeys were routinely monitored by veterinary staff, housed according to American Association for Laboratory Animal Care (AALAC) standards in individual cages, allowed ample feed and were provided with psychological enrichment.

2.3.2 Experimental Design

Animals underwent PET with the radiopharmaceutical 3-(3-(3[^{18}F]fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([^{18}F]FP-TZTP) while anesthetized with isoflurane. A total of 18 experiments, six with each animal, were conducted on separate days with each experiment separated by 20-180 days. On each study day, two dynamic PET scan sessions were collected, including an initial baseline scanning session with saline administration followed by a second with continuous infusion of procaine, at one of six doses, ranging from zero, 0.01562, 0.03125, 0.0625, 0.125, to 0.5 mg/kg/min (active drug doses referred to as dose 1 to dose 5, respectively). The order of procaine doses was randomized. These doses were chosen based on self-administration studies (Ford and Balster, 1976;

Hammerbeck and Mitchell, 1978), while being safely under the seizure-inducing levels (Babb et al., 1979). These infusion rates were determined from procaine infusion rates from self-administration studies of 1 mg/kg per injection at an average rate of 24 injections per hour (Ford and Balster, 1976) and 4 mg/kg per injection at an average rate of nine injections per hour (Hammerbeck and Mitchell, 1978).

2.3.3 Radiopharmaceutical

[^{18}F]FP-TZTP (Kiesewetter et al., 1995, 1999) was developed as an extension of the structural class of M_2 agonists made available by Nova Nordisk (Sauerberg et al., 1992). FP-TZTP exhibits modest selectivity for M_2 (2.2 nM) over M_1 (7 nM) receptors. Studies of cross reactivity with other neurotransmitter systems showed low affinity for all biogenic amine systems evaluated. FP-TZTP exhibited affinity for sigma-1 (62 nM) and 5-HT_1 receptor (2 μM) (Kiesewetter et al., 1995). The uniform uptake of [^{18}F]FP-TZTP across the brain resembles the distribution of M_2 receptors, which is consistent with M_2 selective binding. In rats, [^{18}F]FP-TZTP displays high uptake and high specific binding as determined by ex vivo autoradiography (Kiesewetter et al., 1999). In muscarinic knockout mice, the M_2 knockout mouse is the only one that shows significantly reduced [^{18}F]FP-TZTP uptake in all brain tissues regions (Jagoda et al., 2003). Taken together, these data support [^{18}F]FP-TZTP preferential M_2 binding in vivo. Tracer kinetic modeling for [^{18}F]FP-TZTP has been developed by Carson et al. (1998), so that PET data can be converted into parametric images of total binding (V, volume of distribution) and radioligand delivery (K_1). The binding of [^{18}F]FP-TZTP has been shown to be decreased by administration of

physostigmine, and thus the binding was sensitive to endogenous acetylcholine (Carson et al., 1998). While other potential ligands, such as the nonselective muscarinic agonist CI-979 (milameline; Hartvig et al., 1997) or nonselective muscarinic antagonist [^{11}C]-scopolamine (Frey et al., 1992), and [^{11}C]NMPB ([^{11}C]N-methyl-4-piperidyl benzilate; Zubieta et al., 2003) could contribute additional information pertaining to the profile of in vivo actions of potential agents on muscarinic receptors, [^{18}F]FP-TZTP was chosen over others due to its preferential binding to M_2 receptors. Furthermore, the study design was conducive to using an ^{18}F compound, particularly because a single synthesis generated the total radiotracer required for both phases of each study day.

2.3.4 Procedure

On the morning of the each procedure, the fasted animal was initially given 0.5 mg ketamine and 5 cc of 2.5% sodium pentathol intramuscularly to produce quasi-anesthesia (light anesthesia) for the placement of three i.v. lines, usually in both radial veins and a femoral vein, and intubation for eventual general anesthesia. The animal was transported to the PET suite and placed in the PET scanner and general anesthesia was induced with inhalation of 1-2% isoflurane using a Stevens-Johnson anesthesia machine.

The head was positioned in a stereotactic headholder for coronal image acquisition. An arterial line was attached to a permanent subcutaneous arterial port (Model 21-Y036, Sims Deltec, St Paul, MN) implanted in the femoral artery, a temperature probe was inserted into the rectum, and cardiopulmonary monitoring

equipment leads were placed on the animal to monitor their cardiovascular, pulmonary, and thermoregulatory functions throughout the entire procedure (electrocardiogram (EKG), blood pressure, respiration rate, end-tidal pCO_2, and temperature). Once cardiopulmonary measures were stable under anesthesia, the experimental procedures began.

After a transmission scan, two [^{18}F]FP-TZTP dynamic scan sessions were acquired. First, a series of dynamic scans were collected over 180 min with saline infusion. After the primary data collection period of 90 min, procaine administration began and continued for the remainder of the experimental procedure. Sterile 10% procaine hydrochloride solution (Sanofi Winthrop Pharmaceuticals) was diluted with saline to desired concentrations on each experimental day and kept in the dark until experiments began, as procaine is light sensitive.

Procaine was administered i.v. in a continuous fashion with a Harvard pump. The syringe and i.v. lines were wrapped with aluminum foil to limit light exposure. The procaine dosing strategy involved a two-step infusion rate; a loading dose, which was double the target dose, was administered for 40 min and was followed by the target or maintenance dose for the remainder of the scanning session. At 40 min after beginning the target dose, the second set of dynamic scans was acquired over 90 min, while the constant procaine infusion continued (mean interval between scans 185±2 min). Upon completion of the experiment, the intravenous and arterial lines and monitoring equipment were removed, the animal was taken out of the PET scanner, returned to its home cage and allowed to recover from anesthesia.

[^{18}F]FP-TZTP was synthesized for each study day according to previously published methods (Kiesewetter et al., 1995, 1999). The product from a single radiosynthesis was split for two injections. The mean activity injected was 1.0±0.1 mCi for the first injection (saline scan) and 3.5±1.6 mCi for the second injection (procaine scan); mean [^{18}F]FP-TZTP mass was 0.4±0.2 nmol for the first injection and 4.5±2.1 nmol for the second injection; mean specific activity injected was 2700±1000 mCi/mol for the first injection and 840±310 mCi/mol for the second injection. More radioactivity was given for the second injection to attenuate the effect of the residual radioactivity from the first injection.

Arterial blood samples were drawn from the indwelling arterial port throughout the scanning procedure. A total of 29 blood samples (0.5 ml) taken over the scanning period were centrifuged and 0.1 ml plasma aliquots were counted in a calibrated gamma counter to generate time activity curves. Seven 1 ml blood samples were obtained at 0,3,8,15,30,50 and 90 min after injection for determination of the unmetabolized radiotracer fractions by thin layer chromatography (TLC) according to methods previously described (Carson et al., 1998).

Procaine levels were determined from three 2 ml blood samples taken at 0, 15, and 45 min after the second injection of the radiotracer (40, 55, and 85 min after maintenance dose was initiated); two drops of sodium arsenite per milliliter blood were added immediately to inhibit procaine metabolism by butyrylcholinesterases in blood. The samples were assayed for procaine levels by gas chromatography measuring the free procaine level in plasma (National Medical Services, Inc., Willow Grove, PA). Steady-state plasma concentrations were achieved through the

loading/maintenance dose strategy, with procaine concentrations having a coefficient of variation of 9%. No trends were observed over time among the three procaine samples. The mean plasma concentrations achieved are presented in Table 3.

2.3.5 Imaging Data Collection and Analysis

Image collection and analysis followed the methods of Carson et al. (1998). Images were acquired with the General Electric (GE) Advance tomograph (DeGrado et al., 1994) in three- dimensional mode, which collects 35 slices simultaneously with a 4.25 mm interslice distance and a 6 mm isotropic reconstructed resolution. Reconstructed scans were corrected for attenuation, scatter, random emissions, and deadtime, and calibrated in nCi/ml.

[^{18}F]FP-TZTP functional images of delivery rate from the plasma (K_1 (ml plasma/min/ml tissue)) and equilibrium volume of distribution (V (ml plasma/ml tissue)) or total binding were computed from the dynamic scans collected over the initial 45 min, utilizing a kinetic model with an arterial input function that has been corrected for metabolites (Carson et al., 1998). Initially, the time delay between brain and blood sampling (t) was determined by a one-compartment model fitting three parameters (t, V, K_1). Functional images of V and K_1 were created by first generating a pixel by pixel time activity curve adjusting for the global t, and then fitting to a two-parameter (K_1 and V) model.

For the second injection, the data were adjusted for residual radioactivity and residual metabolites remaining from the first injection (baseline scanning). Images were corrected for residual activity and metabolites from the first injection with

identical methodology as that of Carson et al. (1998). Briefly, the model equation for the second injection was modified to include a nonzero initial radioactivity concentration (C_0), which clears exponentially (exp(-k2*t*)), where *k*2 is the clearance rate constant of the second injection. For each pixel, C_0 was estimated by averaging the pixel value for the 30 min preceding the second injection (corrected for decay). This extrapolated background radioactivity amounted to less than 10% of total activity for the second injection. The magnitude of metabolites remaining from the first injection was estimated in a similar manner, and affected the early portion of the input function for the second injection.

Regions of interest (ROIs) were defined on coronal magnetic resonance imaging scans (MRIs) using the rhesus monkey atlas of Paxinos et al. (2000). T1 weighted images were acquired on a GE Signa (1.5 Tesla), with a sequence 3D SPGR (X=1 mm, Y=1 mm, Z=0.39 mm), while sedated with ketamine and robinol on a separate day from the PET studies. The V and K_1 images were coregistered to the structural MRIs using the Automated Image Registration (AIR) algorithm (Woods et al., 1998) that transformed and resliced the functional images into the coordinate system of the MRIs. Primary ROIs were anterior cingulate, amygdala, and basal forebrain structures ventral striatal and pallidal nuclei that were particularly activated in the human blood flow studies. Additional ROIs were defined in prefrontal, parietal, occipital, temporal cortices, thalamus, striatum, posterior cingulate, hippocampus, cerebellum, and brainstem. Primary sensory (V1 [visual], A1[auditory] and S1[somatosensory]) and motor cortex (M1) were also measured, but not included into statistical inference testing as they were encompassed in some of the previously

mentioned regions. The mean V and K_1 levels were calculated from baseline and procaine scans for each ROI and a cortical average (prefrontal, anterior cingulate, posterior cingulate, parietal, occipital, and temporal cortices) was obtained.

The V values measured at baseline (Vbase) and with procaine (Vproc) administration were corrected for nonspecific binding by subtracting a uniform value of 7 mL/mL (rationale explained below) taken from preblocking studies with [^{18}F]FP-TZTP (Carson et al., 1998), yielding binding potential (BP) values for each scan (BP1=Vbase-7; BP2=Vproc-7). Percent blockade was calculated by

$$\Delta BP = 100((BP1 - BP2) / BP1).$$

The relationship of average cortical blockade (BP) to procaine dose was evaluated with repeated measure ANOVA with procaine dose as the one within factor, (SuperAnova, v1.11, Abacus Concepts, Berkeley, CA).

IC_{50} (inhibitory concentration for 50% blockade) values were calculated by two methods using Graphpad Prism software (v3.0a for Macintosh, Graphpad Software, Inc., San Diego, CA), and were compared for best fit. The first method determined IC_{50} from the percent blockade measures (BP) with the two-parameter model equation:

$$\Delta BP = \Delta BP_{max} [L] / (IC_{50} + [L])$$

where ΔBPmax is the maximum percent blockade achievable with procaine. Ligand concentrations [L] were based on mean procaine plasma levels acquired during each scanning period. The second fitting method used a sigmoidal dose-response model fitting three parameters to the equation

$$BP2 = BP_{max} (1- (\Delta BP_{max} /100) [L] / (IC_{50} + [L]))$$

where BPmax is the binding potential in the absence of procaine. These models differ in that the three-parameter model only used data from the second scan of each day and assumed a fixed value for BPmax on all days for all animals. The two-parameter model used the baseline results (BP1) measured for each animal on each experimental day to directly calculate BP. The three-parameter model also included the second-scan information on the zero-dose experimental day. Regional variations of IC_{50} values were assessed with repeated measure ANOVA with two within factors, region and procaine dose.

The statistical inferential methods for K_1 values were essentially identical to those described to assess BP measures. K_1 ROI values (without any corrections) measured at baseline ($K_{1\text{-base}}$) and with procaine ($K_{1\text{-proc}}$) administration were used to calculate percent change by

$$\Delta K_1 = 100((K_{1\text{-proc}} - K_{1\text{-base}}) / K_{1\text{-base}}).$$

The relationship of average cortical change of K_1 to procaine dose was evaluated with repeated measure ANOVA with one within factor, procaine dose. Regional variations of K_1 change were assessed with repeated measure ANOVA with two within factors, region and dose. When appropriate post hoc means comparisons were conducted, as in the case of testing the a priori hypothesis of selective anterior paralimbic, K_1 increases. Variability of peripheral measures was also examined with repeated measures ANOVA, with two within factors of dose and times.

Owing to the relative uniformity of muscarinic M_2 receptor distribution across the brain, nonspecific binding measures were estimated in the following way. Since individual values were not available, a constant value for the nonspecific distribution

of volume was used. Several constants were evaluated (6, 7, 8, and 8.7 ml/ml (the mean cortex value reported in Carson et al. (1998) from preblocking studies)), but the IC_{50} estimates, regardless of constant value, remained essentially identical. For example, for the mean cortex IC_{50}, a value of 1.34 μM was obtained when using a constant value of 8.7 ml/ml as compared to 1.31 μM obtained with 7 ml/ml for nonspecific distribution volume estimates. The constant value of 7 ml/ml, approximately the mean value of the cerebellum from preblocking studies was chosen for the analyses, under the assumption that nonspecific binding is uniform across the brain. Note that the underestimation of nonspecific binding in this study would have the effect of underestimating the maximum percent blockade. Again using the cortex average as an example region, the BPmax using 8.7 ml/ml was 100.1%, while 7 ml/ml produced a value of 88.4%.

2.4 Results

2.4.1 Control Values

On one day, each animal received saline administration during the second scanning period, in lieu of procaine, to establish test-retest reliability. Cortical [^{18}F]FP-TZTP BP did not differ significantly on retest (mean±SD saline1, 21.4±2.9 ml/ml; saline2, 23.1±3.4; F=1.48, df=1,2, p=ns). However, there was a slight tendency for regional [^{18}F]FP-TZTP BP changes from saline1 to saline2, as indicated by a region X scan interaction (F=2.28, df=12,24, p=0.04). Most regions increased slightly 1.0-2.4 ml/ml (average change 5.9%), but the thalamus, cerebellum, and the brainstem remained at the same level (<0.8 ml/ml change). This slight trend of BP

with time on anesthesia was also seen in the work of Carson et al. (1998) and will tend to result in an underestimation of BP.

Cortical values for K_1 also showed a slight, but nonsignificant increase (5.7%) from saline1 to saline2, a nonsignificant increase (mean±SD saline1, 0.42±0.09; saline2, 0.45±0.13; F=0.53, df=1,2, p=ns). Again there was significant regional variation (F=2.32, df=12,24, p=0.04) from saline1 to saline 2. Most regions increased 0.02-0.133 ml/min/ml (average change 11.2%), but parietal and occipital cortex remained at the same level. These increases in K_1 are consistent with the work of Carson et al. (1998) and most likely reflects time-related CBF increases associated with isoflurane administration (McPherson et al., 1994). These small increases in K_1 measures indicate that any procaine-induced flow increases may be slightly overestimated.

2.4.2 Peripheral Measures

There was no significant effect of procaine administration dose on heart rate (HR: F=1.21, df=5,10, p=ns), systolic or diastolic blood pressure (SYS: F=0.90, df=5,10, p=ns; DIA: F=1.04, df=5,10, p=ns;), respiration rate (RR: F=1.26, df=5,10, p=ns), or pCO_2 (pCO_2: F=1.46, df=5,10, p=ns). Although most measures significantly decreased with time (HR: F=11.27, df=5,10, p=0.000; SYS: F=6.47, df=5,10, p=0.000; DIA: F=2.45, df=5,10, p=0.030; RR: F=6.64, df=5,10, p=0.000; pCO_2: F=8.37, df=5,10, p=0.000), a drug X time interaction was only observed with pCO_2 which was significantly increased on the highest dose (0.500 mg/kg/min) after procaine administration and returned to baseline levels after cessation of the loading

phase (HR: F=0.51, df=5,10, p=ns; SYS: F=0.64, df=5,10, p=ns; DIA: F=0.94, df=5,10, p=ns; RR: F=0.83, df=5,10, p=ns; pCO_2: F=1.59, df=5,10, p=0.016).

2.4.3 Global Effects

The IC_{50} values generated by the two fitting methods (two-parameter and three-parameter) differed by approximately a factor of 2. For example, the average cortical ROI IC_{50} values were 1.31 μM and 0.75 μM for the two- and three-parameter models, respectively. The percent coefficient of variation of IC_{50} from the fits was comparable at ~30%, thus model selection could not be performed based on goodness-of-fit. In subsequent results, the two-parameter results were chosen over the three-parameter results, because the former model directly accounted for day-to-day and animal-to-animal variability of baseline [^{18}F]FP-TZTP binding potential (Table 3).

Table 3. The Effects of Procaine Administration on the Functional Response of the Cortex

Procaine		[^{18}F]FP-TZTP Specific Binding			Tracer Delivery		
Dose	Plasma Level	BP (ml/ml)			K_1 (ml/min/ml)		
(mg/kg/min)	(μM)	Baseline	Procaine	% Decrease	Baseline	Procaine	% Increase
0.000	0.00	20.7 ± 3.5	22.4 ± 3.9	-8.5	0.42 ± 0.09	0.45 ± 0.13	5.7
0.016	0.65	20.9 ± 6.2	13.2 ± 2.5	35.1	0.40 ± 0.17	0.62 ± 0.31	51.7
0.031	1.19	16.9 ± 1.8	10.3 ± 0.8	39.1	0.48 ± 0.09	0.62 ± 0.13	31.5
0.063	2.92	16.3 ± 4.5	7.7 ± 2.1	52.5	0.38 ± 0.11	0.48 ± 0.20	22.6
0.250	12.24	12.6 ± 0.6	2.7 ± 0.8	78.1	0.36 ± 0.04	0.43 ± 0.10	17.9
0.500	21.37	20.1 ± 9.7	2.3 ± 1.0	88.6	0.36 ± 0.05	0.42 ± 0.02	19.5

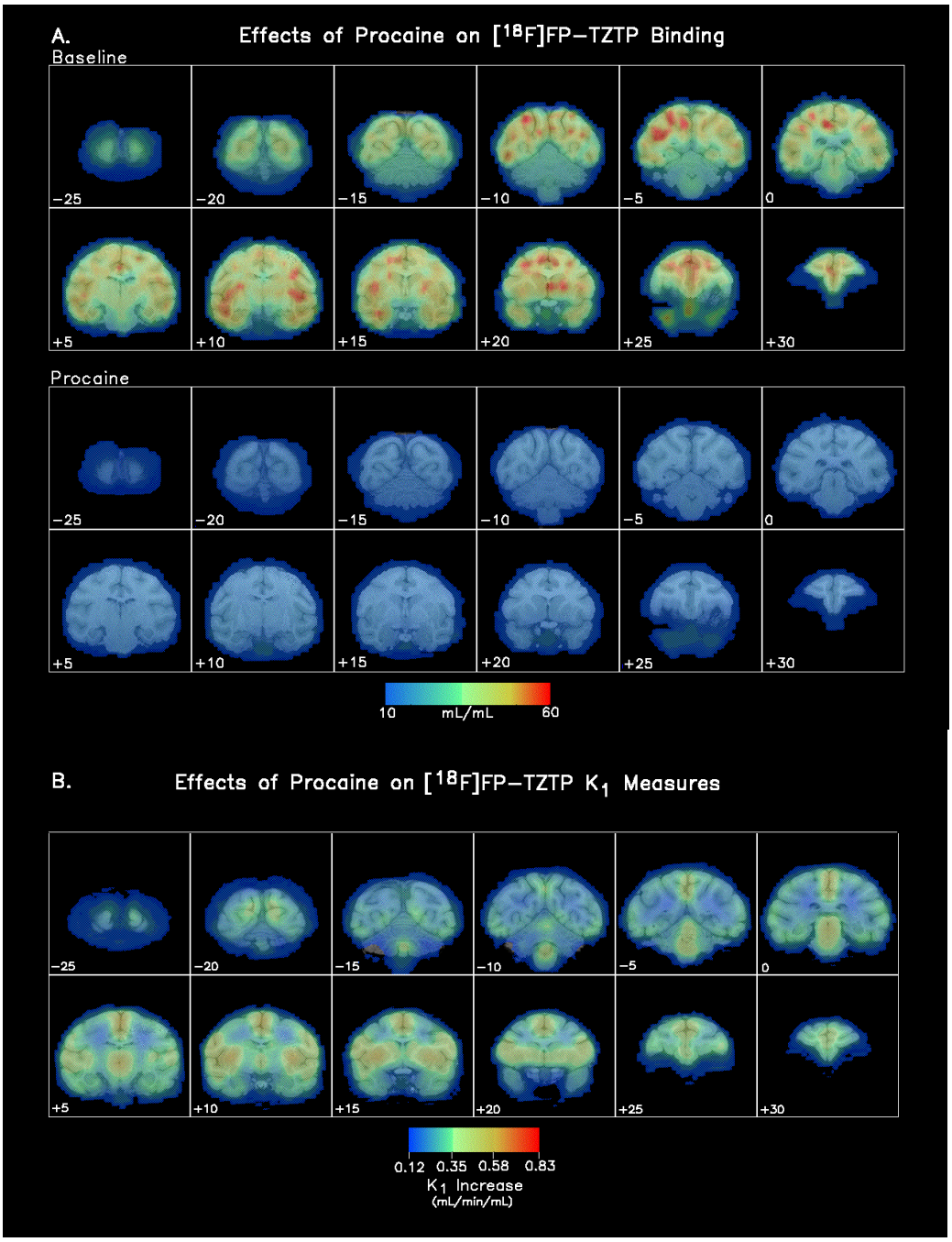

Figure 13. Effects of Procaine on [^{18}F]TZTP Binding and Flow
(A.) Coronal slice presentation of volume of distribution images (V) from a single animal displaying the significant reduction in specific binding of [^{18}F]FP-TZTP by procaine. Global reduction in specific binding in this subject was 86.9%. Upper panel: baseline binding is fairly uniform; lower panel: procaine administration (0.5 mg/kg/min) is essentially identical to preblocking studies with FP-TZTP (Carson et al., 1998). (B.) Procaine increased K_1 measures the greatest in the anterior cingulate, striatum, and basal forebrain nuclei. Data represents a subtraction image in a single monkey (0.016 mg/kg/min procaine minus baseline). The PET images were registered to the animal's MR images; coordinates are mm rostral (+) and caudal (-) to ear canals.

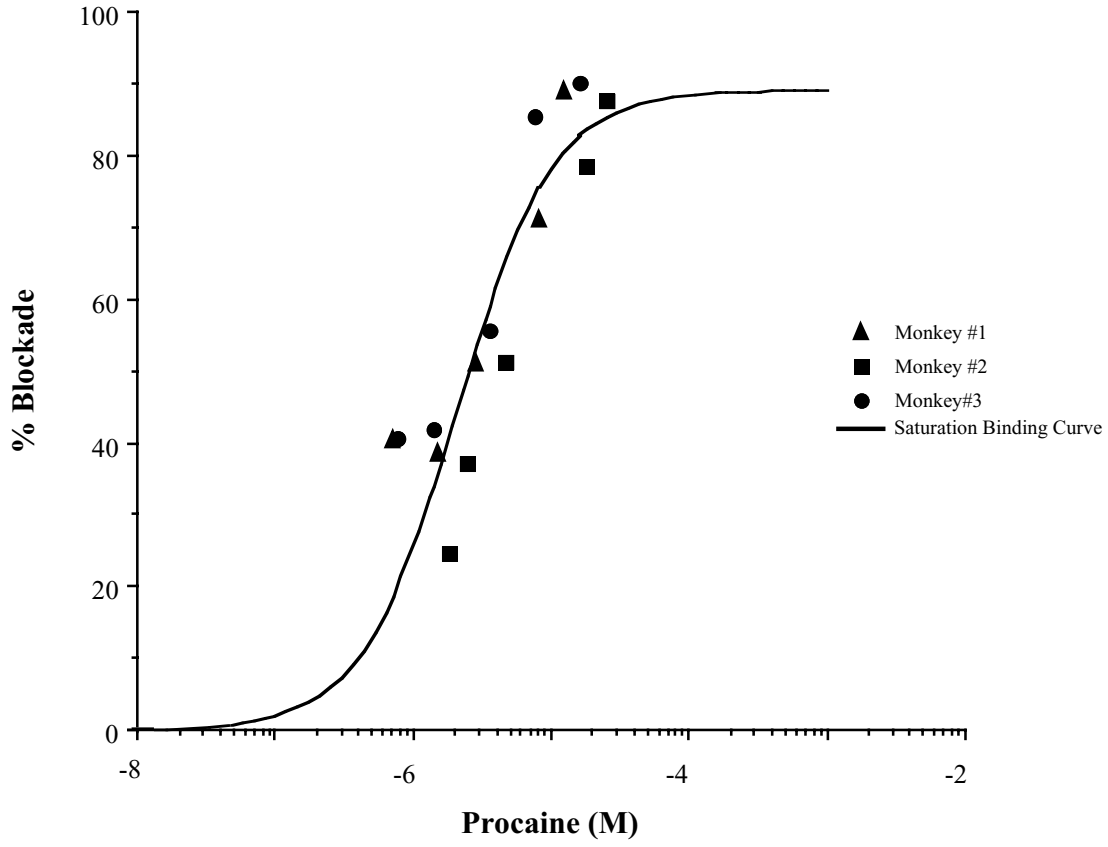

Figure 14. Dose-Response Relationship of Procaine and Global [^{18}F]FP-TZTP Binding

Procaine blocks cortical [^{18}F]FP-TZTP binding potential in a dose-related fashion that follows the saturation binding curve (solid line) with an IC_{50} of 1.31 μM with an estimated accuracy of ±0.41 μM. The maximum blockade was 87% and minimum was 23%.

Figure 13 depicts (top and middle panels) representative [^{18}F]FP-TZTP *V* images with and without procaine co-administration in a single animal at the highest dose of 0.5 mg/kg/min. Baseline and procaine global [^{18}F]FP-TZTP specific binding is reported in Table 3. Procaine, in a dose-related manner, blocked average cortical [^{18}F]FP-TZTP total specific binding (F=51.45, df=5,10, p=0.0001; Figure 14).

Procaine significantly increased average cortical [^{18}F]FP-TZTP K_1 values (F=6.23, df=2,10, p=0.0071), however, not in a sigmoidal dose-related manner (Table 3). The peak change (52%) in K_1 occurred at the low dose of 0.016 mg/kg/ml (mean procaine plasma level=0.65 mM; see Figure 13 bottom panel) and had successively smaller increases with each higher dose, although still increased by 20% at the highest dose of 0.5 mg/kg/min. The post hoc analysis of dose revealed all doses combined resulted in significantly higher K_1 values compared to the baseline study (baseline: 5.7±17.0%; procaine: 28.6±17.3; F=11.28, df=1, 10, p=0.007). This effect was attributable primarily to 0.016 mg/kg/min dose with a 51.7% increase (dose 1 vs baseline: F=27.16, df=1, 10, p=0.002) and also to the 0.031 mg/kg/min dose with a 31.5% increase (dose 2 vs baseline: F=23.54, df=1, 10, p=0.02).

2.4.4 Regional Effects

The dose-response relationship of [^{18}F]FP-TZTP specific binding across the regions mirrored the global findings (Table 4). The regional IC_{50} did not vary significantly across the areas measured and ranged from 1.00 to 2.25 μM (F=0.88; df=12,24; p=ns).

[^{18}F]FP-TZTP K_1 (Table 4) varied significantly across ROIs (F=5.58; df=2,12; p=0.0001) and across procaine doses (F=5.61; df=4,8; p=0.02); however, there was no interaction of region X dose (F=0.79; df=60,120; p=ns). In the regional post hoc analysis, the K_1 of the anterior paralimbic areas (including the amygdala, anterior cingulate, basal forebrain nuclei, hippocampus, and prefrontal cortex, mean K_1=34.7%) was significantly increased compared with other regions (including posterior cingulate, occipital cortex, parietal cortex, cerebellum, and brainstem; mean K_1=21.0%, F=29.05, df=1,24, p=0.0001).

Table 4. Regional [^{18}F]FP-TZTP Parameter Values

	Specific Binding			Delivery Rate	
Region	Baseline BP (ml/ml)	IC_{50} (μM)	ΔBPmax	Baseline K_1 (ml/min/ml)	K_1 Increase (Maximal)
Cortex Average	17.9 ± 5.4	1.31	88.4	0.40 ± 0.09	51.7
Striatum	19.5 ± 5.8	1.48	88.9	0.48 ± 0.12	73.6
Basal Nuclei	17.4 ± 5.2	1.51	93.2	0.47 ± 0.11	72.6
Ant Cingulate	21.4 ± 6.7	1.00	85.4	0.43 ± 0.10	68.4
Hippocampus	17.0 ± 5.1	1.65	92.0	0.46 ± 0.11	57.5
Amygdala	15.2 ± 5.0	1.74	94.2	0.43 ± 0.10	55.9
Thalamus	15.6 ± 5.0	1.72	83.9	0.46 ± 0.12	55.1
Prefrontal	17.0 ± 5.1	1.11	88.5	0.36 ± 0.09	54.8
Temporal	17.4 ± 5.4	1.48	91.5	0.41 ± 0.10	54.2
Brainstem	9.2 ± 3.3	2.25	97.7	0.41 ± 0.12	51.6
Post Cingulate	19.1 ± 5.7	1.36	85.2	0.40 ± 0.09	50.0
Cerebellum	8.9 ± 2.9	2.12	100.4	0.62 ± 0.17	49.4
Parietal	18.8 ± 5.8	1.24	84.9	0.35 ± 0.07	40.3
Occipital	14.9 ± 4.4	1.52	93.3	0.38 ± 0.11	34.4
Primary Sensory (V1)	14.9 ± 4.5	1.51	92.7	0.41 ± 0.12	37.5
(A1)	21.6 ± 6.2	1.37	87.6	0.48 ± 0.11	61.8
(S1)	15.2 ± 4.8	1.45	91.4	0.33 ± 0.07	52.1
Primary Motor (M1)	16.7 ± 5.5	1.19	89.9	0.34 ± 0.07	66.6

2.5 Discussion

In this PET study of anesthetized monkeys, procaine blocked the binding of a muscarinic ligand in a dose-related manner, globally and uniformly across the primate brain. The IC_{50} for the cortex was estimated to be 1.31 μM, which corresponds to plasma levels achieved between infusion doses 0.0312 and 0.0625 mg/kg/min procaine. This is in the proximity of in vitro assessments of M_2 muscarinic receptor Kd of 4 μM as determined in rat hippocampal slices and guinea-pig ileum (Hisayama et al., 1989; Sharkey et al., 1988). Given the nearly 100% blockade achieved, these results support a direct interaction of procaine with the same muscarinic receptors to which [^{18}F]FP-TZTP is binding. Furthermore, the *V* images of procaine administration at the maximal dose in this study were essentially identical to the *V* images obtained during the preblocking experiments with [^{18}F]FP-TZTP studies (Carson et al., 1998).

Although procaine is known to selectively increase limbic electrophysiological activity in animals and anterior paralimbic perfusion in humans (Heynen et al., 1995; Ketter et al., 1996; Munson et al., 1970; Parekh et al., 1995; Post, 1981; Post et al., 1984; Racine et al., 1975, 1979; Wagman et al., 1967), the lack of regional variation in IC_{50} values suggests that the competition of procaine with [^{18}F]FP-TZTP binding sites is similar across the brain. Furthermore, these data suggest that procaine's limbic selectivity is most likely not a result of limbic muscarinic receptors having enhanced regional sensitivity to procaine over that of other brain regions.

Nonetheless, the selective activation of paralimbic structures by procaine could still involve cholinergic mechanisms. Amygdalar neurons via muscarinic mediation have been associated with bursting phenomena (Yajeya et al., 1997), postsynaptically (Washburn and Moises, 1992) and presynaptically (Sugita et al., 1991). Bursting activity was further tied to a complement of presynaptic muscarinic receptors that were estimated to be comprised of 50% M_3, 30% M_2, and 20% or less M_1, but most likely not due to the well characterized M-current (Yajeya et al., 1997). Moreover, similar neuronal depolarization responses due to cholinergic mechanisms have been implicated in other paralimbic structures such as hippocampus, entorhinal, and piriform cortices and anterior cingulate (Benson et al., 1988; Colino and Halliwell, 1993; Hasselmo and Bower, 1992; Klink and Alonso, 1997; McCormick and Prince, 1986), as well as septum, basal forebrain, striatum, thalamus, and neocortical structures (Hasuo et al., 1988; Hsu et al., 1995; McCormick and Prince, 1987; Szerb et al., 1994). Thus, it is possible that procaine acting on M_2 receptors could initiate bursting activity and enhance firing in limbic regions. These results suggest that the interaction of muscarinic receptors with the local neuronal environment could contribute to procaine's limbic effects.

It is likely that procaine is acting as an agonist since: cholinomimetics induce kindling similar to procaine (Wasterlain et al., 1981); and physostigmine weakly facilitates procaine-induced kindling while atropine substantially slows this process (Heynen et al., 1995). Moreover, the clinical effects of physostigmine share similarities to procaine (Janowsky et al., 1986).

Conversely, however, antagonist activity is suggested by procaine competitively inhibiting acetylcholine-induced contraction (Ishii and Shimo, 1984) of guinea-pig cecum. Furthermore, procaine inhibits opening of cation channels on guinea-pig ileal smooth muscle cells thus affecting the acetylcholine-induced cationic currents; GTPγS currents are inhibited in a similar manner (Chen et al., 1993). Lastly, procaine is known to have a biphasic effect on neuronal excitation with anticonvulsant activity at lower concentrations and proconvulsant activity at higher concentrations (De Jong, 1994; Foldes et al., 1960, 1965), which could reflect both agonist and antagonist cholinergic activity.

Cerebral blood flow, as measured by K_1, significantly increased globally and regionally in the anterior paralimbic regions with all procaine doses, but the relationship of procaine dose and K_1 increase appears to be more complex than that observed with the binding data. The most robust increase was observed at the lowest dose of procaine and all subsequent higher doses resulted in less of an increase above baseline saline condition. The largest K_1 changes occurred at plasma levels near the IC_{50}. This suggests that the K_1 effects associated with the lower doses could possibly be related to M_2 muscarinic blockade. However, the apparent reduction of the K_1 increase at the subsequently higher doses might be explained by procaine's effects on sigma or other receptors, or be a direct effect of the M_2 receptors yielding a biphasic relationship of procaine dose on K_1. Allosteric modulation of the muscarinic receptor has been documented with cocaine (Flynn et al., 1992), and given the structural similarities between procaine and cocaine, such modulation could account for a smaller change in K_1 at higher doses. Another potential contributor to the complex K_1

changes observed in this study could be the reduced ability of the model to fit K_1 with higher receptor occupancy (Carson et al., 1998), resulting in an underestimation of K_1. This would yield lower flow changes than would be expected with a direct receptor blockade to flow relationship.

While it would be enticing to suggest that these K_1 changes reflect actual blood flow alterations, caution should be exercised. Despite the strong correlation of cerebral blood flow and K_1 (r=0.85; Carson et al., 1998), the relationship of blood flow changes to procaine administration in this study can only be inferred. However, the regional pattern of K_1 increases in this study is similar to the blood flow increases observed in humans (Ketter et al., 1996). The K_1 data indicate that procaine binding to cholinergic M_2 receptors may contribute to the overall activation of anterior paralimbic regions. This suggestion is supported by preferential reductions in prefrontal cortex blood flow after scopolamine injection in humans in xenon-133 PET studies (Honer et al., 1988), which are reversed by physostigmine (Prohovnik et al., 1997). Also, carbachol injection into the substantia inominata increases blood flow globally and in many limbic areas in rats (Barbelivien et al., 1999).

Procaine can have autonomic effects, such as increases or decreases in heart rate or blood pressure, when administered i.v. in humans (Foldes et al., 1965, 1960; Haasio et al., 1988; Scott, 1975). In this study, the minimal autonomic decreases observed are consistent with known effects related to time on anesthesia. It is notable that significant cerebral effects occurred while the peripheral effects were minimal.

There are several limitations to this study. Few animals were studied; despite this, the dose-response relationship of procaine blockade of [^{18}F]FP-TZTP was robust

and consistent across each monkey (Figure 14). Further, the use of only rhesus monkeys suggest caution in extrapolation to man.

General anesthesia precluded the collection of behavioral measures during procaine administration restricting the scope of the study; the ability to relate the degree of blockade to behavioral measures would be a key element in determining a clearer role of the muscarinic system in the emotional and sensory effects of procaine. The general anesthesia (Durieux, 1996) may have confounded the K_1 changes observed, or interacted with procaine to alter the muscarinic activity (Brett et al., 1988; Dilger et al., 1992, 1993). Ketamine-muscarinic interactions (Durieux, 1995) may have also confounded the binding changes observed, but these effects would be present at baseline and with procaine. Anesthesia may have also limited the physiological changes potentially induced by procaine, either directly or indirectly.

Finally, different methods from those used in the human studies were necessary for the acquisition of the binding potential data, including the [^{18}F]FP-TZTP measurement after plasma procaine levels had leveled off, as well as the use of a constant infusion paradigm rather than a bolus administration. This may have resulted in assessing the procaine-flow relationship under different physiological conditions than in the human studies, limiting the ability to extrapolate from this study to the human studies.

With these caveats in mind, considerable evidence suggests a role for the cholinergic system in normal and pathological emotions. An enhanced ratio of cholinergic to adrenergic function has been hypothesized to play a role in mania and depression (Fritze, 1993; Janowsky et al., 1972a). Cholinergic challenge studies lend

support to this theory. For example, physostigmine can reduce manic symptoms and exacerbate depressive symptoms in bipolar patients (Janowsky et al., 1972b). Furthermore, physostigmine administration results in relapse of depressive symptoms in bipolar patients successfully treated with lithium, while healthy controls do not develop depressed mood. These patients have concomitant hormonal disturbances and emotional arousal expressed as dysphoria (Janowsky et al., 1986).

Arecoline, a nonselective muscarinic agonist, has also induced dysphoria in mood disorder patients whether or not they were currently depressed; this was greater in individuals with a family history of depression compared to those without such history (Gillin et al., 1991; Nurnberger et al., 1989). Both arecoline (Sitaram et al., 1980) and donepezil, a cholinesterase inhibitor (Perlis et al., 2002) decrease REM latency in patients with major depression, but not in healthy controls.

In a [^{15}O] blood flow study analyzing the functional associativity of cholinergic forebrain regions with and without procaine administration, some abnormalities were found to normalize with procaine (Benson et al., unpublished data). At baseline, patients with mood disorders compared to healthy controls show significantly weaker positive relationships among the cholinergic forebrain regions, which became stronger and similar to controls with procaine. Thus, commonly reported alterations in prefrontal, anterior cingulate, temporal, striatal, and cerebellar brain regions in mood disorders (see reviews; Dougherty and Rauch, 1997; Ketter et al., 1997; Drevets, 1998) may have a cholinergic component to their dysregulation.

In conclusion, these data demonstrate that intravenous procaine administration in anesthetized monkeys was associated with a dose-related blockade of the M_2

muscarinic ligand, [^{18}F]FP-TZTP. This is the first demonstration of muscarinic cholinergic binding of procaine in primates, in vivo. In addition, cerebral blood flow (K_1) was significantly increased in limbic regions of the brain. If significant receptor occupancy occurs at comparable plasma levels in humans, these data suggest a possible cholinergic contribution to the robust emotional and sensory effects of procaine and its ability to selectively activate amygdala and closely related anterior paralimbic structures. Thus, based on the binding and flow data presented here, procaine could prove to be another way of assessing muscarinic receptor tone in limbic areas in patients compared to healthy volunteers. Further studies to explore the relationship of muscarinic receptor activity with behavioral assessments, and use of agonist and antagonist ligands could delineate a clearer role of the cholinergic system in the effects of procaine on emotion.

Acknowledgements

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2.6 Afterword: Linking Preclinical and Clinical Data

To recapitulate the major findings of this study, procaine blocked the muscarinic PET ligand in a dose-related manner that suggests direct binding with the same M_2 site. Moreover, the flow of the ligand was increased with procaine administration most with the dose closest to the *in vivo* IC_{50} and in the same areas as demonstrated in humans (Ketter et al., 1996).

The potential for interaction between muscarinic receptors and the anesthetics used in this study needs clarification. Sodium pentathol does not modulate muscarinic receptor activity (Weber et al., 2004), but more likely will affect nicotinic, glutamate and GABA receptors. However, the findings in the control studies support two notions concerning general anesthesia effects. First, on the zero dose of procaine [^{18}FP]-TZTP binding was higher with the second scan, possibly indicating an interaction with time on anesthesia. These results agree with the work of Durieux (1996); he suggests that isoflurane decreases the dissociation rate from muscarinic receptors, which results in increased binding of muscarinic agents with muscarinic receptors. As noted in the paper, increased binding with time on anesthesia would have the effect of under-estimating the difference in binding between baseline and procaine scans on all non-zero doses. Second, it is well-known that cerebral blood flow increases with general anesthesia. This was also observed in the control studies, and would have the effect of over-estimating the difference in the flow of the ligand between baseline and procaine scans.

An outstanding question centers on whether the IC_{50} determined here corresponds to a clinically relevant dose of procaine. However, two potential

confounds limit the successful extrapolation of doses used in this study to those used in the human studies. The classic approach of allometry (Chappell and Mordenti, 1991) assumes faster drug metabolism in species smaller than in humans, which is not the case for procaine; rhesus monkeys ($t_{1/2}$= 45 minutes; Reidenberg, 1972) metabolize procaine at least four times slower than humans ($t_{1/2}$= 7.69 minutes; Seifen et al., 1979). Further complications arise from the conversion from bolus to constant infusion administration methods, and the resulting difference in the physiologic response to the two administration styles. With these caveats in mind, inter-species scaling of doses could be as follows. Assuming a conversion factor from monkey to human dose is 4.5 (Reidenberg, 1972) and converting a constant infusion rate back to a bolus injection by reversing the method applied to establish the doses tested in this study, the IC_{50} of procaine in humans could be equivalent to a dose between 0.71 and 1.94 mg/kg procaine, depending on the rate of injections. If the conversion from bolus administration dosing in the self-administration studies to constant infusion doses in this study produced somewhat equivalent plasma procaine levels, then the doses used in this study could be behaviorally or physiologically relevant.

Procaine levels of 0.11 μg/ml were obtained with an bolus dose of 1.84 mg/kg in the study of Ketter et al. (1996), however, the plasma samples were collected 5 minutes post-injection. Given the short half-life of procaine, these levels most likely do not reflect the procaine concentrations at the time of peak intensity of emotional and sensory experiences. Green and colleagues (1974) reported plasma levels of 3.6-11 μg/ml, equivalently 15-47 μM, with injections of procaine-penicillin that were associated with panic attacks. The procaine levels in these studies are in the same

range as the IC_{50} determined in this study. Taken together, these findings suggest that procaine binding to muscarinic receptors is viable explanation for the emotional and sensory experiences induced by procaine administration.

The question remains as to the relationship of the muscarinic system to the functional response of procaine, i.e., is muscarinic modulation contributing in the emotional and sensory experiences that procaine induces. This study will adopt an exploratory approach to confirm cholinergic pathways and potential relationships between the blood flow and emotional responses to procaine. Functional connectivity, as defined as the correlative strengths between regions of interest (ROIs), will serve as a method to confirm cholinergic pathways. The relationship between rCBF and emotional responses will be examined with multivariate multiple regression methods as outlined by the cholinergic model in Figure 9. Regional blood flow in core cholinergic brain regions will be measured utilizing an existing CBF PET data from patients with mood disorders and healthy volunteers who participated in the procaine study. Multivariate multiple regression can describe the strength, direction and nature of the relationships between brain regions and behavioral outcome.

Chapter 3: Alterations of Regional Cerebral Blood Flow with Procaine Administration in Bipolar Illness

3.1 Introduction

The local anesthetic procaine has unique neuropsychopharmacological properties that make it useful as a discrete probe of the limbic system and its relationship to emotion modulation. Procaine and related local anesthetics are known to selectively increase limbic perfusion, metabolism and electrophysiological activity in animals (Benson et al. 2004; Heynen et al. 1995; Munson et al. 1970; Post 1981; Post et al. 1984; Racine et al. 1975; Racine et al. 1979; Wagman et al. 1967). Furthermore, procaine has been used as an affective challenge in healthy controls (Ketter et al., 1996; Servan-Schreiber et al., 1998), and patients with mood disorders (Ketter et al., 1993) and panic disorder (George et al., 1993), as well as individuals abusing alcohol (George et al., 1990) and cocaine (Adinoff et al., 2001).

Procaine, when administered intravenously (i.v.) in healthy controls, results in brief intense emotional and sensory experiences in association with increased global and regional anterior paralimbic cerebral blood flow (CBF) as measured with [^{15}O] water PET (Ketter et al. 1996); the amygdala, insula and anterior cingulate are particularly activated. In healthy individuals, 1.84 mg/kg procaine induces the following: an array of brief intense emotional responses ranging from dysphoria (fear and anxiety) to euphoria; visual and auditory sensory illusions/hallucinations (Kellner et al. 1987); hormonal changes such as increased ACTH, cortisol, and prolactin secretion (Kling et al. 1994); and increased temporal lobe fast activity EEG (Parekh et

al. 1995). Furthermore, the degree of right amygdala activity on PET correlated with the degree of affective arousal, while the left amygdala CBF correlated positively with the degree of dysphoria and inversely with the degree of euphoria (Ketter et al. 1996). Increases in the mesial occipital CBF were associated with the occurrence of visual hallucinations.

Although classically associated with the blockade of voltage-gated sodium channels, procaine also interacts with many neurochemical systems. However, it has high affinity to the muscarinic cholinergic receptor. In vitro studies have shown procaine competitively displaces the non – specific muscarinic antagonist, QNB (quinuclidinyl benzilate), at putative M_2 receptors (K_i = 4 μM; Hisayama et al. 1989) on guinea pig ileum and M_1 and M_2 receptors (K_i = 4 μM; Sharkey et al. 1988) in rat hippocampal membranes.

Moreover, procaine has recently been shown to have *in vivo* action on muscarinic M_2 receptors in the anesthetized rhesus monkey utilizing a radiotracer with preferential binding to these receptors (Benson et al., 2004). In that study, procaine blocked the M_2 ligand (TZTP) in a dose-related manner with an *in vivo* IC_{50} (1.31 μM) that mirrored estimates determined from in vitro studies (Sharkey et al., 1988; Hisayama et al. 1989). Furthermore, peak increases in the flow of the muscarinic radiotracer were near this IC_{50}. These results suggest that the binding of procaine to muscarinic receptors may contribute to the emotional and sensory effects observed in man (Ketter et al., 1996).

Such a cholinergic effect would be consistent with the work of Janowsky, et al. (1972a, 1972b, 1986), in which they proposed that there exists an imbalance of

cholinergic-adrenergic activity in patients with mood disorders. Indirect and direct cholinergic agonists, such as physostigmine and arecoline, can produce dysphoria in manic patients (Janowsky et al., 1972b). The patients have concomitant hormonal disturbances with this emotional arousal (Janowsky et al., 1986). These findings have led Janowsky to hypothesize that the cholinergic-noradrenergic balance is shifted towards cholinergic activity with depression and with noradrenergic activity with mania (Janowsky et al., 1972a). More recently, Overstreet and colleagues (1998) have suggested a cholinergic-serotonergic imbalance in mood disorders in which they argue that a cholinergic overactivity predisposes one to depression and serotonergic mechanisms induce depressive episodes.

Procaine studies have not included patients with bipolar disorder despite the putative role of amygdala and cholinergic substrates in this disorder. This study was undertaken to assess the CBF response to procaine in a group in which emotion dysregulation is one of the hallmark symptoms. Bipolar disorder is characterized by baseline hyper-metabolism in several subcortical limbic regions, including the amygdala, and striatum and hypo-metabolism in cortical limbic structures such as the dorsolateral prefrontal cortex and anterior cingulate at baseline (Ketter et al., 2001). Preliminary results suggest patients with mood disorders (unipolar and bipolar) exhibit blunted anterior paralimbic CBF response to procaine compared to healthy controls (Ketter et al., 1993). This study postulates that a blunted response to procaine in bipolar disorder will be confirmed in this larger and diagnostically more pure sample. In addition, with patients primarily in the depressed phase of the illness, cholinergic regions may be overactive compared to controls. More specific

hypotheses relating changes in cholinergic activity to other brain regions are examined in a companion paper on functional connectivity of the basal forebrain cholinergic region in patients and controls before and after procaine.

3.2 Methods

3.2.1 Subjects

Healthy controls (HC; n=32; 17 men, 15 women; mean ± SD age, 30.1 ± 7.5; range 18 to 45 years) and patients with bipolar illness (BPD; n=15; 7 men, 8 women; gender $\chi^2 = 0.17$, p = ns, df =1; mean ± SD age, 39.2 ± 10.4; range 24 to 53 years; between groups age comparison: t = 9.2, df = 45, p = 0.001) participated in PET study using $H_2{}^{15}O$ water after obtaining written informed consent (Ketter et al., 1996). The subjects were informed that procaine had various transient emotional and sensory effects, ranging from minimal to intense and from pleasant to unpleasant. They were told they could stop the procedure at any time, and that diazepam was available if needed. All subjects were determined to be physically healthy by history and physical examination, including neurological exam, electrocardiogram (EKG), routine serum chemistry studies (with HIV), comprehensive drug screening, and magnetic resonance imaging (MRI).

Diagnosis of Bipolar Disorder was confirmed with Schedule for Affective Disorders and Schizophrenia – Lifetime Anxiety Version (Mannuzza et al., 1986) by trained physician. Except for one BPI patient (at some point experienced moderate to sever mania), all were BPII (n=14; at some point experienced hypo-mania or mild mania), most with a confirmed history of rapid cycling (n=11; defined as

experiencing 4 or more cycles of mania and depression). A fuller description of the illness characteristics was derived from data on the prior course of each patient's illness gathered from retrospective life charts from 12 patients (Leverich and Post, 1996, 1998). Age of onset and number of hospitalizations for depression or mania were directly transcribed, while, number of episodes for depression or mania, episodes per year, number of weeks ill, percent of life ill for entire life and since age of onset, and duration of illness (current age minus age of onset) were determined using prescribed criteria. Previous successful and unsuccessful medication trials were also examined in 12 patients, from whom a refractoriness index was computed on a 0 to 100 scale. A failed trial of each anti-depressant of a different class was assigned a rating of 10, ECT a 30, adjunctive treatments such as thyroid a 5, and other treatments a 2.5 (Post et al. 2001, unpublished rating scale, available upon request).

3.2.2 Procaine Procedure and Behavioral Measures

Regional cerebral blood flow (rCBF) was measured using $H_2{}^{15}O$ PET during intravenous saline and procaine administration in a single-blind design, where they were instructed to expect saline, low-dose or high-dose procaine. Subjects were injected with saline (sham scan), saline (baseline scan; BL), low-dose procaine (0.46 mg/kg), high-dose procaine (1.84 mg/kg; PR) and saline (recovery of baseline scan) in the same order. This order was chosen to ensure progressing to the higher dose was safe and to remove possible artifacts from high-dose exposure to subsequent scans. There was twenty minutes between each scan allowing for time to return to baseline. Analysis of low-dose procaine in previous studies of healthy controls and

preliminary analysis in patients revealed little to no effect on blood flow or behavioral ratings; for these reasons, only high dose procaine results will be presented.

Baseline behavioral measures of mood, including clinician and self-ratings, were collected before the scanning session began (Table 5). Clinician ratings included Hamilton Depression Rating scale (Hamilton, 1960; [HDRS]), Young Mania Rating Scale (Young et al., 1978; [YMRS]), and Spielberger State Anxiety Scale (Spielberger et al., 1970; [SSAS]), and self-ratings included Beck Depression Inventory (Beck, et al., 1961; [BDI]). These assessments, as well as the life chart variable measures, indicate that the patients were moderately depressed (mean HDRS = 16.9), predominately not manic (two subjects YMRS > 10), and treatment-refractory (multiple medication trials). Likert-based ratings of emotional and sensory experiences were collected at baseline and immediately after each scan. Demographic, clinician and self-ratings data were analyzed using paired (within group drug comparisons) and unpaired t-tests (between-group comparisons) or repeated measures ANOVA with between and within group factors, as deemed appropriate.

Table 5. Subjects Demographics and Illness Characteristics

Variable	N	Mean	SD	Range
Healthy Controls				
Gender (17 M / 15 F)				
Age (years)	32	30.0	7.5	18-45
Hamilton Depression Rating Score	32	1.0	1.4	0-5
Patients with Bipolar Disorder				
Gender (7 M / 8 F)				
Age (years)	15	39.5	14.2	20-65
Hamilton Depression Rating Score	15	16.9	9.6	0-33
Spielberger Anxiety Scale	14	44.5	11.7	25-69
Beck Depression Score	15	14.8	9.8	0-37
Young Mania Rating	13	4.1	5.1	0-17
Age of Onset (years)	12	18.8	8.1	6-30
Duration of Illness (years)	12	20.4	10.7	8-43
Number of Hospitalizations	12	2.6	3.0	0-10
Number of Episodes (depression)	12	208.9	562	3-1983
Number of Episodes (mania)	12	186.3	565	1-1981
Number of Weeks Depressed	12	246.8	276.6	42.7-988.5
Number of Weeks Manic	12	84.9	152.5	1.3-457.9
Number of Failed Medication Trials	12	9.2	4.1	1-13
Refractory Index Score (0-100)	12	14.1	28.9	1.75-55.5

3.2.3 PET Acquisition and Data Analysis

After placement of an arterial-line in the radial artery and an intravenous line placed in the opposite arm, the subjects were taken to the PET suite and asked to lie supine on the bed of the PET scanner. A thermoplastic mask with openings for the eyes, ears, nose, and mouth placed over the face and fastened to the scanner bed restricted head movement (Smith and Nephew, Madison, WI). A transmission scan from a germanium 68 / gallium-68rotating pin source was obtained to allow correction for attenuation by the skull and cerebrum, and to ensure optimal placement in the scanner. This was followed by the series (saline / procaine) of scan acquisitions described above. The subjects were instructed to lie quietly with eyes closed and monitor their sensory and emotional experiences during the scans. Upon completion of the scan, emotional and sensory ratings, as well as reports of any unusual cognitions or somatic / visceral sensations, were collected.

As a preventative measure, each subject's heart rate, blood pressure and chemistry were monitored during the entire procedure. To correct for pCO_2 effects on CBF, the pixel values were adjusted for pCO_2 levels at baseline and with procaine with the following formula suggested by Grubb et al. (1974):

$$rCBF_{adj} = 1.8\ (40 - rCBF).$$

All results reported in this study incorporate this adjustment.

Brain images were collected on a Scanditronix tomograph (PC2048-15B, Scanditronix Inc, Uppsala, Sweden) with 6.9-mm in-plane and 5- to 6-mm axial-full-width-half maximum resolution over a four minute period. Fifteen seconds after procaine/saline administration, the radiotracer consisting of 1110-MBq bolus of

$H_2{}^{15}O$ water was injected. Emission data were collected in 15 planes spaced 6.5 mm apart beginning at the canthomeatal line and started when the radiotracer bolus reached the head. Continuous radial arterial blood sampling for blood radioactivity levels was used to generate radiotracer time-activity curves. Regional CBF was calculated from the dynamic tracer-kinetic modeling of the PET scan data and the time-activity curve using least-squares method (Herscovitch et al. 1983; Raichle et al. 1983).

Image processing was performed on a Unix workstation (SUN Ultra 10 Sparc station, SUN Microsystems Inc, Mountain View, CA) and statistical analysis was performed on a Macintosh G3 and Windows 2000™ personal computers. Image display and region definition were performed using Analyze imaging software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN).

After CBF scans were visually inspected for image quality and artifacts, automated image processing was performed using SPM99 (statistical parametric mapping; Friston et al., 1994). Images were first co-registered across conditions. This was followed by reorientation parallel to intercommissural plane, and resizing, reslicing and rescaling to yield sterotatically normalized images (Friston et al., 1989, 1995) corresponding to the human brain atlas of Talairach and Tourneaux (1988). Images were then smoothed to a resolution of 10-mm transaxial and 6-mm axial yielding a final reconstructed resolution of 12 mm isotropic.

Global cerebral blood flow (gCBF) was calculated as the mean of all grey-matter voxels. For grey-matter determination, the background noise threshold (above which a voxel is presumed to be in-brain) was set at one-eighth of the mean of the

entire image space, and the whole-brain mean was calculated using only these suprathreshold voxels. Voxels exceeding 70% of the whole-brain mean were assumed to be in grey matter.

Statistical inference testing on absolute and globally normalized rCBF data was performed to examine potential differences between groups, conditions and their interaction using SPM 99 (Friston et al., 1990). The relationship between mood and procaine-induced blood flow response was assessed by correlating HDRS scores with change in blood flow.

The resulting Z-maps correspond to the raw probabilities in each voxel of the null hypothesis (H_0: Z=0). To correct for multiple comparisons, these Z-maps were then submitted to cluster analysis within SPM99 (Friston et al. 1994). Cluster analysis considers the smoothness of the Z-map, arising from the low-pass filtering applied to the input images, as well as inherent spatial autocorrelation in the underlying brain function, in determining the effective independence of the multiple comparisons contained in the total brain volume analyzed. A spatial extent threshold is generated from this smoothness estimate and the Z threshold specified. A voxel is retained as significant only if it falls within a cluster of contiguous suprathreshold voxels that is larger that this extent, which should occur by chance only 5% of the time (for a cluster probability threshold set at .05) in a Z-map of that smoothness.

The cluster analysis corrects for multiple comparisons by dropping voxels that are not robustly represented. Thus, the final cluster size is a combination of spatial extent and significance level. For purposes of depicting topography of the findings in the figures, raw p-values are displayed for all voxels falling in clusters deemed

significant by this analysis. Regions with significant findings, as well as the local maxima, were identified according to the atlas of Talairach and Tourneaux (1988). When possible, Brodmann's areas are also indicated.

3.3 Results

3.3.1 Emotional and Sensory Response to Procaine

Despite the expected significant baseline differences between the groups in ratings of depression, fear, anxiety, anger, calmness, and overall bad/good feeling, the brief emotional and sensory responses were generally equivalent in the procaine condition, exhibiting a simple main effect of procaine (Table 6 and Figures 15-19). This included emotional ratings of euphoria, anxiety, calmness, tiredness, and sensory ratings for visual and auditory changes. Anger and overall feeling of bad/good did not significantly increase with procaine in either group.

The remainder of the emotion ratings changed in more complex ways. Depression exhibited a significant drug x group interaction, due to the significantly greater baseline depression ratings in the patients (HC: 0.0 ± 0.0; BPD: 1.73 ± 1.5; t=-4.38, p=0.001, df=14; unequal variances: F=114.07, p=0.000, df=1, 45), which became nonsignificant with procaine (HC: 0.63 ± 1.7; BPD: 1.27 ± 1.7; t=-1.20, p = ns, df=45). Patients rated fear significantly higher than controls at baseline and with procaine, and each group rated fear higher with procaine compared to baseline, such that there was an overall main group and drug effect respectively, but no significant drug x group (DXG) interaction.

In both groups, ratings of anger at baseline, with procaine or delta-condition were not normally distributed. In addition, only patient ratings of depression at baseline or with procaine were normally distributed. Thus, correlations involving anger and depression (baseline, procaine, or delta) should be viewed with caution, and delta-ratings were not included in the results of the multivariate analyses described below.

Table 6. Emotional and Sensory Reponses at Baseline and with Procaine

			Descriptive Statistics			Student's t-test		Repeated Measures ANOVA			
Rating	Drug	Group	Mean	SD		t	p	Source	F	P	Wilk's λ
Depression	Saline	HC	0.00	0.00	*	-4.38	0.001	Drug	0.11	ns	0.998
		BPD	1.73	1.53				Group	11.92	0.001	
	Procaine	HC	0.63	1.70		-1.20	ns	D X G	5.01	0.030	
		BPD	1.27	1.71							
Anger	Saline	HC	0.00	0.00	*	-2.36	0.033	Drug	3.45	ns	0.929
		BPD	0.60	0.99				Group	1.86	ns	
	Procaine	HC	0.66	1.60		-0.30	ns	D X G	0.98	ns	
		BPD	0.80	1.32							
Euphoria	Saline	HC	0.44	0.98		0.35	ns	Drug	34.07	0.000	0.569
		BPD	0.33	0.90				Group	0.35	ns	
	Procaine	HC	2.34	2.57		-0.81	ns	D X G	0.94	ns	
		BPD	3.00	2.59							
Bad/Good	Saline	HC	3.13	0.71		3.58	0.001	Drug	0.14	ns	0.997
		BPD	2.27	0.88				Group	2.00	ns	
	Procaine	HC	2.66	2.18		0.28	ns	D X G	0.86	ns	
		BPD	2.47	2.26							
Fear	Saline	HC	0.13	0.42	*	-2.77	0.013	Drug	49.07	0.000	0.478
		BPD	0.73	0.80				Group	7.20	0.010	
	Procaine	HC	2.25	2.49		-2.06	0.045	D X G	1.61	ns	
		BPD	3.80	2.21							
Anxiety	Saline	HC	0.69	1.06		-2.11	0.041	Drug	65.64	0.000	0.407
		BPD	1.40	1.12				Group	1.07	ns	
	Procaine	HC	3.63	2.18		-0.26	ns	D X G	0.67	ns	
		BPD	3.80	2.21							
Calmness	Saline	HC	3.72	1.40		2.29	0.027	Drug	13.34	0.001	0.771
		BPD	2.73	1.34				Group	1.01	ns	
	Procaine	HC	1.78	2.06		-0.23	ns	D X G	2.30	ns	
		BPD	1.93	2.25							
Tiredness	Saline	HC	1.44	1.46		0.24	ns	Drug	9.45	0.004	0.826
		BPD	1.33	1.23				Group	0.72	ns	
	Procaine	HC	0.41	0.80	*	-1.57	ns	D X G	3.28	ns	
		BPD	1.07	1.53							
Visual	Saline	HC	0.31	0.74		0.17	ns	Drug	29.00	0.000	0.608
		BPD	0.27	1.03				Group	0.36	ns	
	Procaine	HC	2.72	2.52		0.58	ns	D X G	0.25	ns	
		BPD	2.27	2.43							
Auditory	Saline	HC	0.06	0.35		0.68	ns	Drug	322.72	0.000	0.122
		BPD	0.00	0.00				Group	0.07	ns	
	Procaine	HC	4.66	1.83		-0.40	ns	D X G	0.27	ns	
		BPD	4.87	1.36							

* unequal group variance as indicated by Levene's Test for Equality of Variances

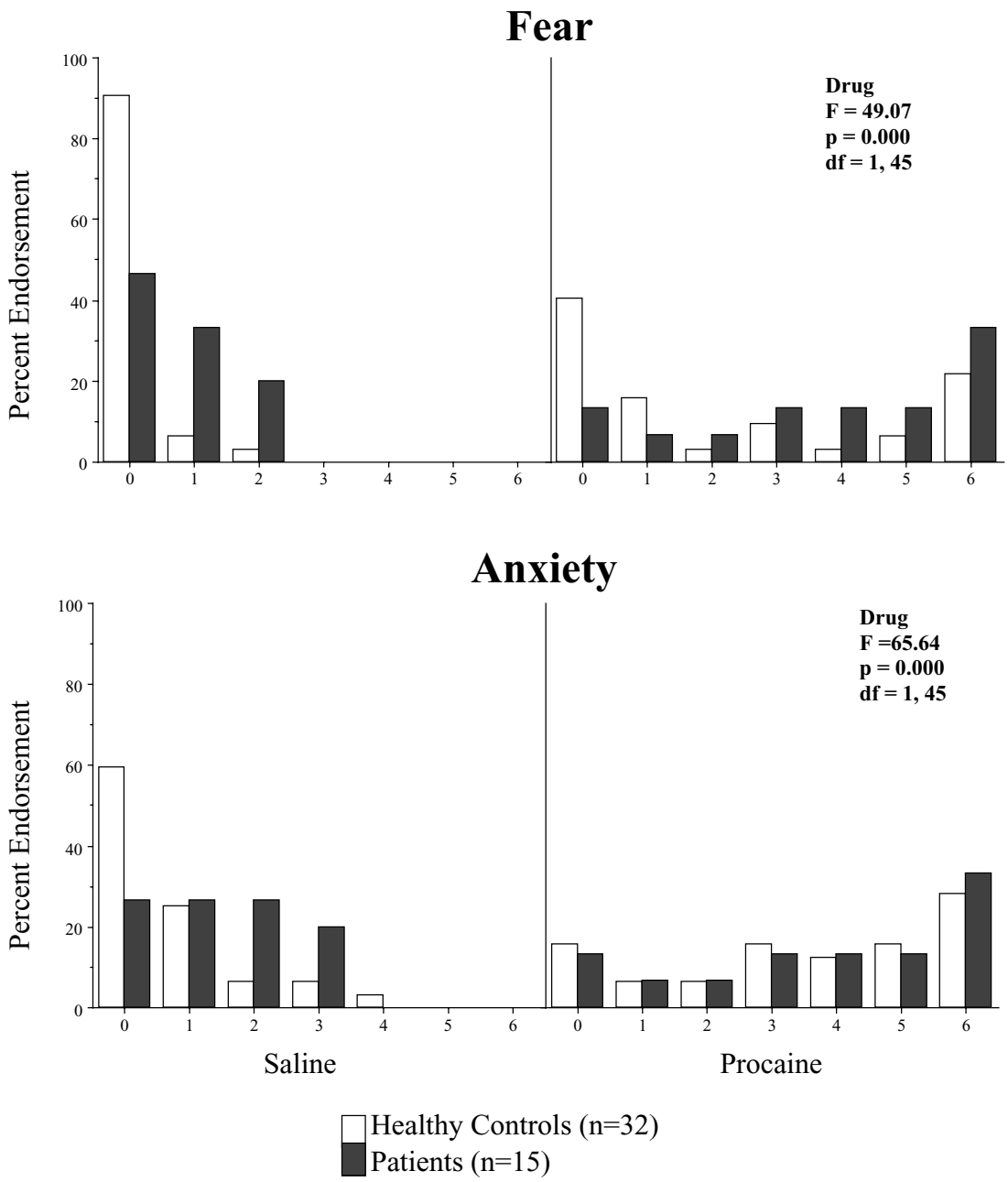

Figure 15. Procaine-Induced Emotions: Fear and Anxiety
At baseline, patients with bipolar disorder have significantly higher fear and anxiety ratings than healthy controls. Procaine-induced increases in these ratings were the same on average and in distribution in both groups.

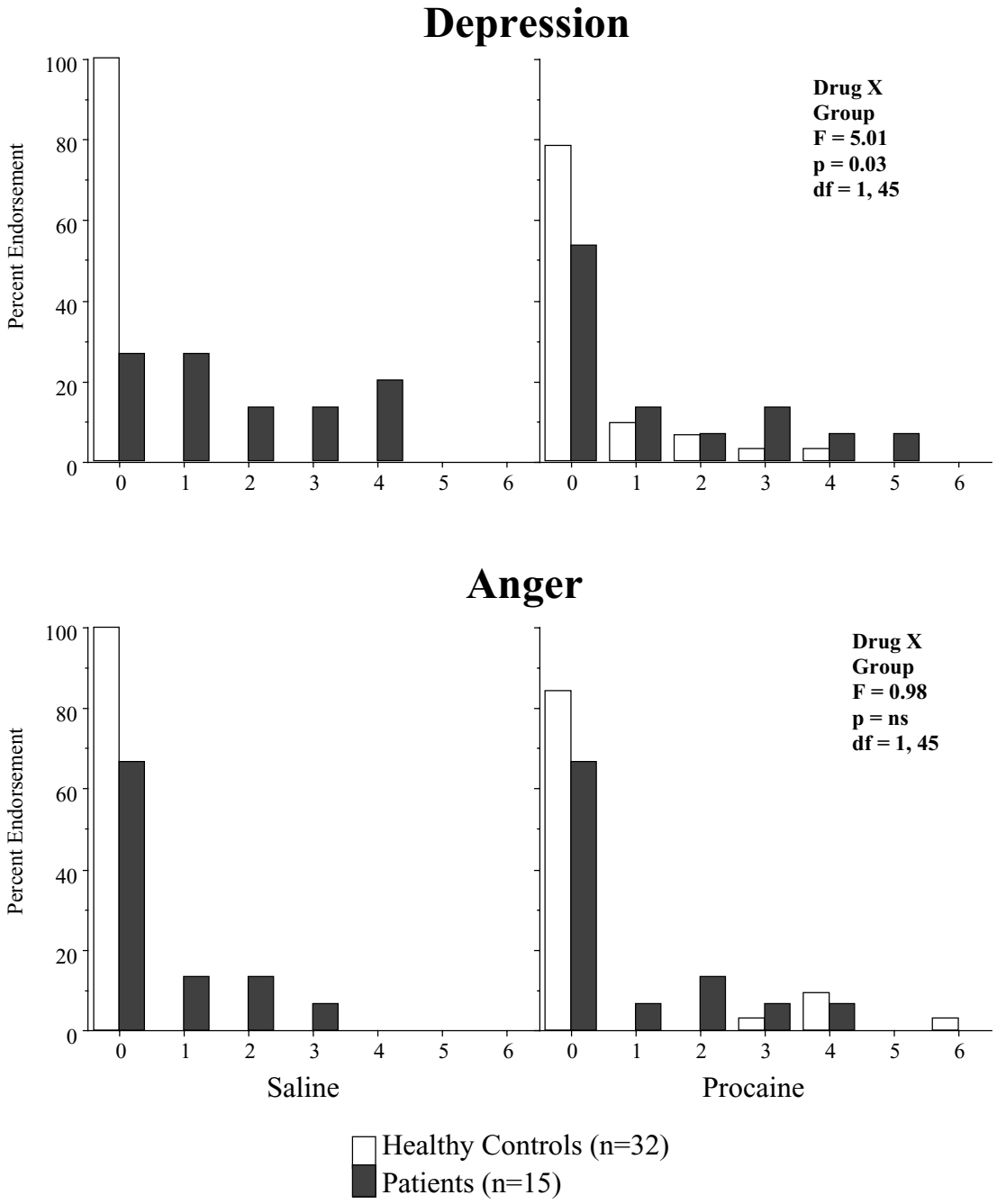

Figure 16. Procaine-Induced Emotions: Depression and Anger
At baseline, patients with bipolar disorder have significantly higher depression and anger ratings than healthy controls. There was a significant interaction of drug and group in ratings of depression, suggesting that the patients depressed mood at baseline did not change with procaine, as seen in the controls. Procaine-induced increases in anger ratings were the same on average and in distribution in both groups. Neither depression nor anger ratings exhibited a normal distribution, as most were scored as zero.

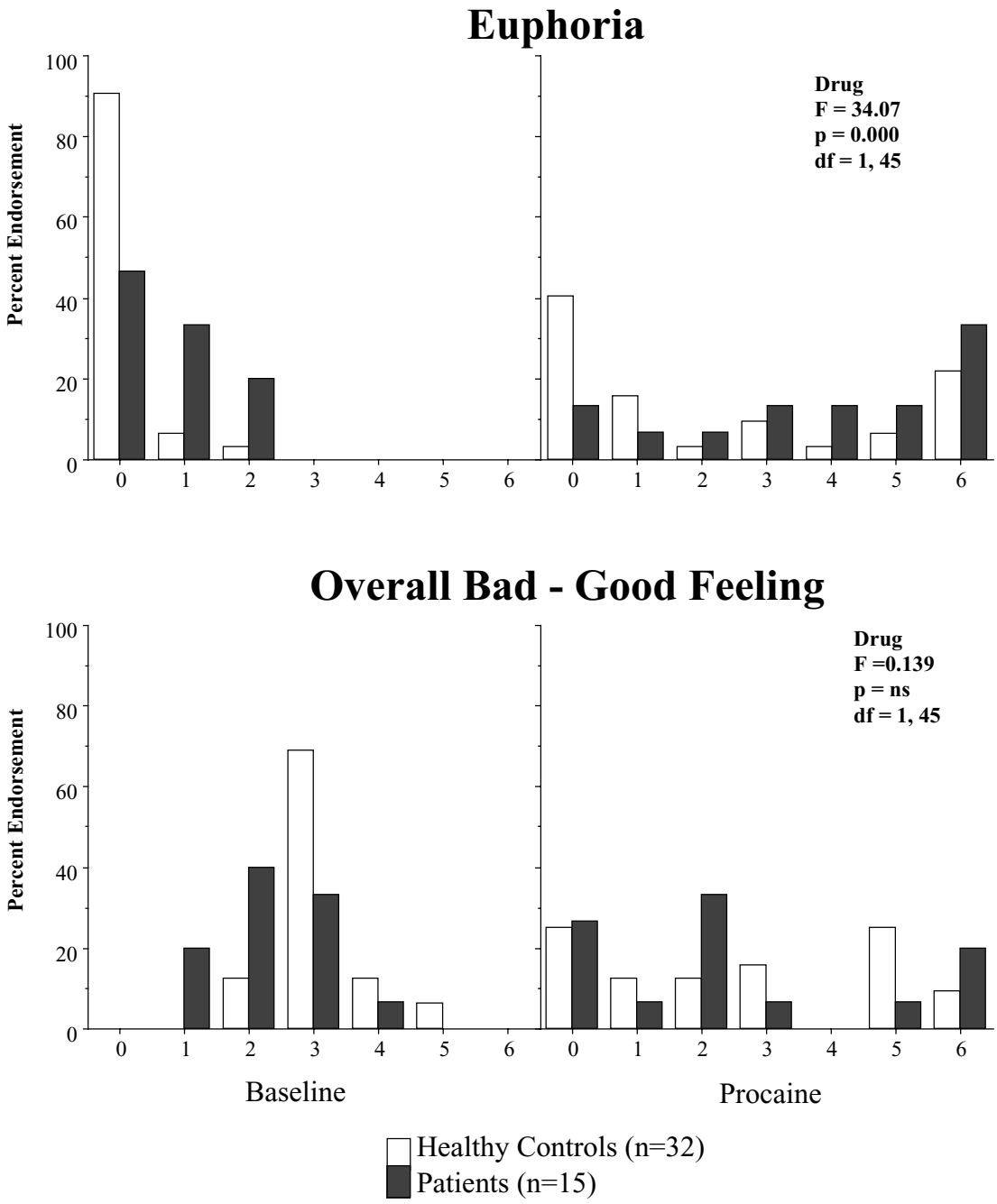

Figure 17. Procaine-Induced Emotions: Euphoria and Good-Bad Feeling
At baseline, patients with bipolar disorder and healthy controls have similar euphoria and overall feeling of good or bad ratings than healthy controls. Procaine-induced increases in these ratings were the same on average and in distribution in both groups.

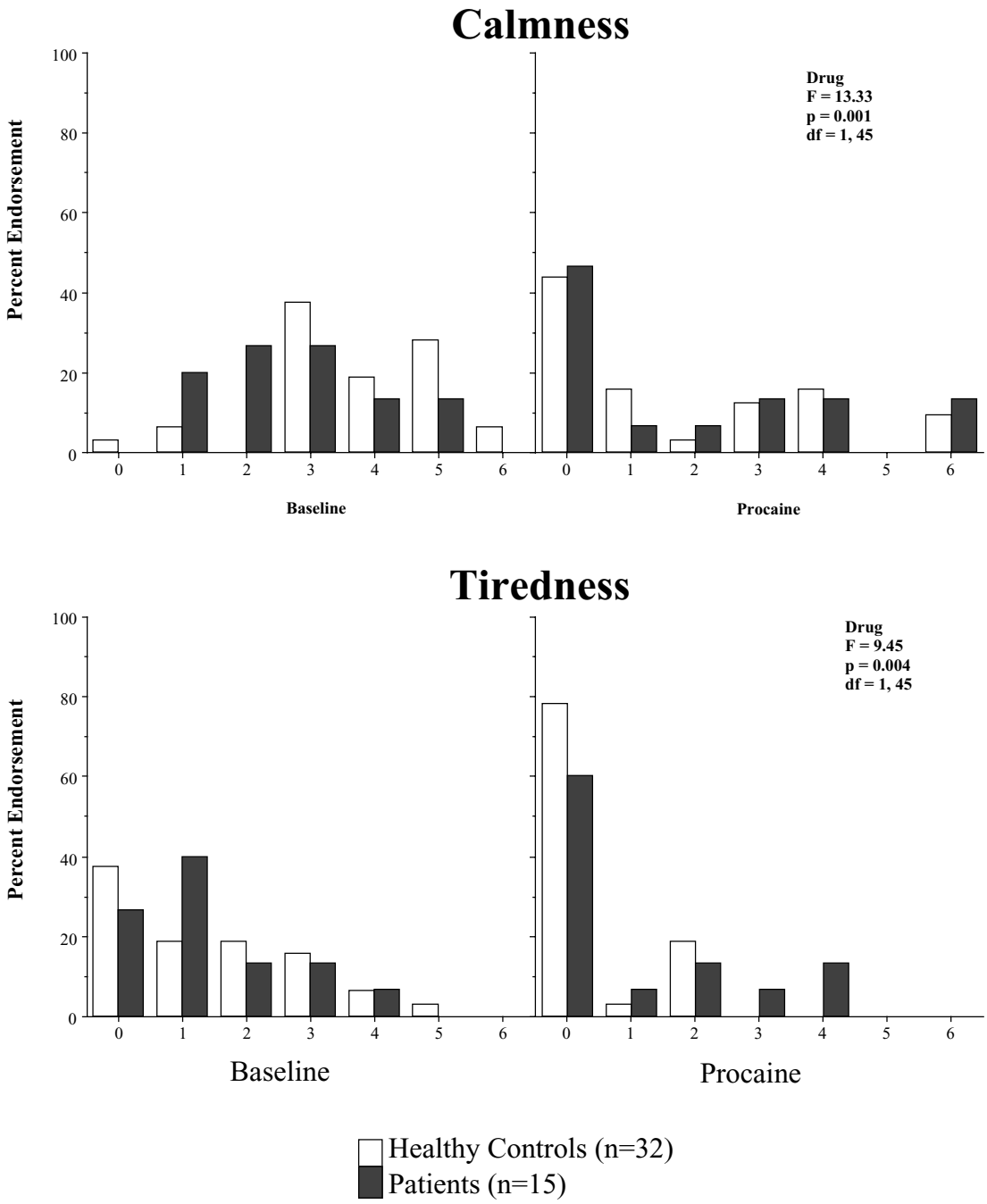

Figure 18. Procaine-Induced Emotions: Calmness and Tiredness
At baseline, patients with bipolar disorder and healthy controls have similar calmness and tiredness ratings than healthy controls. Procaine-induced increases in these ratings were the same on average and in distribution in both groups.

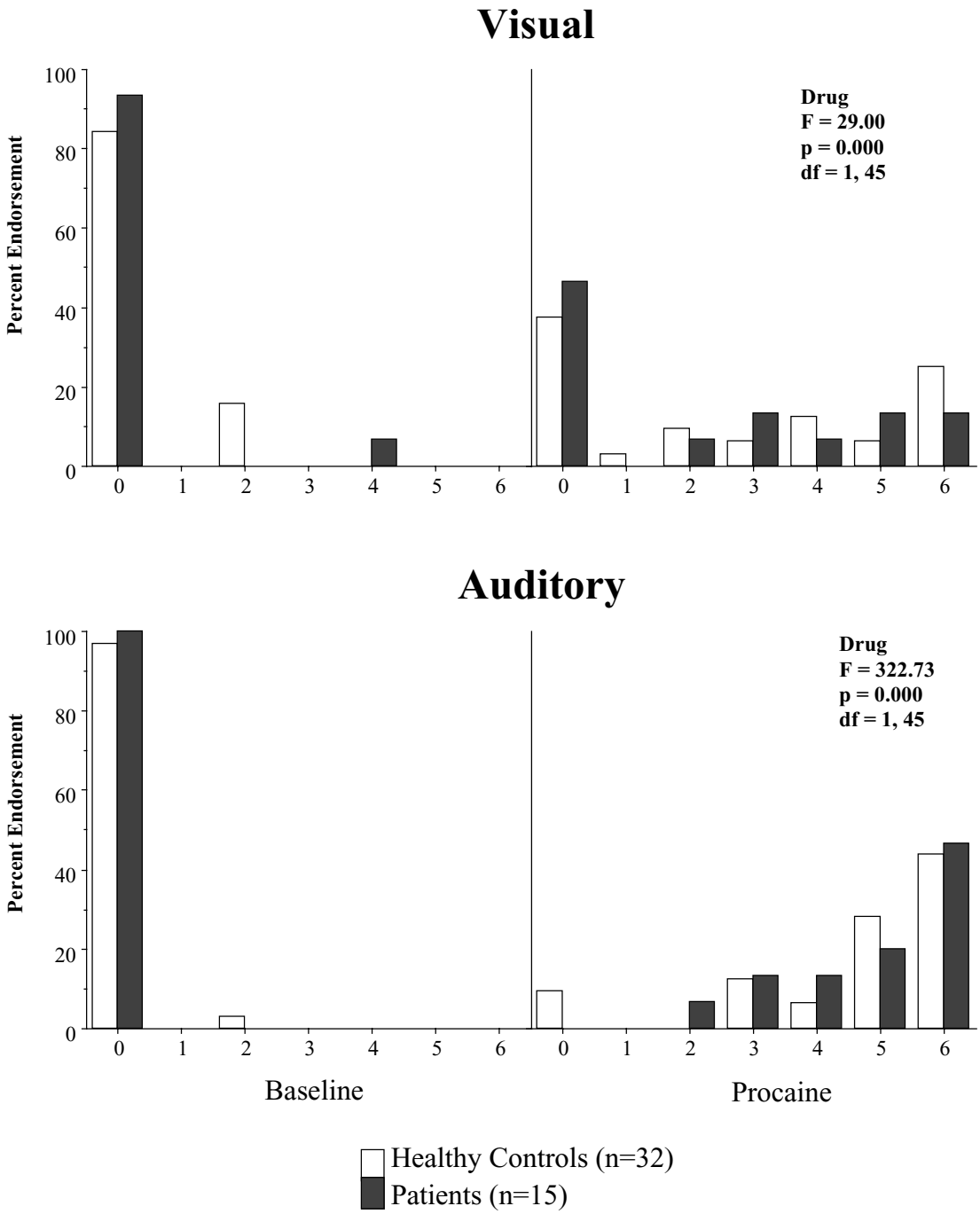

Figure 19. Procaine-Induced Sensations: Visual and Auditory
At baseline, patients with bipolar disorder and healthy controls have similar visual and auditory sensory ratings than healthy controls. Procaine-induced increases in these ratings were the same on average and in distribution in both groups.

3.3.2 Activation Studies

3.3.2.1 Healthy Controls

Since the CBF response to procaine administration in healthy controls has been published (Ketter et al., 1996), only a short synopsis of those results follows. A robust global and relative blood flow increase is associated with procaine administration (Figure 20). Procaine increased gCBF by 20.3% (baseline [BL]: 42.2 mL/min/100g; procaine [PR]: 50.8 mL/min/100g; $t = 8.57$, $p = .0004$, $df = 31$). In an analysis of absolute rCBF with procaine compared to baseline, all regions of the brain are affected, such that the resultant cluster of significant increases with procaine compared to baseline encompassed the entire brain with a maximal difference in the anterior cingulate (Figure 21; Table 7). There were no significant absolute decreases in rCBF with procaine compared to baseline.

Normalized rCBF increases occur in the anterior paralimbic structures, with a large cluster including the anterior cingulate, anterior insula, medial (amygdala) and anterior temporal lobe, the basal forebrain cholinergic area, the dorsal and ventral striatum, and the medial orbitofrontal cortex (Figure 21). Significant decreases in rCBF with procaine compared to baseline were observed in mostly posterior regions of the brain, including bilateral cerebellum, inferior parietal, fusiform cortex, and posterior temporal cortex. However, significant decreases were also observed in the left DLPFC (BA 46), which may encompass Broca's area. Due to the data normalization process, the normalized decreases reported in both the healthy controls and patients are relative to the average blood flow of the brain. In both groups, procaine robustly increased rCBF globally and no area exhibited decreased blood

flow. Therefore, any significant decreases in rCBF represent less robust increases than significant rCBF increases.

Table 7. Significant Findings in Regional CBF Identified by Comparative Analyses

Analysis			Region	Talairach Location X	Y	Z	Cluster Size (k)	Z
Healthy Controls (HC)								
Procaine vs Baseline								
	Absolute	↑ L	Pregenual AC	-2	+22	+20	46610	5.56
		↓	none					
	Normalized	↑ L	Pregenual AC	-2	22	16	10798	5.87
		↑ R	Supragenual AC	+8	+12	+28		5.67
		↑ L	SI/Hypothalamus	-2	-2	0		5.56
		↑ R	Middle Temporal	+58	-40	+4	76	3.40
		↑ L	Somatosensory	-54	0	+8	20	3.22
		↑ R	Somatosensory	+56	-4	+16	86	4.00
		↓ L	Cerebellum	-12	-78	-16	9148	4.57
		↓ R	Cerebellum	34	-62	-12		4.91
		↓ L	Inferior Parietal	-42	-36	38	262	3.59
		↓ R	Inferior Parietal	46	-26	36	302	3.59
		↓ L	DLPFC (BA44)	-32	14	28	159	3.53
		↓ L	Cuneus	-14	-52	8	60	3.45
Bipolar Disorder (BPD)								
Procaine vs Baseline								
	Absolute	↑ L	Pregenual AC	-4	28	+12	52855	3.69
		↑ R	Supragenual AC	+2	+10	+12		3.44
		↑ L	Amygdala	+22	0	-20		3.69
		↑ R	Amygdala	-18	+6	-20		3.55
		↑ L	SI/Hypothalamus	-2	+4	0		3.38
		↓	none					
	Normalized	↑ R	Supragenual AC	+2	+4	+36	1515	4.37
		↑ R	Pregenual	+6	+28	+16		4.25
		↑	SI/Hypothalamus	0	2	0		3.25
		↑ R	Anterior Insula	+28	+8	-8	22	3.44
		↑ L	Amygdala	20	0	-16	4422	4.42
		↑ R	Amygdala	-16	6	-20		5.08
		↑ L	Parahippocampal	-18	-42	+4	143	3.20
		↑ L	Occipital	-10	-84	+28	48	3.26
		↓ L	Cerebellum	-40	-62	-28	2075	3.52
		↓ R	Cerebellum	2	-56	-24	363	4.87
		↓	Medulla	2	-28	-28	148	3.76
		↓ R	Inferior Parietal	46	-38	40	513	3.37
		↓ R	DLPFC	30	6	32	171	3.43

Table 7. Cont'd

Analysis			Region	Talairach Location X	Y	Z	Cluster Size k	Z
15 BPDs vs 32 HCs - Baseline								
Absolute	↑		none					
	↓		none					
Normalized	↑	R	Medial Frontal	16	48	8	315	3.35
	↑	R	Amygdala / Hippocampus	26	-12	-20	143	3.89
	↑	L	Posterior Temporal	-36	-50	20	120	4.25
	↑	R	Posterior Temporal	34	-50	20	351	3.37
	↑	L	Cerebellum	-54	-8	-12	43	3.48
	↓	R	Subgenual AC / SI	2	8	-12	94	3.16
	↓	L	DLPFC	-38	24	32	514	2.97
15 BPDs vs 32 HCs - Procaine								
Absolute	↑		none					
	↓		none					
Normalized	↑	L	Posterior Temporal	-36	-48	16	73	2.99
	↑	R	Posterior Temporal	36	-36	24	135	3.02
	↑	L	Occipital	-32	-84	4	413	3.08
	↑	L	Cerebellum	-10	-66	-4	1752	3.41
	↑	R	Cerebellum	14	-58	-24	1752	3.22
	↓	L	DLPFC	-28	26	40	94	3.03
	↓	R	GP/Putamen/Insula	30	-10	4	400	3.01
	↓	L	Posterior Temporal	-54	-30	0	225	3.37
	↓	L	Inferior Parietal	-50	-52	28	80	3.17
15 BPDs vs 32 HCs - Drug X Group								
Absolute	↑		none					
	↓		none					
Normalized	↑	L	Cerebellum	-16	-52	-16	1912	3.15
	↑	R	Cerebellum	+18	-76	-24	621	3.11
	↑	L	Parahippocampal Gyrus	-16	-48	4	1912	3.68
	↓	R	Amygdala	+14	-18	-20	139	3.85
	↓	R	Dorsal AC	+10	-26	+36	45	3.42
	↓	R	Supragenual AC	+14	+24	+40	61	3.15
	↓	R	Temporal Pole	+38	+8	-20	199	2.97

For $Z > 2.97$ $p<0.001$; for $Z > 3.89$ $p<0.0001$

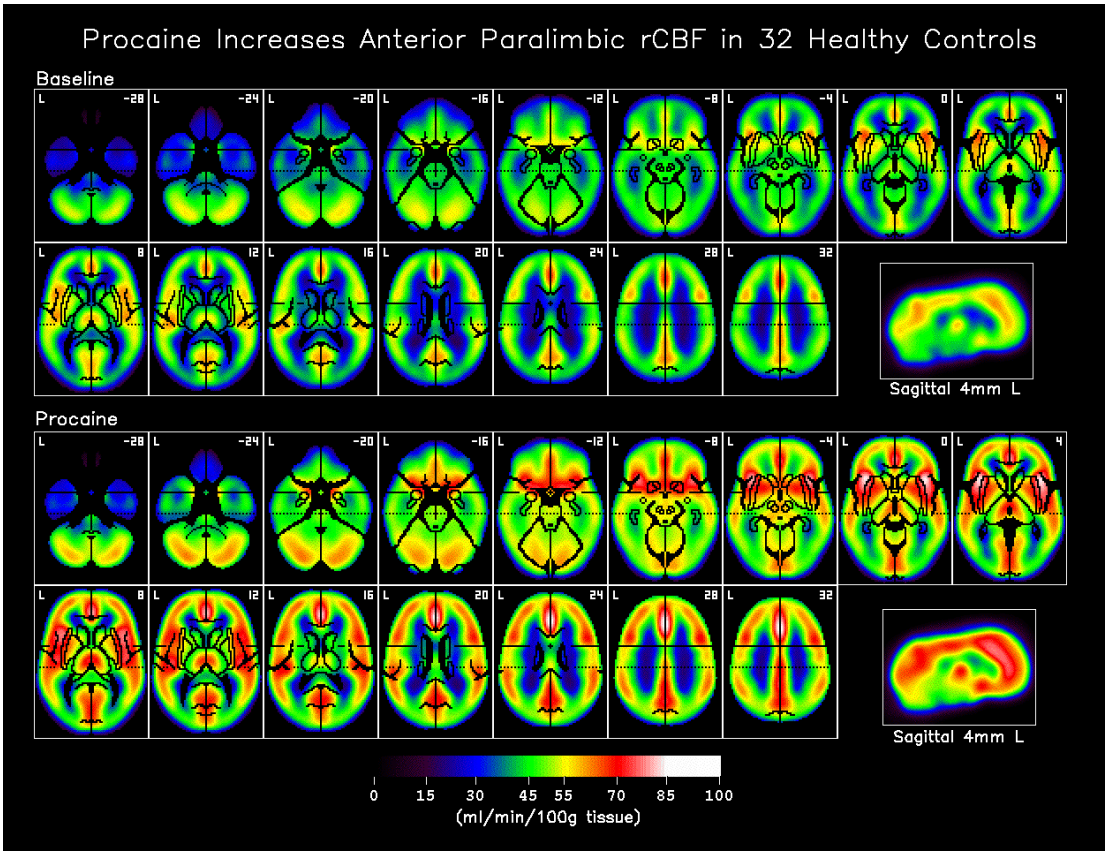

Figure 20. Effects of Procaine on rCBF in 32 Healthy Controls
Mean regional cerebral blood flow (rCBF) at baseline (top) and with procaine (bottom) in 32 healthy controls. Procaine increases rCBF throughout the brain with the most robust increase (indicated in red) in anterior paralimbic regions, such as the anterior cingulate, amygdala, insula, medial orbitofrontal and prefrontal cortex, and ventral and dorsal striatum. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: numbers indicate Talairach Z plane; L=left. Note: the sagittal views illustrate the limits of the data collection range, i.e., the extreme dorsal and ventral areas of the brain were not in the imaging space.

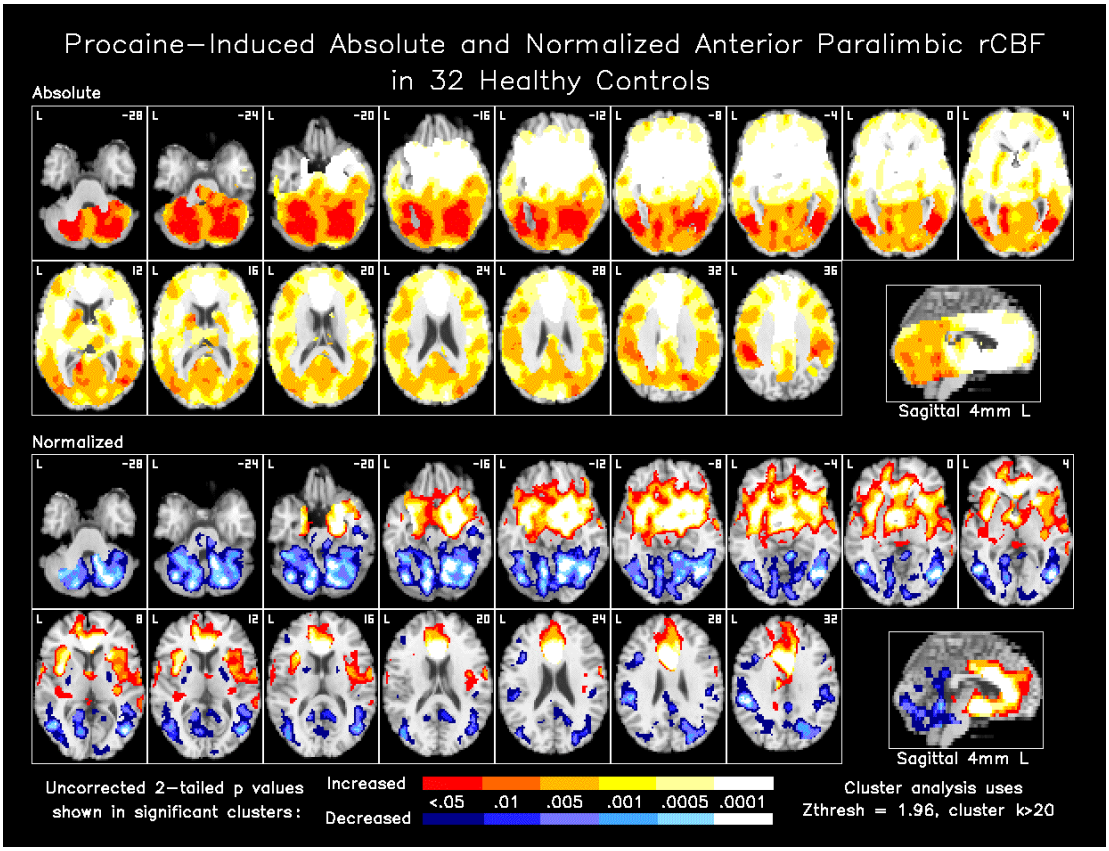

Figure 21. Procaine Increases Anterior Paralimbic rCBF in 32 Healthy Controls Procaine significantly increases anterior paralimbic rCBF compared to baseline in both absolute and normalized statistical comparisons. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: L=left; Zthresh=Z threshold; k=number of voxels; numbers indicate Talairach Z plane. Note: the sagittal views illustrate the limits of the data collection range, i.e., the extreme dorsal and ventral areas of the brain were not in the imaging space.

3.3.2.2 Patients with Bipolar Disorder

In these moderately depressed (mean HDRS = 16.9) patients with bipolar disorder, gCBF significantly increased by 27.4% (BL: 43.4 mL/min/100g; PR: 55.2 mL/min/100g; $t = 2.91$, $p = .01$, $df=14$) with procaine compared to baseline (Figure 22). These increases were not significantly different from healthy controls (Group: $F = 0.74$, p= ns, $df = 1, 45$; Drug: $F = 23.71$, $p < .0001$, $df = 1, 45$; DXG: $F = 0.62$, p= ns, $df = 1, 45$). As seen in healthy controls, only significant increases in absolute rCBF with procaine compared to baseline occurred in the patients (Figure 23; Table 7). However, the significance level was diminished, most likely due to the smaller sample size. Absolute CBF was significantly increased across the entire imaging space, with maxima centering in the anterior cingulate, amygdala and basal forebrain cholinergic area.

In the normalized rCBF analysis, procaine yielded anterior paralimbic increases, and posterior cortical and cerebellar decreases compared to baseline. This pattern was similar to that observed in the healthy controls, however, the spatial extent of the changes was somewhat diminished. Regions that were significantly increased with procaine compared to baseline included bilateral anterior cingulate, amygdala, the basal forebrain cholinergic area, anterior insula, and right medial orbitofrontal cortex. Significant decreases in rCBF with procaine compared to baseline occurred in bilateral cerebellum, inferior parietal, and right DLPFC.

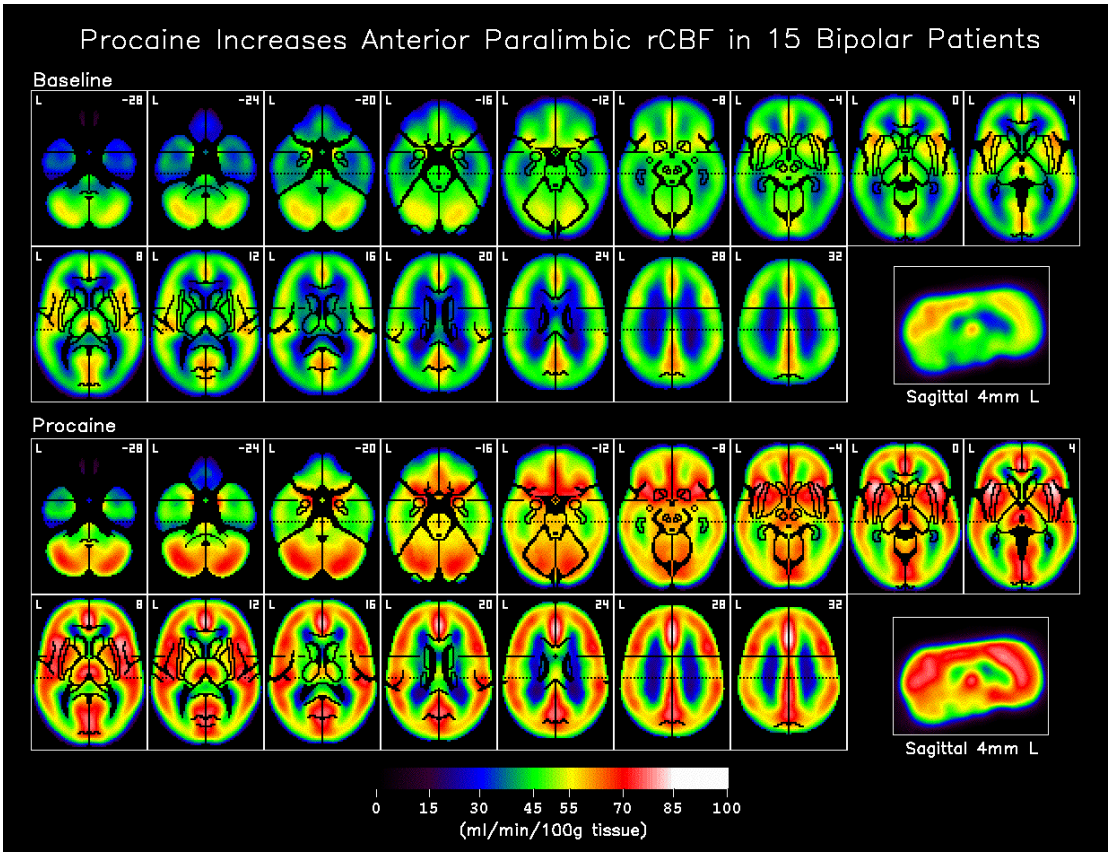

Figure 22. Effects of Procaine on rCBF in 15 Patients with Bipolar Disorder
Mean regional cerebral blood flow (rCBF) at baseline (top) and with procaine (bottom) in 15 patients with bipolar disorder. Procaine increases rCBF throughout the brain with the most robust increase (indicated in red) in anterior paralimbic regions, such as the anterior cingulate, amygdala, insula, medial orbitofrontal and prefrontal cortex, and ventral and dorsal striatum. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: numbers indicate Talairach Z plane; L=left. Note: the sagittal views illustrate the limits of the data collection range, i.e., the extreme dorsal and ventral areas of the brain were not in the imaging space.

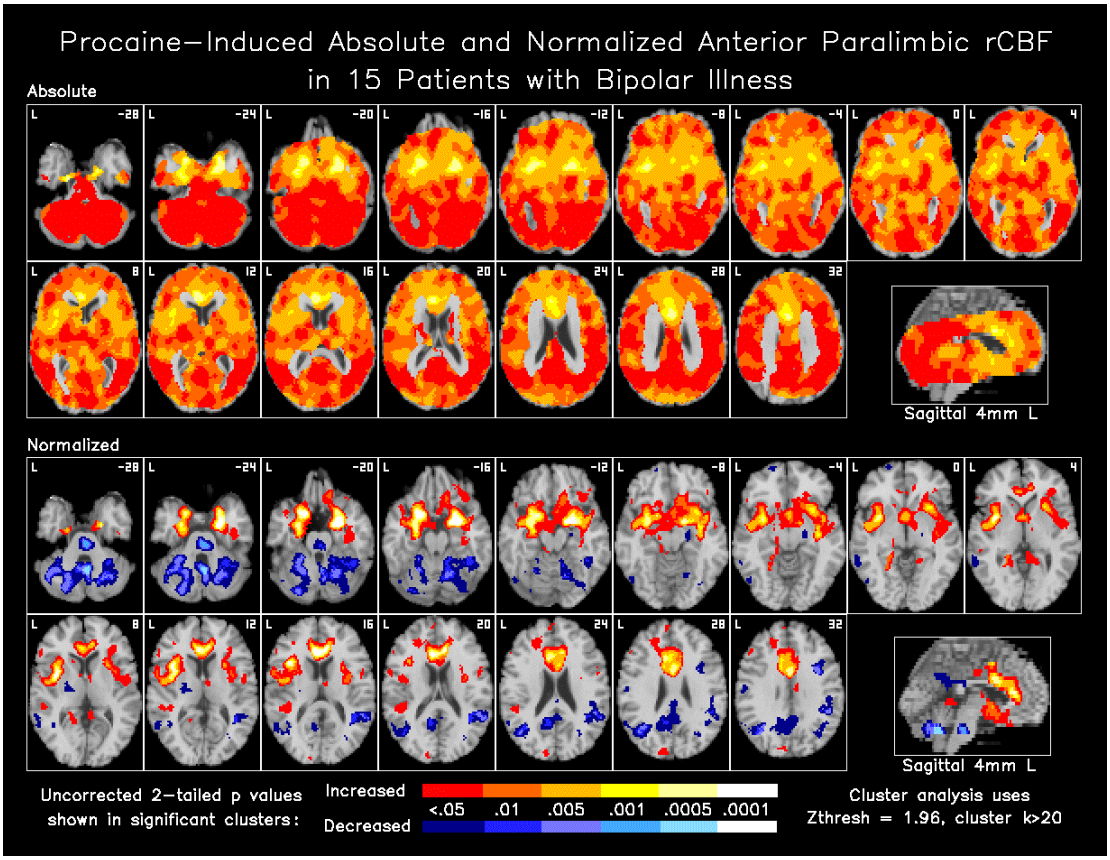

Figure 23. Procaine Increases Anterior Paralimbic rCBF in 15 Patients with Bipolar Disorder

Procaine significantly increases anterior paralimbic rCBF compared to baseline in both absolute and normalized statistical comparisons. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: L=left; Zthresh=Z threshold; k=number of voxels; numbers indicate Talairach Z plane. Note: the sagittal views illustrate the limits of the data collection range, i.e., the extreme dorsal and ventral areas of the brain were not in the imaging space.

3.2.3.3 Patient versus Controls Comparisons

There were no significant differences between the patients and controls at baseline in gCBF (HC: 42.2 mL/min/100g; BPD: 43.4 mL/min/100g; t = 1.40, p = ns, df = 45) or in the absolute analysis (not shown). At baseline, in the normalized analysis compared to controls, rCBF in the patients was higher in the right peri-amygdalar region, and bilateral MOFC and MPFC, posterior temporal cortex and cerebellum (Figure 24; Table 7). The DLPFC and VLPFC, and right subgenual and left pregenual anterior cingulate were significantly decreased in the patient compared to the controls. The subgenual cluster extended into the basal forebrain substantia inominata area.

With procaine, gCBF (HC: 50.8 mL/min/100g; BPD: 55.2 mL/min/100g; t = 1.40, p = ns, df = 45) and absolute rCBF were not significantly different between patients and controls (not shown). In the normalized analysis, many posterior areas elevated at baseline in patients versus controls remained after procaine, such as in bilateral posterior temporal, occipital, parietal cortex, and cerebellum. Patients showed significant normalized rCBF decreases (less rCBF increases) than controls in bilateral DLPFC (BA 10), the left supragenual anterior cingulate cortex, right putamen/insula, right anterior temporal, and left medial temporal cortices, as well as left inferior parietal cortex (Figure 24; Table 7).

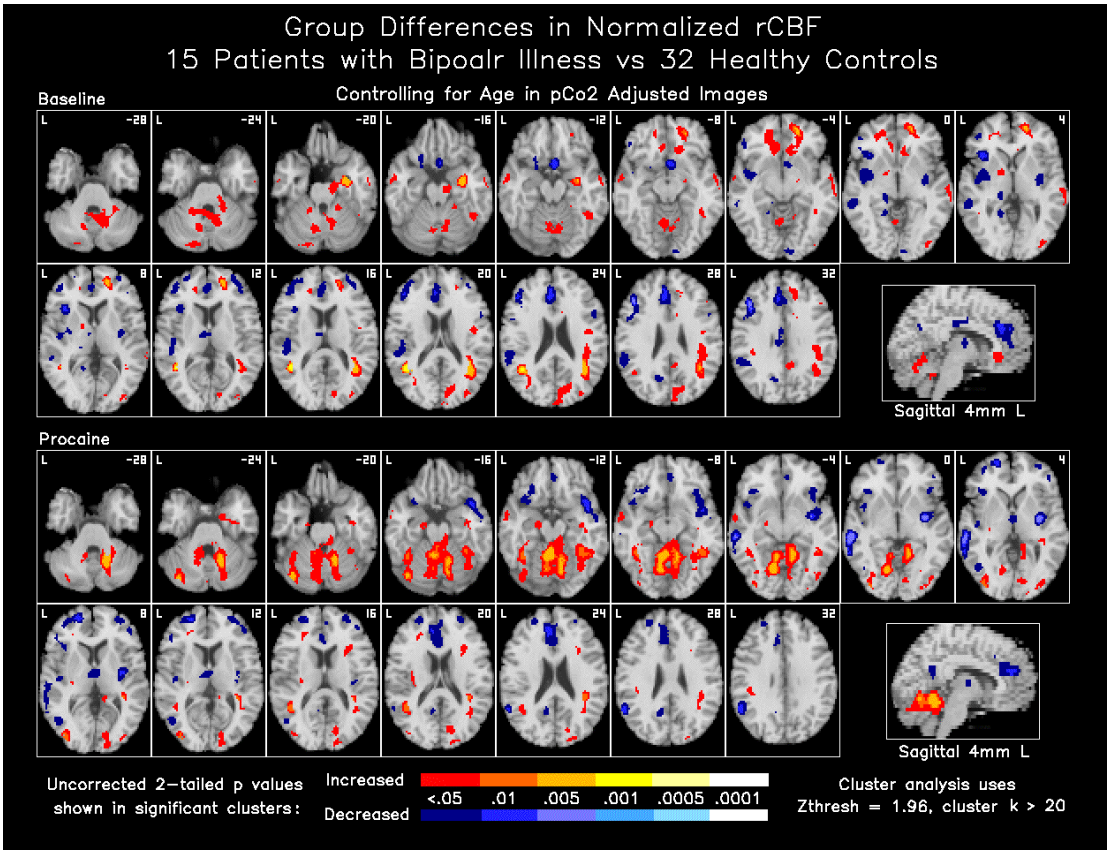

Figure 24. Group Differences in Normalized rCBF at Baseline and with Procaine Patients with Bipolar Disorder exhibit hypoperfusion in the DLPFC and pregenual anterior cingulate (PGAC), and hyperperfusion in the subgenual anterior cingulate (SGAC) compared to healthy controls at baseline. The baseline abnormalities in the DLPFC and PGAC persist while the SGAC activity normalizes. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: L=left; Zthresh=Z threshold; k=number of voxels; numbers indicate Talairach Z plane.

Regions showing a significant group x drug interaction were distributed throughout the brain (Figure 25; Table 7), but revealed a general pattern of attenuated anterior paralimbic and enhanced posterior-cerebellar CBF responses to procaine in patients compared to controls. Specifically, the right subgenual and pregenual anterior cingulate, right medial and anterior temporal, and left inferior parietal cortices were significantly less activated by procaine in the patients than in the controls. In contrast, regions significantly more activated by procaine in the patients compared to controls included the left fusiform / posterior parahippocampal region, and, occipital, and cerebellum. However, this pattern was not completely uniform; the anterior regions bilateral DLPFC (BA 46) and left pregenual anterior cingulate were also significantly more activated by procaine in the patients compared to controls.

3.3.3 Mood and Change in CBF Correlations in Patients

Hamilton depressions ratings (HDRS) significantly correlated positively with the change blood flow (procaine-baseline) in the right amygdala and left substantia inominata, and bilateral medial orbitofrontal, hippocampus, posterior temporal, occipital, parietal and cerebellar cortices in patients (Figure 26). Also, significant negative correlations were observed in bilateral subgenual and pregenual anterior cingulate, VLPFC and MPFC, and right DLPFC.

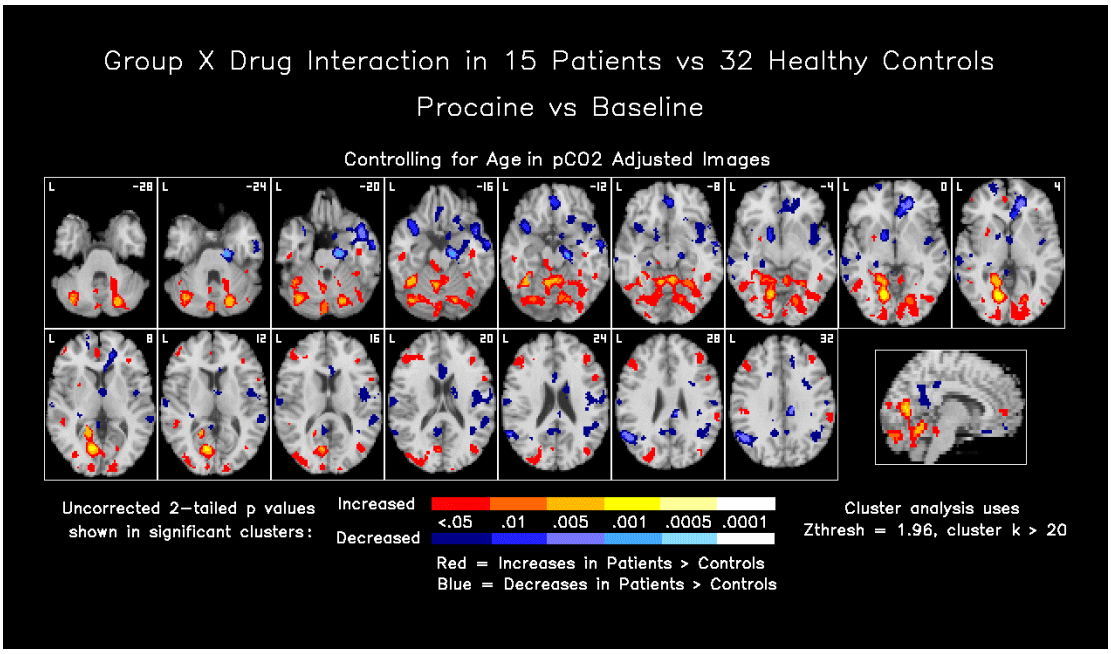

Figure 25. Group / Drug Interaction: 15 Patients vs 32 Controls, Procaine vs Baseline
The DLPFC and cerebellum activated significantly more in the patients than in controls, while the pregenual anterior cingulate activated significantly less so. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: L=left; Zthresh=Z threshold; k=number of voxels; numbers indicate Talairach Z plane.

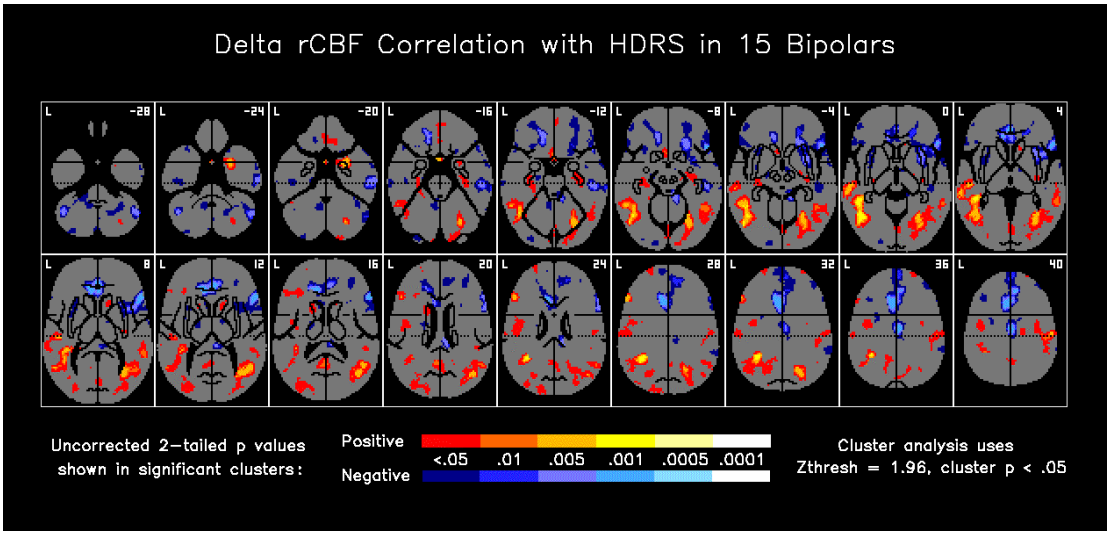

Figure 26. Mood Correlation with Change in rCBF in 15 Patients
Worsening of mood was associated with procaine-induced increased right amygdala, left ventral striatum, MOFC, left DLPFC, and posterior rCBF, and decreased in anterior cingulate, VLPFC and MPFC rCBF.

3.4 Discussion

As in healthy controls (Ketter et al., 1996), moderately depressed treatment-refractory patients with bipolar disorder showed regional CBF increases in the amygdala, anterior cingulate, basal forebrain, ventral striatum, and insula with procaine compared to baseline. However, this anterior paralimbic activation was less robust compared to controls. Moreover, regions previously implicated in the pathophysiology of bipolar illness, including DLPFC and subgenual anterior cingulate, exhibited a differential CBF response in patients and controls with procaine compared to baseline.

Despite similar robust global cerebral blood flow responses to procaine in patients and healthy controls, differences in normalized rCBF exhibited many of the expected differences at baseline and with procaine. At baseline compared to controls, patients with bipolar disorder exhibited hypoperfusion in the DLPFC and anterior cingulate, while orbitofrontal, temporal lobe and cerebellar regions showed hyper-perfusion. Of note, the basal forebrain region was hypo-perfusional in patients compared to controls at baseline. The DLPFC and anterior cingulate hypoperfusion and posterior hyperperfusion persisted during the procaine condition, suggesting abnormal functional activity of the anterior paralimbic regions in these patients across different states of emotional arousal (at baseline and during procaine-induced emotional experiences). These alterations in these regions may be trait markers for bipolar disorder. That is, they occur across mood states as well as across emotional states. However, reversal of hypo-frontality, but not cerebellar and striatal hyper-activity, is associated with euthymia (Ketter et al., 2001). The lack of significant

changes in the depression ratings from baseline to procaine conditions suggests the level of moderately depressed mood in the patients at the time of the study remained relatively unchanged and is supported by depression ratings collected during the study. This may help explain the persistent hypo-frontality at baseline and with procaine in this study. In addition, the procaine challenge yields primarily affective, rather than cognitive activation, consistent with less expected effects on DLPFC activity.

Some areas of baseline abnormalities in the patients were corrected (normalized) by procaine, such as the increase in MOFC activity, while accompanied by an equivalent emotional response as seen in controls. This contrasts with the response of the DLPFC, and implies the function of this region may be dependent on some facet of the current emotional state. Modulation of the MOFC, a cortical region with heavy direct cholinergic innervation, could reflect procaine's muscarinic cholinergic actions.

Further analysis revealed DLPFC and several other regions exhibited differential normalized CBF responses in the patients compared to the healthy controls with procaine-induced changes. Some parts of the DLPFC (mostly BA 9/10; X=-40, Y=55, Z=12) remained hypo-perfusional in both conditions, while BA 46 (X= -45, Y=32, Z=20) within the DLPFC exhibited blood flow increases that were significantly greater in the patients compared to controls. A second area of interaction occurred in the subgenual and pregenual anterior cingulate region; this area activated significantly less in the patients than in the controls. DLPFC and the anterior cingulate, which have been implicated in the pathophysiology of bipolar

disorder (see reviews, Ketter et al., 1997: Drevets, 1998; Dougherty and Rauch, 1997), thus exhibited differential blood flow responses to procaine in patients compared to controls, although these groups experienced generally similar levels of affective arousal. These findings extend the current understanding of the (dys)function of the DLPFC and anterior cingulate in mood disorders by suggesting that a functional adjustment, hyper- or hypo-activity, respectively, assisted in the attainment of an emotional experience similar to controls.

According to Davidson (2002), both the anterior cingulate and the DLPFC may function in the resolution of conflicts between the internal state and external information, and often these regions act reciprocally (Benson et al., 2000). In this context, a study paradigm in which strong emotional and sensory experiences are occurring in a laboratory environment may raise considerable emotional/cognitive conflicts and re-appraisals, and thus, the expectation would be increased activity in both regions. Relative to controls, hypo-activation in DLPFC converted to hyper-activation and the pregenual AC activation showed further hypo-activation during a chemically driven emotional experience. These differences under procaine activation may represent functional adjustments in patients with bipolar illness during conflict resolution, and further reveal dysregulation of these substrates in bipolar disorder compared to controls. Moreover, the lack of activation of the subgenual AC could reflect the neural and glial abnormalities reported in this region in those with bipolar illness (Öngur et al., 1998, Drevets et al., 1997; Rajkowska et al., 1999; Rajkowska, 2000).

Another region of group/drug interaction occurred in the cerebellum, where elevated rCBF observed at baseline remained higher in the patients compared to the controls during procaine. This differential baseline and functional response to procaine in patients compared to controls with concomitant emotional responses further implicates the cerebellum in mood dysregulation, although causal relationships remain to be determined. Although, historically the cerebellum has been associated with motor activity and movement disorders, recently Schmahmann (1998) has described the cerebellar-mood dysregulation. Several investigators have shown increased activity in the cerebellum in bipolar (Ketter et al., 2001) and unipolar (Kimbrell et al., 2002) disorder.

The cholinergic forebrain regional CBF was significantly reduced at baseline in the patients compared to the controls, and both groups showed increased blood flow in this area with procaine without a significant drug X group interaction. These results suggests that despite the region's reduced blood flow at baseline in the patients compared to controls, the region seems to activate similarly with procaine, and supports a cholinergic mechanism of procaine's actions.

Arecoline, a nonselective muscarinic agonist, has also induced dysphoria in mood disorder patients whether or not they were currently depressed; this was greater in individuals with a family history of depression compared to those without such history (Gillin et al. 1991; Nurnberger et al. 1989). Procaine's ability to induce dysphoria and sensory responses could be more directly related to its cholinergic properties, while euphoria may occur through cholinergic interactions with other neuromodulatory systems, such as dopamine (Graybiel et al., 2000). Other

mechanisms cannot be excluded as procaine binds with sigma and serotonin receptors with similar affinities.

This study has several limitations. As noted above, the sample in this study was relatively small group of patients with bipolar illness, primarily BPII subtype experiencing rapid-cycling, and considered treatment resistant. At the time of the study most of the patients were not severely depressed, manic or euthymic, but mostly mildly depressed. However, despite the small cross-section of bipolar illness in this sample, the regional abnormalities observed at baseline were similar to that reported in the literature (see reviews, Dougherty and Rauch, 1997; Ketter et al., 1997; Drevets, 1998). In any case, examination of the functional response to procaine in patients with bipolar illness during different phases (euthymic, severe depression, or mania) of the illness could provide a more complete picture of limbic and cholinergic dysregulation in bipolar disorder.

No attempt was made to correlate emotional response with the functional response to procaine. These were explored in the next chapter, where the emotional and sensory ratings were correlated with many ROIs. However, the relationship of baseline mood ratings of depression (HDRS) to change in CBF was evaluated. In this analysis, change (procaine – baseline) in anterior cingulate and parts of DLPFC CBF negatively correlated with HDRS scores indicating the more depressed the subjects showed the least change in CBF in these anterior paralimbic regions. These results parallel the notion that patients with bipolar illness showed a blunted CBF response to procaine in anterior paralimbic areas. Additionally, change in blood flow in a small cluster in the cholinergic forebrain area and amygdala positively correlated with

HDRS, suggesting that depressed mood was associated with increased activity in these regions that have significant cholinergic innervation.

3.5 Conclusion

In conclusion, patients with bipolar disorder exhibit blunted anterior paralimbic CBF response to procaine compared to healthy controls. In addition, differential activation in the DLPFC, subgenual and pregenual anterior cingulate, and cerebellum occurred in conjunction with a generally similar range of affective responses to procaine in the groups. These regions have repeatedly been reported as abnormal in this disorder. Procaine-induced blood flow increases in regions believed to have significant cholinergic innervations (amygdala, MOFC and ventral striatum) were associated with worsening of mood. This is consistent with the work of Janowsky and colleagues (1972). Further studies with other affective challenges (pharmacological or self-induced) and in patients with bipolar disorder with more diverse symptomatology may provide a clearer picture of the dysfunction experienced in bipolar illness.

Chapter 4: Alterations in Functional Connectivity With Procaine Administration in Humans

4.1 Introduction

Procaine has been used as an affective challenge in healthy controls (Ketter et al., 1996; Servan-Schreiber et al., 1998), and patients with mood disorders (Ketter et al., 1993) and panic disorder (George et al., 1993), as well as individuals abusing alcohol (George et al., 1990) and cocaine (Adinoff et al., 2001). Procaine, when administered intravenously (i.v.) in healthy controls results in brief intense emotional and sensory experiences in association with increased global and regional anterior paralimbic cerebral blood flow (CBF) as measured with [^{15}O] water PET (Ketter et al. 1996); the amygdala, insula and anterior cingulate are particularly activated. In healthy individuals, 1.84 mg/kg procaine induces the following: an array of brief intense emotional responses ranging from dysphoria (fear and anxiety) to euphoria; visual and auditory sensory illusions/hallucinations (Kellner et al. 1987); hormonal changes such as increased ACTH, cortisol, and prolactin secretion (Kling et al. 1994); and increased temporal lobe fast activity EEG (Parekh et al. 1995).

In patients with bipolar disorder compared to healthy controls, the procaine-induced anterior paralimbic activation was blunted (see previous chapter). Moreover, the severity of depression ratings correlates inversely with the procaine-induced change in blood flow the anterior cingulate and DLPFC and directly in ventral limbic structures, such as the amygdala and basal forebrain cholinergic regions.

Furthermore, a differential CBF response of the DLPFC and subgenual and pregenual anterior cingulate during procaine compared to baseline.

The mechanism of procaine's limbic activation is unknown. Although classically associated with the blockade of voltage-gated sodium channels, procaine has recently been shown to have *in vivo* action on muscarinic M_2 receptors in the anesthetized rhesus monkey utilizing a radiotracer with preferential binding to these receptors (Benson et al., 2004). In that study, procaine blocked the M_2 ligand (TZTP) in a dose-related manner with an *in vivo* IC_{50} (1.31 μM) that mirrored estimates determined from in vitro studies (Sharkey et al., 1988; Hisayama et al. 1989). Furthermore, peak increases in the flow of the muscarinic radiotracer were near the estimated IC_{50}. These results suggest that the binding of procaine to muscarinic receptors may contribute to the emotional and sensory effects observed in humans if comparable receptor occupancy occurs (Ketter et al., 1996).

Indirect and direct cholinergic agonists, such as physostigmine and arecoline, can produce dysphoria in manic patients and normal controls (Janowsky, 1972b). The patients have concomitant hormonal disturbances with this emotional arousal (Janowsky et al. 1986). These findings have led Janowsky to hypothesize that the cholinergic system is favored over noradrenergic activity during the depressive phase of bipolar illness. Arecoline, a nonselective muscarinic agonist, has also induced dysphoria in mood disorder patients whether or not they were currently depressed; this was greater in individuals with a family history of depression compared to those without such history (Gillin et al. 1991; Nurnberger et al. 1989). These effects are

similar to components of the response induced by procaine administration in patients and healthy controls (Ketter et al., 1996; Ketter et al., 1993).

Several other theories relating the cholinergic system have been suggested more recently. Overstreet and colleagues (1998) have suggested a cholinergic-serotonergic interactions in mood disorders in which they argue that a cholinergic overactivity predisposes one to depression and serotonergic mechanisms induce depressive episodes. Another theory relating the cholinergic system to emotional processing lies in the work of LeDoux (1996). In his model of the general emotion system, amygdala triggered arousal systems interface with basal forebrain cholinergic activity to mediate fear responses; the basal forebrain cholinergic system activates prefrontal cortex when stimulated by the amygdala during emotionally significant events. In addition, this activity can be sustained to maintain attention on emotional stimuli. Damage to NBM interrupts this process.

In summary, cholinergic modulation could contribute significantly to procaine-induced emotional and sensory experiences. This is suggested by procaine's ability to: 1) induce emotions and endocrine changes; 2) most robustly activate anterior paralimbic regions in a pattern similar to the most dense muscarinic cholinergic innervation; and 3) *in vivo* action on M_2 muscarinic cholinergic receptors. The purpose of this study was to explore any potential contribution of cholinergic brain regions to procaine-induced emotional and sensory experiences in patients with bipolar disorder and healthy controls. Several methods were employed to assess these potential relationships.

In this study, functional connectivity and multivariate techniques were used to examine a cholinergic model using procaine challenge data in patients with mood disorders and healthy controls. Functional connectivity, defined as the correlative strengths between two ROIs, offers an alternative perspective to activation studies (t-tests) by providing additional information regarding the relationships between ROIs rather than differences between groups or conditions. rCBF activation with procaine was examined in patients with bipolar disorder compared to healthy controls in a companion paper for comparing and contrasting purposes.

Multivariate multiple regression tested a cholinergic model derived from what is known about the neuroanatomy of the basal forebrain cholinergic system. In this model, the heaviest cholinergic regions, cell bodies and targets (Mesulam, 1995; Amaral et al., 1992), are depicted in Figure 9. The main hypothesis tested in this experiment is that the correlation strengths between regions with cholinergic input would be greater than non-cholinergic pathways, and the former would be enhanced following procaine administration. Following the hypothesis of Janowsky, these correlational strengths will be greater in the patients than in the healthy controls. Additionally, anxiety experiences will be related to enhanced cholinergic drive in the network according the work of LeDoux (1996). Although the study was hypothesis-driven, due to the methods employed here and the retrospective nature of the study this work is viewed as exploratory.

4.2 Methods

4.2.1 Subjects

The subjects, procedure, behavioral measures, PET acquisition and preliminary image processing methods were explained in detail in the previous chapter. Briefly, 32 healthy controls and 15 otherwise medically healthy patients with bipolar disorder underwent PET studies with and without procaine administration after informed consent was obtained. The subjects were informed that procaine had various transient emotional and sensory effects, ranging from minimal to intense and from pleasant to unpleasant, and they were told they could stop the procedure at any time, and that diazepam was available at all times. Diagnosis of Bipolar Disorder was confirmed with Schedule for Affective Disorders and Schizophrenia – Lifetime Anxiety Version (Mannuzza et al., 1986) by trained physician. The majority of the fifteen patients with bipolar disorder were type BPII (n=14) experiencing rapid cycling (n=11; 3 were undetermined due to lack of life chart information).

4.2.2 Procaine Procedure and Behavioral Measures

Regional cerebral blood flow (rCBF) was measured using $H_2{}^{15}O$ PET during intravenous saline and procaine administration in a single-blind design, where they were instructed to expect saline, low-dose or high-dose procaine. Subjects were injected with saline (sham scan), saline (baseline scan), low-dose procaine (0.46 mg/kg), high-dose procaine (1.84 mg/kg) and saline (recovery of baseline scan) in the same order.

Baseline behavioral measures of mood, including clinician and self-ratings, were collected before the scanning session began (Table 5). Clinician ratings included Hamilton Depression Rating scale (Hamilton, 1960; [HDRS]), Young Mania Rating Scale (Young et al., 1978), and Spielberger State Anxiety Scale (Spielberger et al., 1970; [SSAS]), and self-ratings included Beck Depression Inventory (Beck, et al., 1961; [BDI]). Likert-based ratings of emotional and sensory experiences were collected at baseline and immediately after each scan. Demographic, clinician and self-ratings data were analyzed using paired (within group drug comparisons) and unpaired t-tests (between-group comparisons) or repeated measures ANOVA with between and within group factors, as deemed appropriate.

4.2.3 PET Acquisition and Data Analysis

The procedure and preliminary data analysis was described previously in the previous chapter. Statistical inference testing on absolute and globally normalized rCBF data (Friston et al., 1990) was performed with a two-fold purpose, thus two methodological approaches were used: 1) functional connectivity studies examined the relationships of the cholinergic ROIs to themselves and a number of other ROIs located across the brain; and 2) multivariate multiple regression explored the relationship of procaine-induced changes in emotional responses to rCBF. Each of these methods is detailed below.

Functional connectivity was assessed using regional correlation matrices generated in Statview (SAS Institute, Cary, NC) and SPSS (SPSS Inc., Evanston, IL). Correlations presented herein are restricted to those between the four bilateral

cholinergic ROIs (hindbrain and forebrain) and the remaining 26 bilateral ROIs and three hindbrain ROIs as depicted in Figure 27. This resulted in 481 distinct relationships.

Small regions (mean number of pixels = 172.2 ± 136.7; range = 71-609; values exclude cerebellar and temporal ROIs) were defined on the magnetic resonance imaging (MRI) supplied with SPM99 by one of two tracing methods or a center of mass approach, depending on the region. With tracing methods, the area chosen for the ROI was considered to be the most central portion of the region. Based on the MRI, the basal forebrain cholinergic area was divided into two ROIs representing the substantia inominata (SI) and the septal area (SEP). A third forebrain ROI was defined as the conglomerate of the basal forebrain cholinergic region (BF), which included the SI and SEP regions, and some extended amygdala, but not the amygdala proper. Due to the indistinct boundaries of the basal forebrain regions and limits of the resolution of the PET scanning technique, the substantia inominata and the septal region were drawn in an inclusive manner, yielding regions that encompassed what was thought to be the heaviest cholinergic innervation area. The brainstem ROI representing the cholinergic cell bodies in the tegmentum (TEG) was also defined on the MRI supplied by SPM 99.

For some areas of cortex, a center of mass was chosen based on coordinates reported in the literature where significant findings occurred. This method was applied to: 1) the region anterior to the genu with Talairach coordinates X = ±6, Y = 42, and Z=10 corresponds the region described by Mayberg and colleagues (1997) that has differential responsiveness with effective antidepressant treatment; 2) a

region near the subgenual anterior cingulate reported to be hypo-metabolic (coordinates X = ±6, Y = 38, and Z=-6; Drevets, et al., 1997) in depressed mood disorder patients; and 3) the dorsolateral prefrontal cortex (DLPFC; coordinates X = ±40, Y = 22, and Z=22) and anterior insula (coordinates X = ±36, Y = 14, and Z=4) where patients with Bipolar Disorder exhibit hypo-metabolism or hyper-metabolism, respectively, at baseline compared to controls (Ketter et al., 1999).

Other cortical regions were defined by placing a spherical mass in the center of the region guided by the atlas of Talairach and Tourneaux (1988); this method was used for ventrolateral prefrontal (VLPFC), lateral (LOFC) and medial (MOFC) orbitofrontal, inferior parietal, anterior and posterior temporal cortices, and the temporal pole. The anterior cingulate was subdivided into subgenual, pregenual, supragenual and dorsal regions (Benson et al., 2000).

Nuclei regions were manually traced from the MRI supplied by SPM 99, such as the amygdala, hippocampus, caudate, putamen, globus pallidus, medial nucleus of the dorsal thalamus, midbrain, pons, and medulla. The cerebellar folia and nuclei were defined based on the atlas of Schmahmann et al. (2000). The temporal and cerebellar ROIs were the only regions for which there was no rationale for a small targeted region, thus the entire region was included in the ROI.

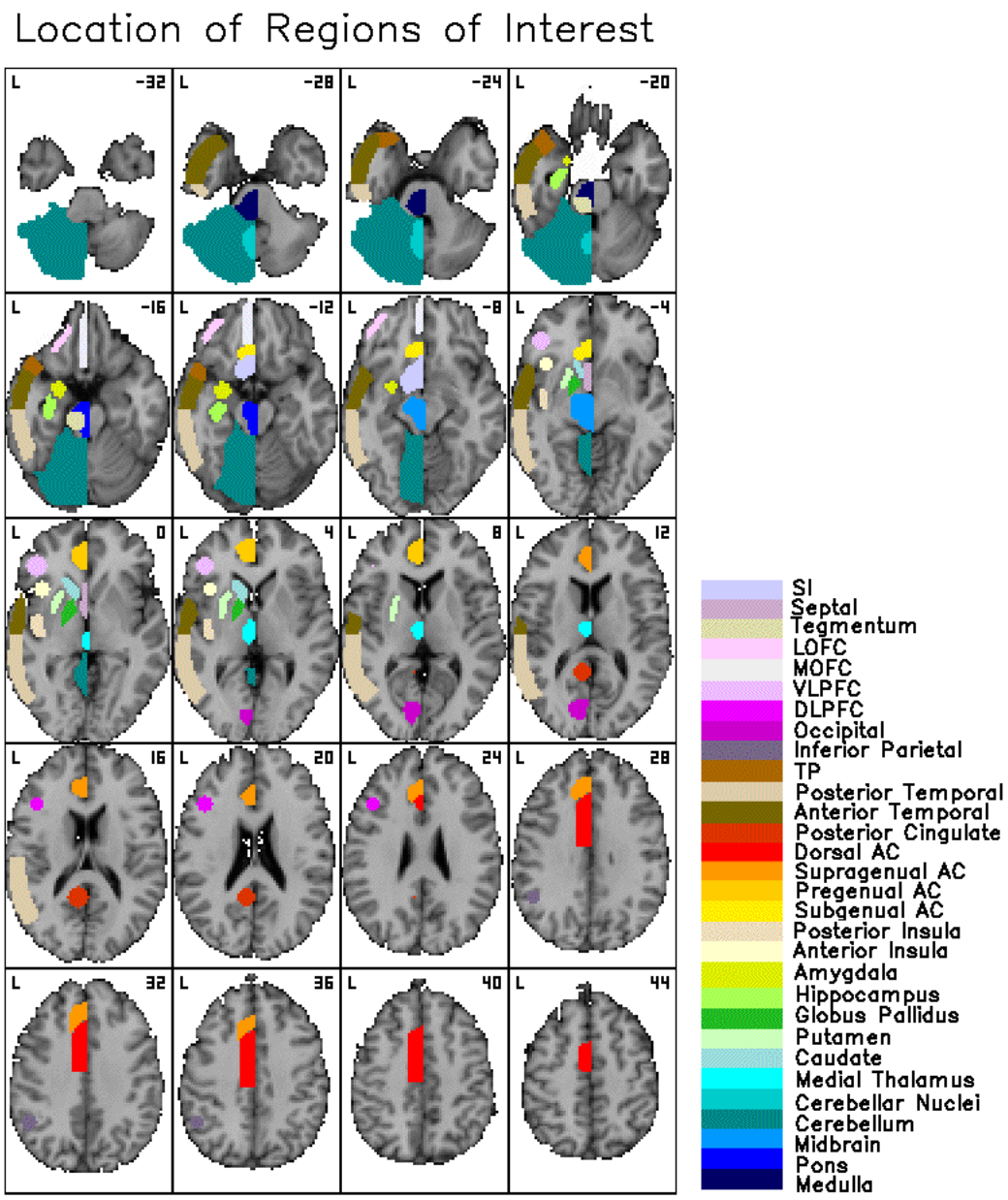

Figure 27. Location of Regions of Interest (ROIs)
Representation of ROI placements on MRI supplied by SPM. Transverse sections through the human brain displayed from left to right moving from ventral to dorsal aspect. Abbreviations: L=left; numbers indicate Talairach Z plane.

Significant differences between groups or drug conditions in functional connectivity were determined by two methods, due to the smaller sample size of the patient group, thus the potential for Type II error. The first method used either statistical comparison across groups by Fisher's r-to-z transformed r values or the methods of Steiger (1980) for comparison across conditions that incorporates within-subject dependence of the conditions. The alpha level for significant correlations and differences between drugs or groups was set at α=0.05. With Steiger's method, the comparisons between conditions in the patient group (n=15) showed few significant differences. This is in sharp contrast to the control group (n=32), where numerous significant differences were found. Given the null hypothesis of no differences between the groups ($r_{controls} = r_{patients}$), the chances of the numerous differences between the groups was suspect and indicated that the likelihood of a Type-II error in the patient group comparisons was high. Tables summarizing the first method can be found in Appendices A and B.

The second method attempted to establish significance less dependent on sample size, while remaining rigorous. This method used the cut-off of r>0.43 or r<-0.43 (critical r for n=15) to determine whether correlations were significantly different from zero in either group or condition. To compare across groups or drugs, the root mean square difference (Δr) was calculated in each case,

$$\Delta r = \sqrt{r_{bd}^2 - r_{hc}^2}, \text{ or}$$

$$\Delta r = \sqrt{r_p^2 - r_c^2},$$

while keeping the sign of correlations intact. The cut-off for significance in the comparison was set r>0.50 or r<-0.50. This second method showed that indeed, in the

healthy control group, the significant differences between conditions were for the most part the same as the first method. However, in the patient group there were many differences between the drug conditions not detected by the first method, and more similar to that observed in the controls. Thus, the results presented in this study refer to this second method.

Due to the retrospective design, the number of regions examined and at times the segmentation of regions close to the resolution of the PET scanning technique, this study is viewed in an exploratory analysis of potential relationships between the cholinergic regions and the remainder of the brain. Owing to the significant age difference between the two groups, regional correlations with and without partialling out age were compared, and none were found not to be significantly different using the methods as outlined below for comparing correlations. No significant differences in the regional correlations were observed when baseline mood, as measured by HDRS, was partialled out. No attempt was made to partial out HDRS in the controls, as controlling for a covariate with no variance is not statistically valid. Thus, the results reported here are correlations without age or HDRS partialled out.

No attempt to correct for multiple comparisons was made with either method in that the aim of the study was to examine the regional patterns of significant changes rather than the depending on any one correlational difference as meaningful. Specific hypotheses in this regard were limited to confirmation in each group of three known neuroanatomical pathways between the cholinergic ROIs and the rest of the brain, i.e., 1) the substantia inominata projection pattern to the amygdala, subgenual and pregenual anterior cingulate, anterior insula, and MOFC; 2) septo-hippocampal

pathway; and 3) tegemental – brainstem pathway. More generally, alterations in the correlative relationships of the cholinergic ROIs with brain regions shown to be hyper- or hypo-active in patients compared to controls would extend and support potential differences in activation studies.

4.2.4 Relationships Between Emotional Responses and rCBF

Two approaches were employed to delineate relationships between procaine-induced emotional and sensory changes and rCBF. First, a focused assessment of the correlations between the change in rCBF in the cholinergic ROIs and the change in emotional and sensory ratings (delta-delta correlations) directly evaluated the cholinergic relationships to emotion and sensory regulation. This was followed by multivariate multiple regression analyses examining the ability of delta CBF cholinergic network (AChNet), as described by Figure 9, to predict the change in emotional and sensory responses, which is detailed below.

Multivariate multiple regression analysis was used to evaluate the degree of associativity between emotional experiences and rCBF activation. Delta ratings were predicted from delta-rCBF according to the cholinergic model in Figure 9 (AChNet). The six ROIs (SI, amygdala, subgenual AC, pregenual AC, anterior insula, and MOFC) with the heaviest cholinergic innervation were chosen to predict positive (euphoria, calmness) and negative (anxiety, fear) emotional responses. Due to sample size limitations, separate analyses were performed for each group and laterality (right and left). A reduced model, which explained the most variance without loosing more than 20% of the total variance, was the result of an elimination process whereby

variables were removed from the full model one by one (similar to stepwise regression, although the automated software function was not used). First, the one emotion with the least variance explained was eliminated; this was followed by elimination of a ROI based on the lowest partial correlation. In most cases, the model was reduced to two to three regions that explained a majority of the variance in the complete model $\Delta R^2 > 0.20$. Subsequently, substitutions in the model were made in an effort to improve the fit of the model. Either singly or in combinations, exchanges included BF for SI, Drevets ROI for subgenual AC, Mayberg ROI for pregenual AC, and DLPFC for MOFC. A significant model was considered to be $R^2 > 0.25$, and significant improvement after substitutions in a model was $\Delta R^2 > 0.20$. Significant differences in models between groups was set at $\Delta R^2 > 0.10$.

4.3 Results

4.3.1 Emotional and Sensory Response to Procaine

Emotional and sensory responses to procaine were reported in the previous chapter, and will only be briefly described. Baseline ratings of depression, fear, anxiety, anger, calmness and overall bad/good feeling were significantly different between the groups. Ratings of euphoria, anxiety, calmness, tiredness, and sensory ratings for visual and auditory changes were essentially identical in the procaine condition. There was a significant group X drug interaction in depression ratings, due to greater depression ratings of the patients at baseline, which became nonsignificant with procaine. Also, Patients rated fear significantly greater in both conditions.

Ratings of anger and depression were generally not normally distributed (mostly zero scores), with the exception of depression ratings by the patients at baseline.

4.3.2 Functional Connectivity Studies

The correlational matrices for each condition (saline or procaine) in the healthy controls and patients are displayed in Tables 8 and 9, respectively. The regions in these tables are listed in the general order from rostral to caudal orientation across the brain, with the cholinergic ROIs heading the table. This method of organization revealed clusters of significant positive and negative correlations across the ROIs, often indicating strong correlative relationships between adjacent or anatomically related regions with the cholinergic ROIs. An example would be the cluster of positive correlations between the forebrain septal area ROI with dorsal thalamus and striatum. The right-hand panel illustrates where there was a significant change in the correlation coefficients across conditions, with red indicating significantly more positive correlation and blue indicating significantly less positive correlation. The color-coding along the right edge of the tables corresponds to three cholinergic pathways, tegemental-midbrain, substantia inominata-forebrain, and the septo-hippocampal pathways.

4.3.2.1 Healthy Controls at Baseline

All of the forebrain cholinergic ROIs were significantly positively inter-correlated in healthy controls at baseline (Tabele 8). However, the tegemental ROI did not always correlate significantly with the forebrain cholinergic ROIs. The

brainstem, including the medulla, pons, midbrain, and cerebellum, ROIs were all significantly positively correlated with bilateral tegmental ROI, but not all the cholinergic forebrain ROIs. Of the cholinergic forebrain ROIs, the septal area (and thus the BF mean ROIs) was positively correlated with the midbrain and hindbrain, while the SI ROIs were not.

Many of the forebrain nuclei, such as the amygdala, hippocampus, striatum and thalamus, exhibited positive correlations with predominately the forebrain, but few of the tegmental cholinergic ROIs. Again, the majority of the forebrain significant correlations were with the septal area ROI, as seen in the hindbrain and midbrain.

Cortical regions displayed both positive and negative correlations with the cholinergic ROIs, although few of the negative correlations were significant. Almost all of the subdivisions of the anterior cingulate ROIs were significantly positively correlated with the SI ROI (mostly on the right), but relatively few were significantly correlated with the septal ROI. The subgenual subdivision correlated with both SI and SEP ROIs.

The temporal pole significantly positively correlated with the forebrain cholinergic ROIs. In contrast, the posterior temporal cortex generally was not correlated with the forebrain cholinergic ROIs, but in a few cases was significantly negatively correlated. None of the temporal cortex ROIs were significantly correlated with the tegemental ROI. In general, the insula was not significantly correlated with the cholinergic ROIs, either forebrain or tegmental; the exceptions were the right

Table 8. Correlative Relationships Between Cholinergic Brain Regions and ROIs Across the Brain in 32 Healthy Controls

	Saline									Procaine									Procaine vs Saline								
	Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean		
ROI	L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF	
L Tegementum		0.51	0.31	0.27	0.56	0.57	0.52	0.50			0.67	-0.34	-0.23	-0.21	-0.18	-0.21	-0.16			0	0	0	↓	↓	↓	↓	
R Tegmentum	0.51		0.05	0.03	0.44	0.37	0.31	0.30		0.67		-0.28	-0.04	-0.04	0.02	-0.12	-0.01		0		0	0	0	0	0	0	
L SI	0.31	0.05		0.61	0.64	0.59	0.81	0.60		-0.34	-0.28		0.80	0.87	0.71	0.95	0.79		0	0		↑	↑	0	0	↑	
R SI	0.27	0.03	0.61		0.43	0.67	0.57	0.76		-0.23	-0.04	0.80		0.74	0.81	0.81	0.94		0	0	↑		↑	0	↑	↑	
L Septal n	0.56	0.44	0.64	0.43		0.85	0.88	0.81		-0.21	-0.04	0.87	0.74		0.86	0.91	0.81		↓	0	↑	↑		0	0	0	
R Septal n	0.57	0.37	0.59	0.67	0.85		0.77	0.91		-0.18	0.02	0.71	0.81	0.86		0.76	0.90		↓	0	0	0	0		0	0	
L BF	0.52	0.31	0.81	0.57	0.88	0.77		0.83		-0.21	-0.12	0.95	0.81	0.91	0.76		0.84		↓	0	0	↑	0	0		0	
R BF	0.50	0.30	0.60	0.76	0.81	0.91	0.83			-0.16	-0.01	0.79	0.94	0.81	0.90	0.84			↓	0	↑	↑	0	0	0		
Medulla	0.56	0.67	0.39	0.34	0.64	0.61	0.59	0.56		0.78	0.57	-0.15	-0.10	-0.02	-0.13	0.00	-0.02		↑	0	0	0	↓	↓	↓	↓	
Pons	0.75	0.77	0.29	0.01	0.63	0.53	0.53	0.42		0.67	0.85	0.00	0.18	0.21	0.24	0.15	0.24		0	0	0	0	↓	0	↓	0	
Midbrain	0.80	0.49	0.41	0.20	0.72	0.62	0.73	0.62		0.31	0.40	0.47	0.50	0.53	0.56	0.61	0.59		↓	0	0	0	0	0	0	0	
L Cerebellum	0.58	0.63	0.18	0.03	0.45	0.33	0.30	0.30		0.77	0.61	-0.40	-0.28	-0.38	-0.41	-0.29	-0.28		↑	0	0	0	↓	↓	0	0	
R Cerebellum	0.61	0.69	0.23	0.00	0.43	0.30	0.31	0.25		0.72	0.56	-0.32	-0.24	-0.33	-0.40	-0.22	-0.23		0	0	0	0	↓	↓	0	0	
L Cerebellum n	0.54	0.59	0.29	0.14	0.46	0.32	0.42	0.36		0.75	0.52	-0.12	-0.02	-0.17	-0.22	-0.03	-0.03		↑	0	0	0	0	0	0	0	
R Cerebellum n	0.49	0.62	0.22	-0.01	0.40	0.23	0.34	0.27		0.62	0.40	0.25	0.22	0.19	0.05	0.35	0.23		0	0	0	0	0	0	0	0	
L m Thalamus	0.52	0.44	0.46	0.28	0.69	0.49	0.74	0.59		0.07	0.08	0.58	0.59	0.61	0.60	0.67	0.65		↓	0	0	↑	0	0	0	0	
R m Thalamus	0.42	0.40	0.29	0.24	0.66	0.56	0.62	0.63		0.12	0.09	0.50	0.58	0.51	0.60	0.53	0.66		0	0	0	↑	0	0	0	0	
L Caudate	0.46	0.03	0.60	0.49	0.60	0.56	0.81	0.67		-0.22	-0.11	0.77	0.75	0.75	0.72	0.78	0.77		↓	0	0	↑	0	0	0	0	
R Caudate	0.32	0.19	0.32	0.57	0.52	0.57	0.61	0.72		0.01	0.06	0.54	0.70	0.63	0.70	0.60	0.74		0	0	0	0	0	0	0	0	
L Putamen	0.06	-0.17	0.31	0.57	0.26	0.37	0.38	0.50		-0.20	-0.18	0.85	0.77	0.75	0.67	0.82	0.77		0	0	↑	↑	↑	↑	↑	↑	
R Putamen	0.04	-0.03	0.45	0.59	0.17	0.28	0.39	0.50		-0.19	-0.13	0.75	0.83	0.66	0.65	0.73	0.83		0	0	↑	↑	↑	↑	↑	↑	
L Globus Pallidus	0.27	0.21	0.18	0.08	0.47	0.38	0.49	0.44		-0.46	-0.42	0.67	0.48	0.58	0.43	0.65	0.47		↓	↓	↑	0	0	0	0	0	
R Globus Pallidus	0.15	0.21	0.37	0.36	0.59	0.50	0.58	0.62		-0.25	-0.20	0.64	0.66	0.62	0.65	0.65	0.74		0	0	↑	↑	0	0	0	0	
L Hippocampus	0.36	0.39	0.38	0.45	0.57	0.52	0.57	0.55		0.24	0.24	0.02	0.05	0.07	-0.09	0.14	0.07		0	0	0	0	↓	↓	↓	↓	
R Hippocampus	0.41	0.34	0.28	0.39	0.53	0.66	0.52	0.62		0.50	0.49	-0.41	-0.34	-0.24	-0.23	-0.30	-0.24		0	0	↓	↓	↓	↓	↓	↓	
L Amygdala	0.54	0.37	0.45	0.30	0.69	0.57	0.67	0.60		-0.05	-0.16	0.50	0.34	0.53	0.41	0.55	0.46		↓	0	0	0	0	0	0	0	
R Amygdala	0.34	0.19	0.28	0.14	0.55	0.47	0.49	0.54		0.00	0.18	0.35	0.48	0.46	0.54	0.41	0.62		0	0	0	0	0	0	0	0	
L Ant Insula	-0.35	-0.46	0.26	0.08	-0.35	-0.32	-0.15	-0.25		-0.32	-0.28	0.72	0.73	0.63	0.62	0.66	0.67		0	0	↑	↑	↑	↑	↑	↑	
R Ant Insula	-0.04	-0.17	0.24	0.46	0.06	0.17	0.12	0.24		-0.25	-0.14	0.60	0.74	0.49	0.63	0.50	0.71		0	0	↑	↑	0	↑	0	↑	
L Post Insula	0.03	-0.07	0.25	0.22	0.07	0.09	0.20	0.24		-0.16	-0.17	0.64	0.55	0.57	0.47	0.60	0.56		0	0	↑	↑	↑	0	↑	↑	
R Post Insula	0.07	-0.09	0.04	-0.05	0.01	-0.06	0.19	0.08		-0.33	-0.20	0.69	0.76	0.62	0.68	0.67	0.78		0	0	↑	↑	↑	↑	↑	↑	
L Subgenual AC	0.03	-0.12	0.66	0.56	0.36	0.30	0.52	0.37		-0.06	-0.07	0.64	0.54	0.58	0.49	0.69	0.51		0	0	0	0	0	0	0	0	
R Subgenual AC	0.20	0.06	0.48	0.66	0.54	0.62	0.50	0.67		0.09	0.14	0.35	0.45	0.43	0.42	0.43	0.45		0	0	0	0	0	0	0	↓	
L Drevet	-0.03	-0.31	0.42	0.51	0.16	0.28	0.19	0.26		-0.31	-0.26	0.69	0.48	0.49	0.30	0.63	0.40		0	0	↑	0	0	0	↑	0	
R Drevet	0.00	-0.18	0.36	0.61	0.19	0.36	0.19	0.40		-0.03	-0.07	0.53	0.61	0.40	0.42	0.45	0.52		0	0	0	0	0	0	0	0	
L Pregenual AC	-0.07	-0.37	0.42	0.68	0.23	0.40	0.30	0.43		-0.36	-0.28	0.66	0.74	0.60	0.63	0.58	0.73		0	0	↑	0	↑	0	0	↑	
R Pregenual AC	-0.12	-0.41	0.25	0.66	0.03	0.25	0.09	0.33		-0.22	-0.31	0.67	0.64	0.53	0.55	0.57	0.59		0	0	↑	0	↑	0	↑	0	
L Mayberg	-0.01	-0.45	0.48	0.68	0.19	0.36	0.31	0.40		-0.28	-0.20	0.61	0.72	0.57	0.64	0.55	0.74		0	0	0	0	↑	↑	0	↑	
R Mayberg	-0.11	-0.45	0.22	0.62	-0.02	0.19	0.07	0.27		-0.24	-0.30	0.66	0.66	0.53	0.58	0.56	0.62		0	0	↑	0	↑	↑	↑	↑	
L Supragenual AC	-0.04	-0.48	0.55	0.62	0.23	0.34	0.31	0.40		-0.45	-0.53	0.71	0.62	0.55	0.54	0.60	0.61		↓	0	0	0	↑	0	↑	0	
R Supragenual AC	0.02	-0.43	0.45	0.69	0.12	0.33	0.27	0.43		-0.46	-0.46	0.76	0.68	0.61	0.60	0.67	0.61		↓	0	↑	0	↑	↑	↑	0	
L Dorsal AC	-0.02	-0.38	0.26	0.65	0.15	0.27	0.33	0.44		-0.23	-0.31	0.63	0.69	0.58	0.68	0.62	0.72		0	0	0	↑	↑	↑	↑	↑	
R Dorsal AC	-0.05	-0.41	0.27	0.56	0.04	0.13	0.26	0.33		-0.22	-0.36	0.62	0.73	0.55	0.69	0.61	0.69		0	0	↑	0	↑	↑	↑	↑	
L Post AC	-0.24	-0.10	0.36	0.19	0.00	0.06	0.20	0.16		0.04	-0.11	0.25	0.25	0.16	0.22	0.32	0.37		0	0	0	0	0	0	0	0	
R Post AC	-0.13	-0.30	0.46	0.33	0.11	0.23	0.26	0.31		-0.02	-0.18	0.16	0.19	0.20	0.33	0.25	0.33		0	0	0	0	0	0	0	0	
L Ant Tenporal	0.00	-0.05	0.30	0.20	0.35	0.40	0.24	0.22		0.02	0.17	-0.14	-0.22	-0.06	-0.14	-0.11	-0.21		0	0	0	0	0	0	0	0	
R Ant Temporal	-0.03	0.02	0.22	0.40	0.07	0.37	0.03	0.28		0.23	0.16	-0.10	-0.02	-0.08	-0.10	-0.07	0.02		0	0	0	0	0	0	0	0	
L Post Temporal	0.16	0.01	0.03	-0.40	0.04	-0.07	-0.01	-0.18		0.15	0.23	-0.08	-0.26	-0.13	-0.32	-0.10	-0.29		0	0	0	0	0	0	0	0	
R Post Temporal	-0.10	0.06	-0.47	-0.47	-0.31	-0.23	-0.43	-0.29		0.15	0.33	-0.31	-0.28	-0.28	-0.27	-0.31	-0.27		0	0	0	0	0	0	0	0	
L TP	0.09	-0.01	0.69	0.62	0.54	0.55	0.63	0.57		0.18	0.12	0.12	0.21	0.15	0.07	0.23	0.21		0	0	↓	↓	↓	↓	↓	↓	
R TP	0.04	0.13	0.36	0.47	0.35	0.50	0.33	0.46		0.23	0.28	-0.02	0.17	-0.01	0.01	0.04	0.20		0	0	0	↓	0	0	0	0	
L Inf Parietal	-0.04	-0.10	0.20	0.19	0.06	0.05	0.13	-0.08		-0.20	-0.23	0.49	0.34	0.34	0.24	0.40	0.21		0	0	0	0	0	0	0	0	
R Inf Parietal	-0.18	-0.06	-0.21	0.05	-0.17	0.00	-0.17	-0.10		-0.26	-0.08	-0.01	0.01	0.02	0.07	-0.05	-0.04		0	0	0	0	0	0	0	0	
L Occipital	-0.12	0.17	0.07	-0.21	-0.12	-0.23	-0.06	-0.25		0.22	0.15	-0.04	-0.13	0.05	0.02	-0.07	-0.08		0	0	0	0	0	0	0	0	
R Occipital	-0.24	-0.29	0.07	-0.30	-0.32	-0.38	-0.16	-0.32		-0.01	-0.14	0.07	-0.04	0.04	0.07	0.00	0.03		0	0	0	0	0	0	0	0	
L DLPFC	-0.25	-0.49	0.17	0.00	-0.06	-0.02	-0.02	-0.06		-0.30	-0.32	0.34	0.18	0.21	0.14	0.16	0.14		0	0	0	0	0	0	0	0	
R DLPFC	-0.35	-0.57	-0.11	-0.16	-0.39	-0.32	-0.33	-0.31		-0.49	-0.38	0.34	0.42	0.22	0.34	0.18	0.34		0	0	0	0	0	0	0	0	
L VLPFC	0.12	-0.27	0.45	0.47	0.13	0.24	0.31	0.32		-0.34	-0.35	0.61	0.37	0.41	0.24	0.51	0.31		0	0	0	0	0	0	0	0	
R VLPFC	0.23	0.01	0.29	0.52	0.32	0.57	0.28	0.56		-0.13	-0.07	0.40	0.44	0.33	0.32	0.37	0.41		0	0	0	0	0	0	0	0	
L MOFC	0.08	-0.18	0.43	0.45	0.16	0.25	0.21	0.24		0.11	0.14	0.28	0.32	0.25	0.16	0.28	0.27		0	0	0	0	0	0	0	0	
R MOFC	0.15	-0.03	0.40	0.60	0.25	0.41	0.26	0.44		0.22	0.19	0.20	0.36	0.14	0.14	0.22	0.28		0	0	0	0	0	0	0	0	
L LOFC	0.40	0.29	0.22	0.35	0.50	0.46	0.46	0.60		0.31	0.25	0.10	0.24	0.01	0.00	0.17	0.21		0	0	0	0	↓	0	0	↓	
R LOFC	0.18	0.22	-0.12	0.18	0.30	0.30	0.18	0.40	Totals	0.13	0.17	-0.13	0.08	-0.17	-0.09	-0.07	0.05	Totals	0	0	0	0	0	0	0	0	
# r > .45	15	8	17	26	22	23	23	24	158	8	7	32	30	30	27	31	30	195	0	0	0	0	0	0	0	0	
# r < -.45	0	4	1	1	0	0	0	0	6	4	2	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	
Total	15	12	18	27	22	23	23	24	164	12	9	32	30	30	27	31	30	201	0	0	0	0	0	0	0	0	
																			Legend						Cholinergic System		
# Positive corrs	40	33	58	55	54	54	54	53	401	26	27	47	49	49	50	47	50	345	r			Δr			Tegmental-Midbrain		
# Negative corrs	22	29	4	7	8	8	8	9	95	36	35	15	13	13	12	15	12	151	>.50		↑	>.50			Substantia Inominata		
Total	62	62	62	62	62	62	62	62	496	62	62	62	62	62	62	62	62	496	<-.50		↓	<-.50			Septo-Hippocampal		

anterior insula was positively correlated with right SI ROI, and the left anterior insula correlated negatively with the left septal and tegmental ROIs.

Significant correlations, of either valence, were generally not observed between prefrontal cortex regions with the cholinergic ROIs. Bilateral VLPFC cortex was generally positively correlated with the forebrain cholinergic ROIs, whereas bilateral DLPFC was negatively correlated with the midbrain tegmental ROI.

Regions lacking significant relationships with any of the cholinergic ROIs were the bilateral posterior insular, lateral orbitofrontal cortex, inferior parietal and occipital cortices, and right anterior and left posterior temporal cortex.

4.3.2.2 Healthy Controls with Procaine

Three major organizational themes emerged in the procaine condition. First, a striking pattern of clustering of positive correlations in the forebrain ROIs was observed with themselves, the anterior cingulate, the insula, and the striatum. This was primarily a result of the addition of the anterior cingulate and the anterior and posterior insula, which were significantly more positive with procaine than at baseline. There were few correlations apart from these clusters. Regions with significantly more positive correlations in the procaine condition than at baseline included the anterior cingulate, anterior and posterior insula, and the putamen.

Second, some correlations were no longer significant resulting in significantly less positive correlations. The correlations with the temporal pole and the hippocampus were either weak and/or negative with procaine, resulting in a significant reduction in correlational strength with the forebrain cholinergic ROIs.

The only significant change in correlative relationships of cholinergic ROIs with the amygdala was a loss of a significant positive correlation between the bilateral tegmental and the left amygdala ROIs.

Third, more often than not, the forebrain and tegmental ROIs exhibited a differential pattern of correlations or significant changes in correlations with procaine compared to baseline with the other ROIs, depending on whether the cholinergic source was brainstem or forebrain. For example, within the cholinergic ROIs, the correlations between forebrain ROIs, whether between contralateral pairs or amongst forebrain ROIs, remained or became significantly more positive with procaine compared to baseline. In contrast, a significant reduction in correlational strength between the left tegmental and cholinergic forebrain ROIs from the baseline condition were observed (Table 8; middle and right sections).

This differential pattern was also observed in the hindbrain, where the medulla, pons and cerebellum lacked significant correlations with the forebrain ROIs while the tegmental ROIs were significantly positively correlated; the pattern in the midbrain was reversed, where the forebrain ROI correlations were significantly positive and the tegmental ROIs were not significant. Significant changes in the correlations with procaine from baseline occurred in the opposite manner also. The correlations of the forebrain ROIs, specifically the septal area ROI, became significantly more negative with the medulla, caudal pons, and cerebellum, while the correlations of the tegmental ROIs were significantly more positive in the medulla, and left cerebellum folia and nuclei; again, the exception is the midbrain. This

differential pattern of significant changes with procaine from baseline also occurred with the caudate, globus pallidus, and supragenual AC.

4.3.2.3 Patients at Baseline

In general, the patients displayed the same overall pattern of significant correlations between the cholinergic and other ROIs as compared to the healthy controls, however, with some significant variations. In some cases, weaker correlations were observed leaving "holes" in the significance pattern (Table 9; left panel). The positive correlations amongst the cholinergic ROIs were for the most part significant, as well as the correlations between the contralateral pairs, with the exception of the SI pair. These positive correlations were in some cases, significantly less positive in the patients compared to the controls (Table 9; left panel). In contrast, the contralateral tegmental correlation was significantly more positive in the patient compared to the controls.

Although some of the correlational patterns appeared somewhat different from the controls, upon assessment of significant differences, often there were few (Table 10). This occurred in correlations between the cholinergic and the brainstem, the dorsal thalamus and striatum ROIs.

Table 9. Correlative Relationships Between Cholinergic Brain Regions and ROIs Across the Brain in 15 Patients with Bipolar Disorder

	Saline									Procaine									Procaine vs Saline								
	Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean		
ROI	L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF	
L Tegementum		0.69	0.36	0.16	0.28	0.55	0.35	0.28			0.62	0.05	-0.11	0.28	0.44	0.27	0.10			0	0	0	0	0	0	0	
R Tegmentum	0.69		0.19	0.12	0.27	0.54	0.17	0.33		0.62		-0.18	-0.13	0.06	0.15	0.03	0.16		0		0	0	0	↓	0	0	
L SI	0.36	0.19		0.31	0.89	0.46	0.82	0.36		0.05	-0.18		0.75	0.80	0.73	0.82	0.75		0	0		↑	0	↑	0	↑	
R SI	0.16	0.12	0.31		0.19	0.57	0.54	0.95		-0.11	-0.13	0.75		0.76	0.64	0.83	0.93		0	0	↑		↑	0	↑	0	
L Septal n	0.28	0.27	0.89	0.19		0.52	0.82	0.33		0.28	0.06	0.80	0.76		0.87	0.96	0.80		0	0	0	↑		↑	↑	↑	
R Septal n	0.55	0.54	0.46	0.57	0.52		0.50	0.73		0.44	0.15	0.73	0.64	0.87		0.83	0.77		0	↓	↑	0	↑		↑	0	
L BF	0.35	0.17	0.82	0.54	0.82	0.50		0.59		0.27	0.03	0.82	0.83	0.96	0.83		0.85		0	0	0	↑	↑	↑		↑	
R BF	0.28	0.33	0.36	0.95	0.33	0.73	0.59			0.10	0.16	0.75	0.93	0.80	0.77	0.85			0	0	↑	0	↑	0	↑		
Medulla	0.34	0.75	0.02	0.14	0.22	0.37	0.23	0.30		0.27	0.68	-0.34	-0.13	-0.06	0.13	-0.14	0.10		0	0	0	0	0	0	0	0	
Pons	0.80	0.85	0.49	0.20	0.58	0.73	0.50	0.40		0.88	0.64	0.34	0.07	0.40	0.55	0.41	0.30		0	↓	0	0	0	0	0	0	
Midbrain	0.60	0.70	0.54	0.23	0.65	0.69	0.62	0.44		0.59	0.23	0.79	0.48	0.72	0.84	0.73	0.63		0	↓	↑	0	0	0	0	0	
L Cerebellum	0.61	0.79	0.15	0.33	0.27	0.57	0.27	0.48		0.68	0.55	-0.21	-0.30	-0.13	0.03	-0.12	-0.12		0	↓	0	0	0	↓	0	0	
R Cerebellum	0.67	0.78	0.24	0.14	0.35	0.72	0.23	0.36		0.57	0.61	-0.18	-0.26	-0.15	0.12	-0.17	0.01		0	↓	0	0	0	↓	0	0	
L Cerebellum n	0.66	0.48	0.51	0.41	0.36	0.70	0.41	0.53		0.31	0.36	0.24	0.12	0.24	0.33	0.25	0.33		↓	0	0	0	0	↓	0	0	
R Cerebellum n	0.43	0.68	0.38	0.36	0.32	0.66	0.25	0.51		0.05	0.35	0.27	0.26	0.16	0.23	0.16	0.46		0	↓	0	0	0	↓	0	0	
L m Thalamus	0.39	0.50	0.59	0.38	0.66	0.53	0.72	0.48		0.36	0.25	0.64	0.42	0.63	0.80	0.66	0.63		0	0	0	0	0	↑	0	0	
R m Thalamus	0.66	0.51	0.64	0.35	0.57	0.69	0.63	0.45		0.36	0.31	0.81	0.63	0.80	0.85	0.80	0.79		↓	0	↑	↑	↑	0	0	↑	
L Caudate	0.19	-0.14	0.56	0.24	0.61	0.18	0.75	0.27		0.23	-0.25	0.58	0.49	0.66	0.43	0.74	0.42		0	0	0	0	0	0	0	0	
R Caudate	0.43	0.01	-0.04	0.50	-0.14	0.29	0.28	0.44		0.35	-0.31	0.51	0.34	0.57	0.53	0.62	0.29		0	0	↑	0	↑	0	↑	0	
L Putamen	0.50	0.21	0.60	0.44	0.49	0.43	0.69	0.49		0.41	0.13	0.77	0.42	0.67	0.77	0.66	0.58		0	0	0	0	↑	0	0	0	
R Putamen	0.02	-0.06	0.10	0.79	-0.11	0.35	0.12	0.72		0.16	-0.04	0.66	0.60	0.55	0.73	0.60	0.71		0	0	↑	↓	↑	↑	↑	0	
L Globus Pallidus	0.38	0.27	0.16	0.51	0.19	0.40	0.43	0.56		0.29	-0.03	0.56	0.37	0.61	0.44	0.66	0.42		0	0	↑	0	↑	0	↑	0	
R Globus Pallidus	0.54	0.46	-0.14	0.33	-0.10	0.42	0.07	0.43		0.04	-0.08	0.62	0.65	0.73	0.69	0.69	0.71		↓	0	↑	↑	↑	↑	↑	↑	
L Hippocampus	-0.15	0.08	-0.01	0.34	0.13	0.22	0.25	0.40		0.34	0.34	0.44	0.26	0.35	0.15	0.33	0.29		0	0	0	0	0	0	0	0	
R Hippocampus	-0.03	-0.46	0.33	0.10	0.18	0.06	0.28	0.07		-0.13	0.10	0.18	0.00	-0.20	-0.02	-0.22	0.06		0	0	0	0	0	0	0	0	
L Amygdala	0.36	0.53	0.46	0.56	0.41	0.75	0.40	0.67		0.38	0.58	0.55	0.55	0.50	0.56	0.53	0.73		0	0	0	0	0	↓	0	0	
R Amygdala	-0.01	0.20	0.68	-0.09	0.76	0.28	0.41	0.06		0.01	0.26	0.55	0.40	0.30	0.40	0.28	0.54		0	0	0	↓	0	0	↑		
L Ant Insula	0.21	0.53	0.23	-0.19	0.43	0.19	0.19	-0.01		0.37	0.31	-0.24	-0.42	-0.14	0.01	-0.27	-0.27		0	0	0	0	0	0	0	0	
R Ant Insula	0.17	0.30	0.22	-0.32	0.22	-0.25	0.06	-0.26		0.29	0.22	-0.14	-0.46	-0.10	-0.07	-0.20	-0.31		0	0	0	0	0	0	0	0	
L Post Insula	0.30	0.24	0.65	0.18	0.51	0.43	0.39	0.28		0.25	0.31	0.40	0.28	0.25	0.37	0.23	0.45		0	0	↓	0	0	0	0	0	
R Post Insula	0.09	0.08	0.64	0.12	0.47	0.24	0.29	0.16		0.17	0.05	0.70	0.43	0.46	0.57	0.43	0.52		0	0	0	0	0	↑	0	↑	
L Subgenual AC	0.14	-0.10	0.11	0.17	0.09	-0.01	0.31	0.07		0.36	-0.12	0.01	0.21	0.37	0.46	0.38	0.18		0	0	0	0	0	0	0	0	
R Subgenual AC	0.12	-0.06	-0.16	0.78	-0.22	0.33	0.22	0.68		-0.09	-0.09	0.25	0.63	0.43	0.26	0.56	0.59		0	0	0	0	0	0	↑	0	
L Drevet	0.45	0.39	0.56	0.41	0.55	0.47	0.61	0.47		0.00	-0.13	0.82	0.75	0.70	0.78	0.71	0.80		0	0	↑	↑	0	↑	0	↑	
R Drevet	0.29	0.20	0.54	0.77	0.49	0.75	0.56	0.81		0.06	0.11	0.76	0.83	0.65	0.58	0.74	0.86		0	0	↑	0	0	0	0	0	
L Pregenual AC	0.50	0.14	0.39	0.68	0.23	0.47	0.55	0.68		-0.10	-0.16	0.79	0.90	0.72	0.71	0.75	0.90		↓	0	↑	↑	↑	↑	↑	↑	
R Pregenual AC	0.34	0.14	0.55	0.72	0.49	0.53	0.76	0.72		-0.04	0.06	0.74	0.76	0.53	0.60	0.65	0.84		0	0	0	0	0	0	0	0	
L Mayberg	0.48	0.03	0.56	0.58	0.42	0.49	0.69	0.58		-0.02	-0.15	0.80	0.91	0.82	0.79	0.84	0.91		0	0	↑	↑	↑	↑	0	↑	
R Mayberg	0.33	0.05	0.60	0.54	0.51	0.36	0.76	0.53		0.04	0.04	0.72	0.75	0.59	0.72	0.68	0.85		0	0	0	↑	0	↑	0	↑	
L Supragenual AC	0.14	-0.23	0.24	0.10	0.13	-0.03	0.42	0.06		0.06	-0.09	0.72	0.78	0.78	0.72	0.74	0.78		0	0	↑	↑	↑	↑	↑	↑	
R Supragenual AC	0.41	-0.06	0.51	0.15	0.35	0.30	0.45	0.17		0.27	-0.10	0.64	0.59	0.68	0.70	0.73	0.67		0	0	0	↑	↑	↑	↑	↑	
L Dorsal AC	0.18	-0.18	0.64	0.25	0.50	0.40	0.48	0.27		0.44	0.18	0.50	0.45	0.48	0.56	0.53	0.57		0	0	0	0	0	0	0	0	
R Dorsal AC	-0.05	-0.17	0.42	0.15	0.40	0.21	0.38	0.20		0.25	0.18	0.53	0.45	0.53	0.61	0.55	0.62		0	0	0	0	↑	0	↑		
L Post AC	0.18	0.24	0.11	0.54	0.03	0.27	0.15	0.54		-0.21	0.08	0.21	0.49	0.01	-0.03	0.14	0.47		0	0	0	0	0	0	0	0	
R Post AC	0.32	0.19	0.09	0.46	-0.04	0.24	0.18	0.40		0.02	0.10	0.06	0.37	0.16	0.07	0.30	0.36		0	0	0	0	0	0	0	0	
L Ant Temporal	-0.26	-0.10	-0.52	-0.41	-0.38	-0.34	-0.38	-0.37		0.09	-0.05	-0.70	-0.36	-0.43	-0.46	-0.42	-0.44		0	0	0	0	0	0	0	0	
R Ant Temporal	-0.18	0.09	-0.38	-0.16	-0.28	-0.20	-0.22	-0.14		0.11	0.17	-0.59	-0.40	-0.43	-0.39	-0.50	-0.39		0	0	0	0	0	0	0	0	
L Post Temporal	-0.52	-0.57	-0.59	-0.48	-0.42	-0.41	-0.54	-0.49		0.06	0.09	-0.88	-0.72	-0.68	-0.60	-0.72	-0.73		↑	↑	↓	↓	↓	0	0	↓	
R Post Temporal	-0.67	-0.32	-0.19	-0.21	0.05	-0.13	-0.30	-0.16		-0.30	-0.04	-0.64	-0.54	-0.69	-0.59	-0.73	-0.54		↑	0	↓	0	↓	↓	↓	↓	
L TP	0.22	0.55	0.26	-0.17	0.43	0.21	0.22	-0.04		0.33	0.42	0.12	0.20	0.22	0.35	0.17	0.34		0	0	0	0	0	0	0	0	
R TP	-0.08	0.36	0.18	-0.29	0.41	0.09	0.16	-0.17		0.33	0.50	0.08	0.17	0.20	0.30	0.15	0.32		0	0	0	0	0	0	0	0	
L Inf Parietal	0.00	-0.14	0.10	-0.16	0.03	-0.02	-0.01	-0.16		0.05	0.06	0.57	0.31	0.39	0.43	0.30	0.37		0	0	↑	0	0	0	0	0	
R Inf Parietal	0.11	-0.31	0.60	0.48	0.29	0.11	0.51	0.35		0.08	0.23	0.71	0.57	0.46	0.48	0.53	0.72		0	0	0	0	0	0	0	↑	
L Occipital	-0.09	0.26	-0.07	-0.44	-0.12	-0.25	-0.44	-0.36		-0.42	-0.29	-0.04	-0.21	-0.47	-0.49	-0.36	-0.25		0	0	0	0	0	0	0	0	
R Occipital	0.36	0.39	0.14	-0.44	0.18	0.02	-0.16	-0.33		0.02	-0.12	0.31	-0.10	-0.13	0.03	-0.04	-0.05		0	0	0	0	0	0	0	0	
L DLPFC	0.22	-0.11	0.28	-0.15	0.23	0.02	0.16	-0.18		-0.03	-0.34	0.55	0.56	0.60	0.53	0.61	0.46		0	0	0	↑	↑	↑	↑	0	
R DLPFC	-0.27	-0.22	0.11	-0.05	0.10	0.07	-0.11	-0.11		-0.14	-0.40	0.77	0.66	0.55	0.60	0.55	0.57		0	0	↑	↑	↑	↑	↑	↑	
L VLPFC	0.19	-0.33	-0.01	0.39	-0.12	0.15	0.27	0.30		-0.04	-0.46	0.48	0.57	0.64	0.54	0.66	0.44		0	0	0	0	↑	↑	↑	0	
R VLPFC	-0.19	-0.38	0.41	0.42	0.34	0.19	0.36	0.31		-0.05	-0.08	0.45	0.25	0.21	0.45	0.20	0.35		0	0	0	0	0	0	0	0	
L MOFC	0.12	0.36	0.09	0.09	0.26	0.22	0.19	0.16		-0.10	-0.24	0.57	0.68	0.64	0.66	0.55	0.61		0	0	↑	↑	↑	↑	↑	↑	
R MOFC	0.09	0.20	0.14	0.66	0.21	0.65	0.25	0.69		-0.18	0.10	0.50	0.85	0.55	0.46	0.58	0.81		0	0	0	↑	↑	0	↑	0	
L LOFC	-0.18	-0.21	-0.40	0.31	-0.38	-0.10	-0.17	0.20		-0.04	0.11	-0.37	0.04	-0.05	-0.09	-0.01	0.01		0	0	0	0	0	0	0	0	
R LOFC	-0.44	-0.58	-0.57	0.09	-0.60	-0.31	-0.44	-0.09	Total	0.06	0.18	-0.56	-0.17	-0.25	-0.28	-0.25	-0.19	Total	0	↑	0	0	↑	0	0	0	
# r > .45	12	15	22	17	17	22	20	20	145	5	7	35	27	32	33	32	32	203	0	0	0	0	0	0	0	0	
# r < -.45	2	3	3	1	1	0	1	1	12	0	1	5	3	3	4	3	2	21	0	0	0	0	0	0	0	0	
Total	14	18	25	18	18	22	21	21	157	5	8	40	30	35	37	35	34	224	0	0	0	0	0	0	0	0	
																			Legend					Cholinergic System			
# Positive corrs	48	42	50	48	50	51	52	48	389	45	39	49	48	48	52	48	52	381	r			Δr		Tegmental-Midbrain			
# Negative corrs	14	20	12	14	12	11	10	14	107	17	23	13	14	14	10	14	10	115	>.50		↑	>.50		Substantia Inominata			
Total	62	62	62	62	62	62	62	62	496	62	62	62	62	62	62	62	62	496	<-.50		↓	<-.50		Septo-Hippocampal			

Table 10. Correlative Relationships Between Cholinergic Brain Regions and ROIs Across the Brain in 15 Patients with Bipolar Illness Compared to 32 Healthy Controls

	Saline									Procaine									Procaine vs Saline								
	Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean	
ROI	L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF	
L Tegementum		0	0	0	0	0	0	0			0	0	0	0	0	0	0			↓	0	0	↑	↑	↑	0	
R Tegmentum	0		0	0	0	0	0	0		0		0	0	0	0	0	0		↓		0	0	0	0	0	0	
L SI	0	0		↓	↑	0	0	0		0	0		0	0	0	0	0		0	0		0	↓	0	0	0	
R SI	0	0	↓		0	0	0	↑		0	0	0		0	0	0	0		0	0	0		0	0	0	↓	
L Septal n	0	0	↑	0		↓	0	↓		0	0	0	0		0	0	0		↑	0	↓	0		↑	0	↑	
R Septal n	0	0	0	0	↓		↓	↓		0	0	0	0	0		0	0		↑	0	0	0	↑		↑	0	
L BF	0	0	0	0	0	↓		↓		0	0	0	0	0	0		0		↑	0	0	0	0	↑		↑	
R BF	0	0	0	↑	↓	↓	↓			0	0	0	0	0	0	0			0	0	0	↓	↑	0	↑		
Medulla	0	0	0	0	↓	0	↓	0		↓	0	0	0	0	0	0	0		↓	0	0	0	↑	↑	↑	0	
Pons	0	0	0	0	0	0	0	0		↑	↓	0	0	0	0	0	0		↑	↓	0	0	0	0	0	0	
Midbrain	↓	↑	0	0	0	0	0	0		↑	0	↑	0	0	↑	0	0		↑	↓	↑	0	↑	↑	↑	0	
L Cerebellum	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	↓	0	0	↑	0	0	0	
R Cerebellum	0	0	0	0	0	↑	0	0		0	0	0	0	0	0	0	0		↓	0	0	0	0	↓	0	0	
L Cerebellum n	0	0	0	0	0	↑	0	0		↓	0	0	0	0	0	0	0		↓	0	0	0	0	0	0	0	
R Cerebellum n	0	0	0	0	0	↑	0	0		↓	0	0	0	0	0	0	0		↓	0	0	0	0	↓	0	0	
L m Thalamus	0	0	0	0	0	0	0	0		0	0	0	0	0	↑	0	0		0	0	0	↓	0	0	0	0	
R m Thalamus	↑	0	↑	0	0	0	0	0		0	0	↑	0	↑	↑	↑	0		0	0	0	0	↑	0	↑	↑	
L Caudate	0	0	0	0	0	↓	0	↓		0	0	↓	↓	0	↓	0	↓		↑	0	0	0	0	0	0	0	
R Caudate	0	0	0	0	↓	0	↓	↓		0	0	0	↓	0	0	0	↓		0	0	0	↓	0	0	↑	0	
L Putamen	↑	0	↑	0	0	↑	0	↑		0	0	0	↓	0	0	0	↓		0	0	↓	↓	↓	0	↓	↓	
R Putamen	0	0	0	↑	0	0	0	↑		0	0	0	↓	0	0	0	0		0	0	0	↓	0	0	0	↓	
L Globus Pallidus	0	0	0	↑	0	0	0	0		↑	0	0	0	0	0	0	0		0	0	0	↓	0	0	0	0	
R Globus Pallidus	↑	0	0	0	↓	0	↓	0		0	0	0	0	0	0	0	0		0	0	0	0	↑	0	↑	0	
L Hippocampus	0	0	0	0	↓	0	↓	0		0	0	0	0	0	0	0	0		0	0	↑	0	↑	↑	↑	0	
R Hippocampus	0	↓	0	0	↓	↓	0	↓		↓	0	0	0	0	0	0	0		0	0	↑	↑	↑	↑	0	↑	
L Amygdala	0	0	0	0	↓	↑	↓	0		0	↑	0	0	0	0	0	↑		↑	0	0	0	↑	0	↑	↑	
R Amygdala	0	0	↑	0	↑	0	0	↓		0	0	0	0	0	0	0	0		0	0	0	0	↓	0	0	0	
L Ant Insula	0	↑	0	0	↑	0	0	0		0	0	↓	↓	↓	↓	↓	↓		0	↓	↓	↓	↓	↓	↓	↓	
R Ant Insula	0	0	0	↓	0	0	0	0		0	0	↓	↓	↓	↓	↓	↓		0	0	↓	↓	↓	↓	↓	↓	
L Post Insula	0	0	↑	0	↑	0	0	0		0	0	0	0	↓	0	↓	0		0	0	↓	0	↓	↓	↓	0	
R Post Insula	0	0	↑	0	0	0	0	0		0	0	0	↓	0	0	↓	↓		0	0	↓	↓	↓	0	↓	↓	
L Subgenual AC	0	0	↓	↓	0	0	0	0		0	0	↓	↓	0	0	↓	0		0	0	0	0	0	0	0	0	
R Subgenual AC	0	0	↓	0	↓	↓	0	0		0	0	0	0	0	0	0	0		0	0	0	0	↑	0	↑	0	
L Drevet	0	0	0	0	↑	0	↑	0		0	0	0	↑	↑	↑	0	↑		0	0	0	↑	0	↑	0	↑	
R Drevet	0	0	0	0	0	↑	↑	↑		0	0	↑	↑	↑	0	↑	↑		0	0	0	0	0	↓	0	0	
L Pregenual AC	↑	0	0	0	0	0	0	↑		0	0	0	↑	0	0	0	↑		0	0	0	↑	0	0	0	0	
R Pregenual AC	0	0	0	0	0	0	↑	↑		0	0	0	0	0	0	0	↑		0	0	0	0	↓	0	↓	0	
L Mayberg	0	0	0	0	0	0	↑	0		0	0	↑	↑	↑	0	↑	↑		0	0	0	↑	0	0	0	0	
R Mayberg	0	0	↑	0	↑	0	↑	0		0	0	0	0	0	0	0	↑		0	0	0	0	0	0	↓	0	
L Supragenual AC	0	0	0	↓	0	0	0	0		0	↑	0	0	↑	0	0	0		0	0	↑	↑	↑	↑	0	↑	
R Supragenual AC	0	0	0	↓	0	0	0	0		↑	0	0	0	0	0	0	0		0	0	↑	0	0	0	0	0	
L Dorsal AC	0	0	↑	↓	0	0	0	0		↑	0	0	↓	0	0	0	0		0	0	↓	0	↓	0	0	0	
R Dorsal AC	0	0	0	↓	0	0	0	0		0	0	0	↓	0	0	0	0		0	0	0	0	0	0	0	0	
L Post AC	0	0	0	↑	0	0	0	↑		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
R Post AC	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
L Ant Temporal	0	0	↓	0	↓	↓	0	0		0	0	↓	0	0	0	0	0		0	0	0	0	0	0	0	0	
R Ant Temporal	0	0	0	0	0	0	0	0		0	0	↓	0	0	0	↓	0		0	0	0	0	0	0	0	0	
L Post Temporal	↓	↓	↓	0	0	0	↓	0		0	0	↓	↓	↓	↓	↓	↓		↑	↑	↓	↓	↓	0	0	0	
R Post Temporal	↓	0	0	0	↑	0	0	0		0	0	↓	0	↓	↓	↓	0		↑	0	↓	↓	↓	↓	↓	↓	
L TP	0	↑	↓	↓	0	↓	↓	↓		0	0	0	0	0	0	0	0		0	0	↑	↑	0	↑	↑	↑	
R TP	0	0	0	↓	0	↓	0	0		0	0	0	0	0	0	0	0		0	0	↑	0	↑	0	↑	0	
L Inf Parietal	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
R Inf Parietal	0	0	↑	0	0	0	↑	0		0	0	↑	↑	0	0	↑	↑		0	0	0	0	0	0	0	↑	
L Occipital	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
R Occipital	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
L DLPFC	0	0	0	0	0	0	0	0		0	0	0	↑	↑	↑	↑	0		0	0	↑	↑	↑	↑	0	0	
R DLPFC	0	↑	0	0	0	0	0	0		0	0	↑	↑	↑	0	↑	0		0	↓	↑	0	0	0	0	0	
L VLPFC	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0		0	0	0	↑	↑	↑	0	0	
R VLPFC	0	0	0	0	0	↓	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	↑	0	0	
L MOFC	0	0	0	0	0	0	0	0		0	0	0	↑	↑	↑	0	↑		0	0	↑	↑	↑	↑	0	↑	
R MOFC	0	0	0	0	0	↑	0	↑		0	0	0	↑	↑	0	↑	↑		0	0	↑	↑	↑	0	↑	↑	
L LOFC	0	0	0	0	↓	0	0	↓		0	0	0	0	0	0	0	0		0	0	0	0	↑	0	0	↑	
R LOFC	0	↓	↓	0	↓	0	0	0	Total	0	0	↓	0	0	0	0	0	Total	0	↑	0	0	↑	0	0	0	
# r > .5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
# r < -.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Legend			
r		Δr	Cholinergic System
>.50	↑	>.50	Tegmental-Midbrain
<-.50	↓	<-.50	Substantia Inominata
			Septo-Hippocampal

In the cortex, the pattern of the correlations between the forebrain cholinergic and the anterior cingulate ROIs in the patients also appeared different from the controls, and in this case many were found to be significantly different. For example, forebrain cholinergic ROIs, as a whole, were significantly positively correlated with ROIs in the pregenual, Mayberg, and Drevets subdivisions of the anterior cingulate, while the remainder of the cingulate, in general, was not; significantly more positive correlations were observed in patients compared to controls in these subdivisions (pregenual, Mayberg, and Drevets) most uniformly with bilateral BF ROI. The correlations between cholinergic forebrain and the subgenual and supragenual subdivision ROIs (SI only) had few significant correlations in either direction in the patients, but when compared to controls, they were significantly less positively correlated.

With the exception of the anterior cingulate, few cortical ROIs were significantly correlated with the most of the cholinergic ROIs. Despite this, there were several cortical areas where significant differences from controls were found in the correlations. The correlations with the anterior temporal cortex and temporal pole ROIs were significantly less positively correlated in the patients compared to the controls. The LOFC ROIs were significantly negatively correlated with the cholinergic ROIs, and were significantly more negatively correlated in the patients compared to the controls.

Some correlational patterns did not look that different and upon significance testing few differences were found between the groups at baseline. The regions that did not have any significant differences between the patients and controls at baseline

were the left thalamus, inferior parietal, and occipital, and right posterior cingulate, posterior temporal, and MOFC. The DLPFC (more positive with right tegmental ROI) and VLPFC (less positive with right septal ROI) only had one correlation significantly between the groups.

4.3.2.4 Patients with Procaine

At first glance, the correlational patterns occurring with procaine in each group appeared more similar than observed at baseline, although this was not always the case. The major organizational themes as described in the controls with procaine were also evident in the patient correlations.

The appearance of clusters of positive correlations in the procaine condition also occurred in the patients. This was similar to controls, albeit not as consistent, and with some meaningful differences (Table 9; middle and right panels). The similarities in the patterns occurred between the forebrain cholinergic ROIs and themselves, the striatum, and anterior cingulate. Most ROIs in these areas showed significantly more positive correlations with procaine compared to baseline.

Nonetheless, when comparing across groups in the procaine condition, often the correlations of the cholinergic ROIs with the caudate and putamen, subgenual subdivision ROIs were significantly less positive, and Drevets, Mayberg, and supragenual ROIs were significantly more positive in the patients compared to the controls. Thus, despite the initial pattern similarities in the striatum and anterior cingulate subdivisions in the procaine condition, the correlational relationships

between the cholinergic ROIs and these regions are changing significantly differently with procaine in the patients compared to the controls.

In the cortex, group differences of the correlational patterns occurred with the bilateral hippocampus, insula, and DLPFC, and left posterior temporal, temporal pole, and VLPFC. In general, there were no significant correlations between any cholinergic ROI and the hippocampal ROIs in either condition, which was significantly different from controls. In most cases, the correlations of the forebrain cholinergic ROIs with the insula were not significantly positively correlated, and in one case it was significantly negatively correlated (right SI ROI). These correlations were significantly less positive in the patients compared to the controls (Table 9; right panel). There were additional clusters between the forebrain cholinergic ROIs and the DLPFC, VLPFC, posterior temporal (left only) and temporal pole ROIs in the patients, which were not observed in the controls. The prefrontal and temporal cortex correlations were significantly more and less, respectively, positively correlated in the patients compared to the controls.

In summary, the correlational strengths were significantly more positive with procaine compared to baseline between the cholinergic ROIs and the anterior cingulate, anterior and posterior insula, the putamen and themselves in the controls. In the patients, significantly more positive correlations were observed with the cholinergic ROIs, the anterior cingulate, DLPFC, VLPFC, MOFC, and striatum with procaine compared to baseline. At baseline, the cholinergic areas were significantly less positively correlated with themselves in the patients compared to controls. In addition, numerous significant correlations, both positive and negative, between the

groups occurred mostly in subcortical forebrain structures, anterior cingulate and temporal cortical area, but not prefrontal cortical areas. With procaine, significantly more positive correlations in the patients compared to the controls occurred between the cholinergic ROIs and the thalamic, pregenual anterior cingulate, DLPFC, and MOFC ROIs. Also observed in the procaine condition, significantly less positive correlations in patients compared to controls occurred between cholinergic ROIs and the striatal nuclei, insula, subgenual anterior cingulate, and temporal cortex ROIs.

4.3.3 Multivariate Analysis of Behavioral and Cerebral Blood Flow Relationships

4.3.3.1 Behavioral and Regional CBF Relationships

In the controls, none of the cholinergic ROIs correlated significantly with any of the emotional or sensory ratings at baseline or with procaine (Appendices C, D). However, in the patients, significant positive correlations occurred between depression ratings and the bilateral BF ROI (Appendix F), and between auditory sensations and right tegemental ROI at baseline. With procaine, all forebrain cholinergic ROIs correlated positively with calmness ratings (Appendix G).

Examination of the delta-delta correlations revealed opposite relationships between cholinergic ROIs and ratings of euphoria and anxiety in both groups in different ways. Specifically, delta anxiety ratings correlated negatively with left BF delta-CBF (r=-0.55) in the patients and non-significantly in the controls (r=.20). Delta euphoria ratings correlated positively with right tegemental delta-CBF(r=.65) in the patients and negatively in the patients (r=-.50). In both cases, the correlations

observed in the patients were significantly different from the controls (Figures 28 and 29).

In addition, significant delta-delta positive correlations occurring only in the patients were observed between calmness and left BF and septal ROIs, overall bad/good and left BF ROI. Also occurring only in the patients, delta auditory sensations and delta bilateral tegmental ROIs were significantly positively correlated.

Regarding non-cholinergic ROIs, significant correlative relationships were sparse in any condition in the controls. In contrast, there were numerous significant correlations in the patients at baseline, with procaine and delta-delta. Examination of these relationships is outside the scope of this study, however, the disparity of the number of CBF/emotion correlations between the two groups is notable.

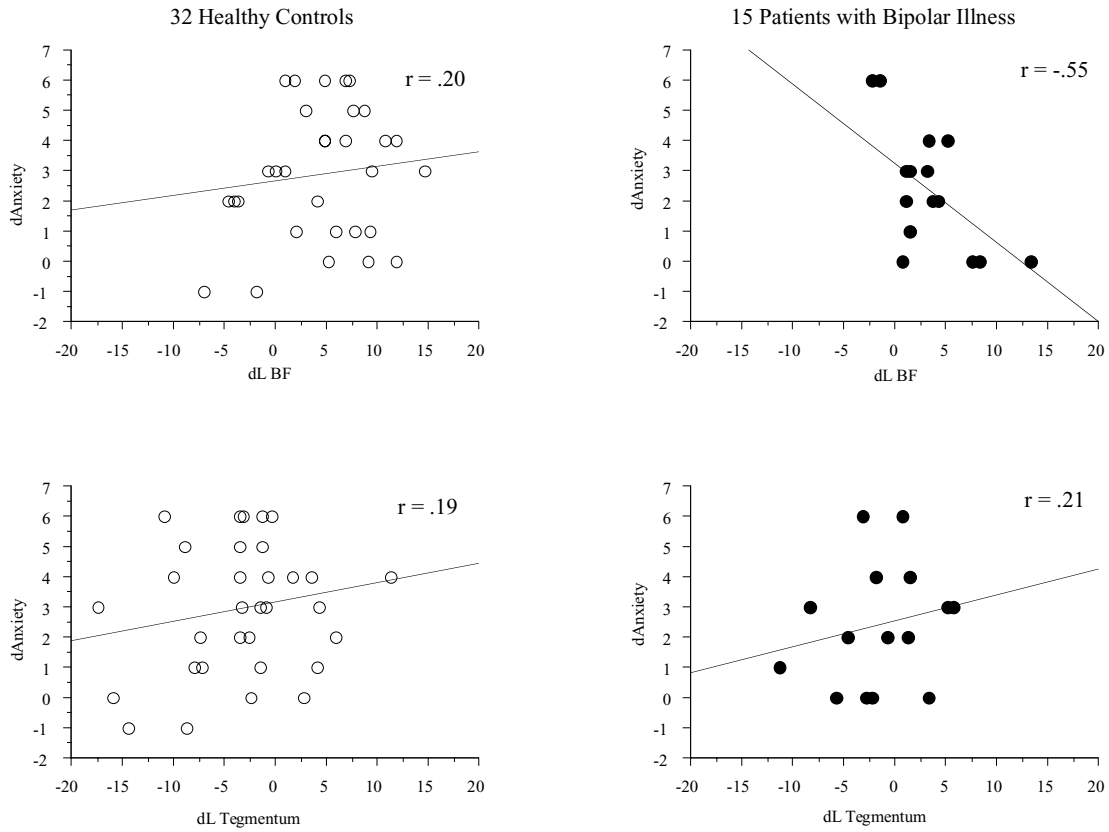

Figure 28. Differential Relationships of Forebrain vs Brainstem rCBF with Anxiety Response to Procaine in Patients vs Controls

rCBF in the BF region correlates inversely with anxiety ratings in patients with bipolar disorder, while this correlation is direct in controls (Fisher's R-to-Z = 2.39). The brainstem relationships were similar in both groups (Fisher's R-to-Z = 0.06).

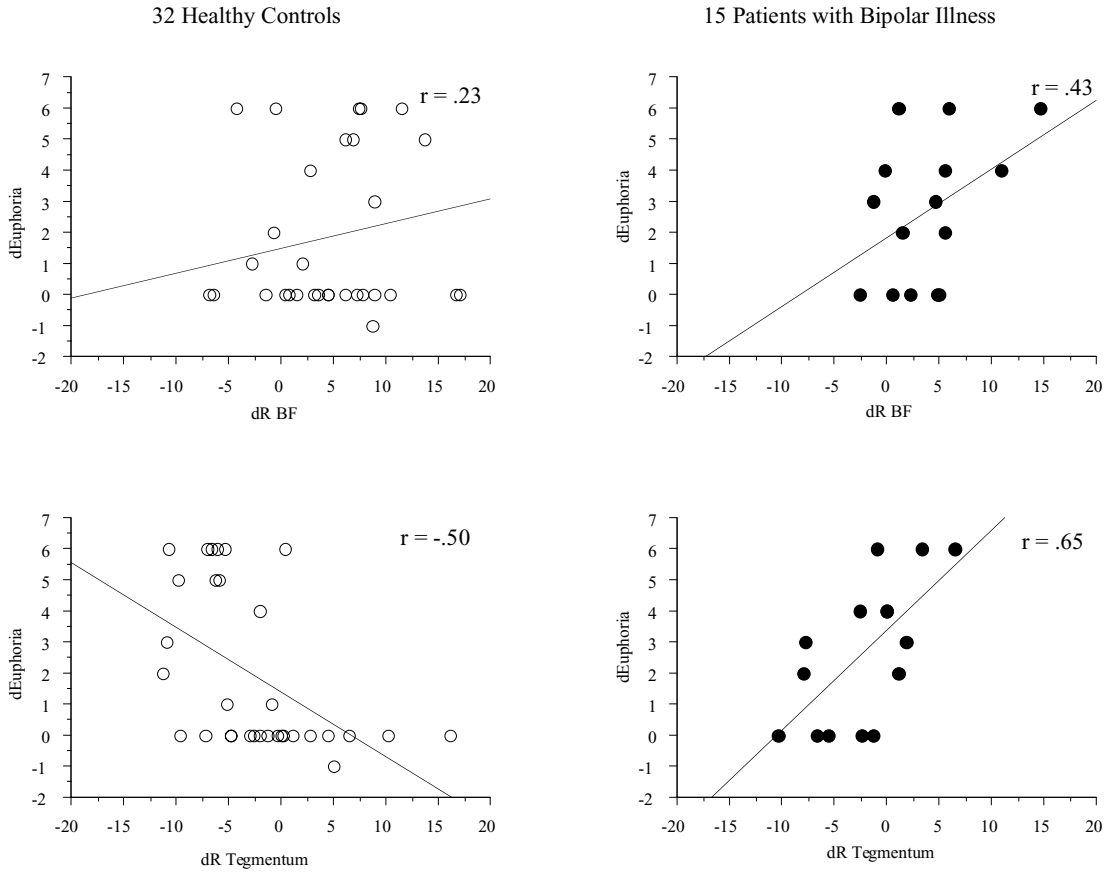

Figure 29. Differential Relationships of Forebrain vs Brainstem rCBF with Euphoria Response to Procaine in Patients vs Controls

rCBF in the tegmentum region correlates directly with anxiety ratings in patients with bipolar disorder, while this correlation is inverse in controls (Fisher's R-to-Z = 3.86). The forebrain relationships were similar in both groups (Fisher's R-to-Z = 0.66).

4.3.3.2 Multivariate Emotion and Cholinergic CBF Network Analysis

The relationship between procaine-induced changes in CBF in the AChNet and changes in ratings, i.e., delta-CBF/delta-rating relationships, was analyzed using multivariate multiple regression separately by group and laterality (Appendix I). Negative emotions were limited to anxiety and fear, due to the non-normal distribution of the depression and anger ratings, and positive emotions included euphoria and calmness ratings. Regions were eliminated one by one to find the core regions responsible for explaining the most variance in the ratings.

In the controls, negative emotions were explained by change in AChNet CBF, as a whole, to a modest degree (HC-L: R^2=0.41; HC-R: R^2=0.42). This was mostly due to anxiety ratings (HC-L: R^2=0.27; HC-R: R^2=0.38), rather than fear ratings (HC-L: R^2=0.18; HC-R: R^2=0.21). Within a reduced AChNet, the variance of delta-anxiety ratings was best explained by delta-rCBF in the amygdala and SI (R^2=0.24) and the amygdala and MOFC (R^2=0.25) in the left and right networks, respectively, in the control group.

Several competing models were tested (in both groups) by substituting BF ROI for SI, Drevets ROI for subgenual AC, Mayberg ROI for pregenual AC, or DLPFC for MOFC, either singly or in combinations. In the controls, the substitution of BF, Drevets, Mayberg and DLPFC ROIs in combination in the network as a whole resulted in the greatest increase in the variance explained in the delta-negative emotions (R^2=0.52), and in delta-anxiety ratings (R^2=0.43). The reduced model of the left BF, Drevets and DLPFC resulted in a significant increase in the variance of the delta-anxiety ratings (R^2=0.41) over the best model with no exchanges (AM and

SI: R^2=0.27; see Figure 30). Exchanges of any combination in the left network did not increase the variance explained in delta-fear ratings in the controls. In addition, in examination of the right network in the controls, none of the substitutions resulted in a significant increase of the variance explained in delta-negative emotions combined or separately.

Turning to the examination of the change of positive emotions, the same methods as outlined with the negative emotions were employed. In the controls, the network in any form, right or left, full or reduced, substitutions or not, did not explain the variance of delta-positive ratings (Figure 31).

In the patients, both left and right networks delta-CBF explained a substantial portion of the variance of the delta-negative emotion ratings (BPD-L: R^2=0.59; BPD-R: R^2=0.61) in the network as a whole, which was significantly more than the controls (ΔR^2 close to .20). Of the negative emotion ratings, further analysis revealed that it was also primarily the delta-anxiety ratings that were explained by the delta-rCBF (BPD-L: R^2=0.29; BPD-R: R^2=0.61) rather than the delta-fear ratings (BPD-L: R^2=0.13; BPD-R: R^2=0.26) as observed in the controls. However, in contrast to the controls, the addition of the pregenual AC ROI along with the amygdala and SI in the left reduced model was needed to explain the most possible variance in the delta-anxiety (R^2=0.23) in the patients. Delta-rCBF in the right AChNet included the same ROIs as in the controls, the subgenual AC and MOFC, to explain 45% of the variance in the anxiety ratings in the patients.

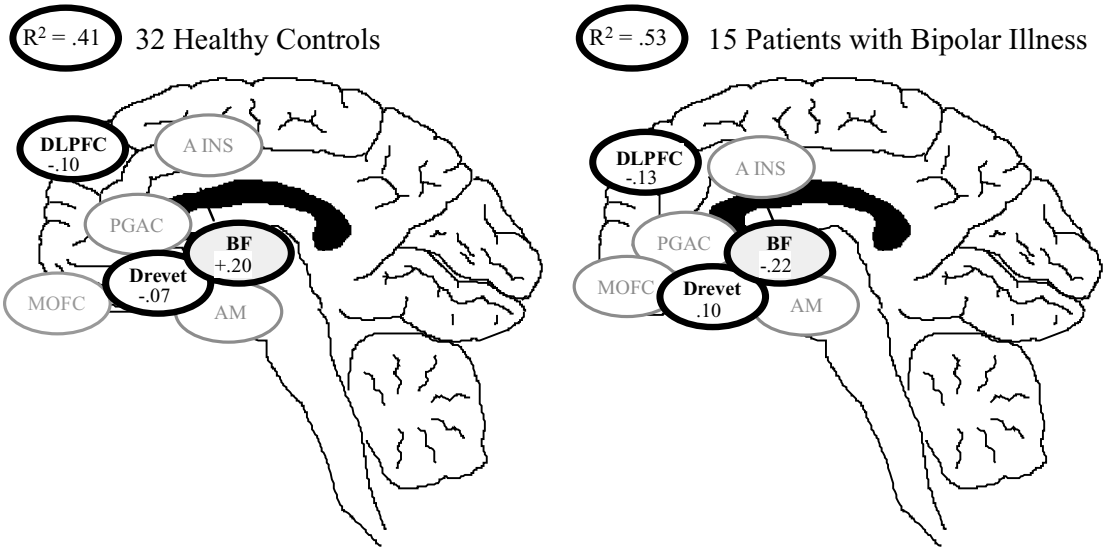

Figure 30. Testing the AChNet Model: Relationship to Anxiety Ratings
Anxiety ratings were explained by rCBF in the left BF, Drevets and DLPFC ROIs slightly more so in the patients compared to the controls. In addition, rCBF in the BF and Drevets ROIs, but not DLPFC, were significantly more negatively related to anxiety ratings in the patients compared to controls (BF: t=-2.94, p=.005, df=45; Drevets: t=2.92, p=.006, df=45; DLPFC: t=-0.35, p=ns, df=45). Abbreviations: AINS= anterior insula; AM=amygdala; BF=basal forebrain cholinergic region; DLPFC= dorsolateral prefrontal cortex; MOFC=medial orbitofrontal cortex; PGAC= pregenual anterior cingulate; SGAC = subgenual anterior cingulate.

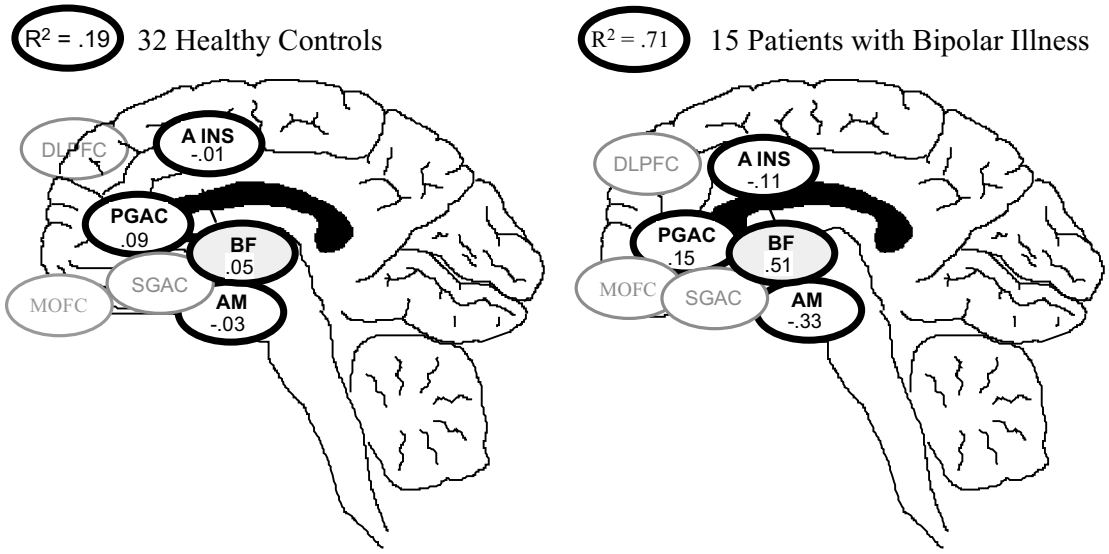

Figure 31. Testing the AChNet Model: Relationship to Euphoria Ratings
Euphoria ratings were explained significantly in the patients but not controls by rCBF in the left BF, AM, PGAC and AINS ROIs . In addition, rCBF in the BF was significantly more positively related to euphoria ratings in the patients compared to controls (BF: t=2.58, p=.01; AM: t=-1.79, p=ns; PGAC: t=0.34, p=ns; AINS: t=-1.72, p=ns). Abbreviations: AINS= anterior insula; AM=amygdala; BF=basal forebrain cholinergic region; DLPFC= dorsolateral prefrontal cortex; MOFC=medial orbitofrontal cortex; PGAC= pregenual anterior cingulate; SGAC = subgenual anterior cingulate.

In the examination of AChNet substitutions in the patients, the exchange of left BF, Drevets, and DLPFC together explained significantly more variance of delta-negative emotions (R^2=0.83) than the basic model (R^2=0.58), and of delta-anxiety ratings (R^2=0.58) than the basic model (R^2=0.29). The left reduced network model explaining the most variance in patient delta-anxiety ratings included the BF, Drevets and DLPFC ROIs (R^2=0.53). Thus, the same substitutions as seen in the controls also resulted in the most increase in the variance explained in the patients. However, the beta weight for the BF ROI was significantly different between the groups (HC: B=.05; BPD: B=.51; t=-2,94, p=.005, df = 45). The variance explained in the delta-fear ratings was improved by substituting DLPFC in the left network as a whole (R^2=0.43), and by the reduced model including the SI, pregenual AC, anterior insula and DLPFC predominately explained (R^2=0.34).

With respect to the right network in the patients, the substitution of Drevets for subgenual AC resulted in the largest increase in the variance explained (R^2=0.79) of delta-negative emotions, while in the reduced model, Drevets and pregenual AC explained 59% of the delta-anxiety rating variance. The variance explained in delta-fear ratings in the patients was best modeled by Drevets, Mayberg and DLPFC substitutions in the complete network (R^2=0.42), however, only 21% of the delta-fear rating variance was explained by those regions alone.

The variance in delta-positive emotions (R^2=0.64) was explained by change in the left AChNet in the patients, which was significantly more than in the controls (R^2=0.25). This was primarily due to delta-euphoria ratings in the whole model (R^2=0.63), and to the left subgenual AC, pregenual AC, anterior insula, and the

MOFC (R^2=0.49) in a reduced model. Substitution of left BF for SI resulted in the greatest increase in the variance explained in delta-positive emotions (R^2=0.84) compared to other alterations of the basic model. The variance in the change in delta-euphoria ratings was explained best by the inclusion of left amygdala, BF, pregenual AC and anterior insula (R^2=0.71) in the model. With substitutions, the variance in the change in delta-calmness ratings was explained best by the inclusion of left BF, subgenual AC, and pregenual AC (R^2=0.44) in the model.

In the right AChNet, 41% of the variance in delta-positive emotions was explained by the original network as whole in the patients, of which, both delta-euphoria ratings (R^2=0.34) and delta-calmness (R^2=0.50) were explained. The best reduced model explaining the variance in delta-euphoria ratings before substitutions included the right subgenual AC, pregenual AC and the MOFC (R^2=0.30). After substitutions, although using Drevets, Mayberg and DLPFC ROI resulted in the greatest increase in the explained variance (R^2=0.69) in delta-positive emotions in a full model, the best reduced model explaining the most variance (R^2=0.45) of the delta-euphoria ratings included BF, Mayberg, and DLPFC CBF. The explained variance in calmness ratings of the patients was by right SI, Drevets, anterior insula and MOFC rCBF (R^2=0.48).

4.4 Discussion

There are two principle findings from this study. First, procaine enhanced the associativity between regions with known neuroanatomical connections via cholinergic pathways in controls. This was replicated in patients, although with

subtle, but meaningful differences. Second, regions of cholinergic cell bodies and their targets were associated with emotional experiences of anxiety in controls. In contrast, anxiety ratings were related to the same cholinergic regions but in an opposite manner in patients. Moreover, feelings of euphoria were positively associated with cholinergic brain regions in patients, as well.

4.4.1 Cholinergic Pathway Confirmation

The functional connectivity analysis of cholinergic brain regions in patients and healthy controls at baseline and with procaine will be discussed in the context of a two-fold purpose: 1) to confirm cholinergic pathways are measurable at baseline and are modulated by procaine administration; 2) to support and extend the activation study findings by providing alternative perspectives from traditional brain imaging methods through examination of associative relationships of the cholinergic regions of the brain with those previously identified by the procaine activation studies.

The main findings of the functional connectivity analysis were that CBF in many of the cholinergic ROIs significantly correlated with CBF in parts of the brain (both brainstem and forebrain) with known cholinergic pathways at baseline in healthy controls and patients. Moreover, when challenged by procaine (an agent that blocks a cholinergic radiotracer; Benson et al., 2004), the functional connectivity of these cholinergic ROIs increased significantly in most of areas with known neuroanatomical connections. For example, in the controls at baseline the SI CBF correlated significantly with CBF in most of the anterior cingulate subdivisions, amygdala, and MOFC, but not the anterior insula. With procaine, significant

increases in correlations were observed between the SI and anterior cingulate and insula CBF. Both at baseline and with procaine, SI functional connectivity reflected a substantial portion of the pattern of what is believed to be the heaviest cholinergic innervation of the forebrain (Amaral et al., 1992; Russchen et al., 1985; Mesulam, 1995). Regions, such as anterior temporal cortex or DLPFC, with less robust or direct (lacking monosynaptic links) cholinergic innervation did not show significant changes in CBF correlative strengths.

In the patients, the SI cholinergic pathways were partially confirmed at baseline, however, they were more so with procaine. At baseline, SI CBF correlated significantly with CBF in the amygdala, most anterior cingulate subdivisions and MOFC, but not with CBF in the anterior insula. With procaine, significantly more positive correlations of CBF in the forebrain cholinergic ROIs occurred for most anterior cingulate subdivisions, and MOFC. While CBF in the amygdala became significantly less positively correlated with septal CBF, the correlations with SI CBF remained unchanged.

The activity in the septo-hippocampal pathway is reflected by significant positive correlation between CBF in these two ROIs in the controls. The procaine-induced significant reduction in correlational strength between septal CBF and hippocampal CBF suggests this pathway may be modulated during intense emotional experiences. These results are interesting in light of the poor memory recall during strong emotional events and are consistent with septo-hippocampal pathway disruption during such times (Dudar, 1975; Smith, 1972). The modulation of

hippocampal systems during emotional processing also is supported by theories of encoding of facial affect and emotional stimuli (Adolphs et al., 1999).

In the patients, hippocampal CBF was not significantly correlated with CBF in any of the cholinergic ROIs at baseline or with procaine, and did not show significant changes in the correlative relationships. Thus, septo-hippocampal function may be disrupted or not revealed by these methods in patients. The significant interaction of group and drug suggests that with procaine the correlative relationships of septal and hippocampal CBF become more positive in patients, while the controls become more negative. This discrepancy is interesting in light the complaints about memory function often expressed by patients. Reduced hippocampal volumes have been reported not only in bipolar disorder (Noga et al., 2001), but also in major depression (Sheline, 1999), post-traumatic stress disorder (PTSD; Bremner et al., 1995), which in some disorders may be a result of hypercortisolemia (Sheline, 2000). These changes in functional connectivity complement the abnormalities reported in traditional brain imaging studies, where hippocampal activity correlated with severity of depression (previous chapter) and metabolism was increased in moderately depressed patients (Ketter et al., 2001).

The temporal pole, a region with an intermediate degree of cholinergic innervation exhibited a reduction in functional connectivity in controls. This complements the hippocampal findings, and is not surprising given the intimate connections of the entorhinal and perirhinal cortex with the hippocampus. However, these changes were not observed in the patients.

Tegmental CBF was predominately positively correlated with brainstem CBF, while having few significant negative correlations with forebrain CBF in the controls. In patients, tegmental CBF correlated with CBF in other brainstem regions, thalamus, globus pallidus, as well as the posterior temporal cortex. The cholinergic tegmental nuclei innervate the medulla, pons, midbrain and globus pallidus (Carpenter, 1991; Krnjevic, 1975). Thus, the predominance of significant changes between the tegmental CBF and CBF in adjacent hindbrain, thalamus, and globus pallidus is consistent with the cholinergic projections traveling in the central, dorsal, and ventral tegmental tract innervating the hindbrain, thalamus, and globus pallidus, respectively.

Discrepancies from known neuroanatomical pathways, however, included the lack of significant changes in correlations of cholinergic ROIs with amygdala and MOFC CBF in controls, and with anterior insula CBF in patients. The amygdala is a small cluster of nuclei, and partial volume effects, scanner resolution, and image processing could contribute to the attenuation of amygdalar signals. Although rCBF in MOFC was significantly increased with procaine in controls in the activation analysis, the lack of change in correlative relationships between MOFC and cholinergic forebrain CBF was not expected. MOFC and SI CBF significantly correlated at baseline, as expected from known pathways, but not with procaine; however, this change was too limited to establish a significant difference in correlations across conditions. Although MOFC receives direct cholinergic innervation from the basal forebrain area, competing influences of different neurotransmitter systems could override the cholinergic input to attenuate the change. Whether cholinergic influences in MOFC are excitatory or inhibitory are unknown,

and furthermore it is not clear whether these influences will result in hyper- or hypo-perfusion.

The presence of significant positive correlations between the cholinergic ROIs and prefrontal ROIs, such as DLPFC, VLPFC, and MOFC in patients provides support for cholinergic dysregulation in bipolar disorder. These correlations were significantly more positive in the patients compared to controls, where significant correlations between cholinergic ROIs and prefrontal cortex were not observed. This suggests that the procaine-induced increases in blood flow in prefrontal cortex may be in some way associated with increased activity in the cholinergic forebrain area in the patients but not the controls. This is consistent with Janowsky's notion of exaggerated responses to cholinergic stimulation in affective disorders compared to healthy controls (1994).

The observed changes in correlative relationships between CBF in cholinergic ROIs and CBF occurred in regions with the heaviest cholinergic innervation; this reinforces the hypothesis that the neuroanatomical distribution of the cholinergic system in limbic structures overlaps with the pattern of selective limbic activation induced by procaine (Ketter et al., 1996). Telencephalic cholinergic projections originate in the basal forebrain nuclei, such as the substantia inominata and nucleus basalis, and send efferents to most of the cortex as well as to the basolateral amygdala and anterior cingulate (Mesulam 1995; Mesulam et al. 1983). Both the amygdala and the anterior cingulate send efferents to the basal forebrain cholinergic nuclei, suggesting feedback modulation of their own afferents (Mesulam, 1995; Heimer, 1994; Záborszky, 1993). These reciprocal connections create an opportunity for

enhancement, diminution, or refinement of neuronal activity related to the valence and saliency of environmental stimuli, thus affecting potential behavioral outcome. The cholinergic drive to the amygdala has been hypothesized to an essential component of the "general emotion system" (LeDoux, 1996). The cholinergic basal forebrain, anterior cingulate, insula, and MOFC may well be other structures integral to emotion regulation.

Cholinergic M_1 and M_2 receptors are present in core limbic areas, such as amygdala and hippocampus, as well as in primary sensory regions. While M_1 receptors have primarily cortical distribution, M_2 receptors have more uniform cortical and subcortical distribution across the brain, (Mesulam 1995; Mesulam et al. 1983). Thus, the distribution of both subtypes of cholinergic receptors in sensory – limbic pathways could allow cholinergic mechanisms to play an important role in emotion.

Two other perspectives cannot be dismissed. Procaine is known to have similar affinities with sigma (Sharkey, 1988), 5-HT_3 (Fan and Weight, 1994), and most recently reported 5-HT_{1A} (Kalipatnapu and Chattopadhyay, 2004). While the distributions of both sigma and 5-HT_3 receptors do not overlap well with the pattern of procaine activation (Ketter et al., 1996), 5-HT_{1A} are distributed in many limbic areas. These receptors do not have any preference for anterior versus posterior cingulate (Neumeister et al., 2004), and PET studies show the highest binding of 5-HT_{1A} antagonists in the hippocampus (Drevets et al., 1999). Neither the posterior cingulate nor the hippocampus were selectively activated with procaine (Ketter et al., 1996).

A second area of consideration is that the cholinergic system is known to interact with many other neurotransmitter systems in the amygdala, hippocampus, ventral and dorsal striatum, and the cortex for starters. For example, GABAergic-cholinergic influences on neural activity in the basal forebrain (Jones, 2004) and the thalamus (Steriade, 2004) may mediate complementary role in arousal and slow wave sleep. Dopaminergic-cholinergic interactions in the dorsal and ventral striatum most likely participate in pleasure / rewards systems (Koob and Nestler, 1997). Cholinergic-serotonergic interactions in the cortex and hippocampus may regulate mood (Overstreet et al., 1998). Thus, considering the functional response of procaine to be solely a cholinergic action would be remiss.

4.4.2 Alternative Perspective of Activation Study Findings

Many of the regions shown to be abnormal in patients in the activation study also exhibited differential functional connectivity as evidenced by significant alterations in the correlative relationships between the cholinergic ROIs and other brain regions. Each of these will be discussed in the context of pertinent findings in the literature.

The functional connectivity of three prefrontal cortical areas, DLPFC, VLPFC and MOFC was significantly altered with procaine in patients, as evidenced by significant increases in positive CBF correlations with the forebrain cholinergic ROIs compared to controls. Furthermore, all three areas exhibited differential responses to procaine in the patients versus the controls in the activation analysis. As mentioned in the previous chapter, baseline DLPFC hypo-activity changed to hyper-activity and

baseline MOFC hyper-activity converted to hypo-activity in patients compared to controls with procaine. Taking the results of the two methods together, the findings suggest that the procaine-induced hyper- or hypo-activity in the DLPFC and MOFC, respectively, may be an expression of altered functional connectivity of the cholinergic forebrain region in patients. Studies of neuronal and glial cell histopathology support prefrontal cortex dysfunction. For example, Rajkowska (2000) has found that a neuronal cell density reduction of 16-22% in layer III, pyramidal cell reduction of 17-30% in layers III and V, and a glial cell reduction of 19% in DLPFC.

In patients compared to controls, CBF in the subgenual anterior cingulate region had distinctly different relationships with CBF in cholinergic ROIs. CBF in bilateral SI and left subgenual ROIs were significantly less positively correlated in the patients compared to the controls, both at baseline and with procaine. Persistent discrepancies could indicate a trait marker. Indeed, the neuronal and glial cell loss of the subgenual region in mood disorder patients compared to controls (Drevets et al., 1997; Öngur et al., 1998; Rajkowska, 2000) is considered one of the first clear-cut documented neuroanatomical abnormalities described at a neuronal level in a psychiatric disease; nerve growth factor (NGF) is known to be co-localized with acetylcholine in basal forebrain neurons (Mesulam, 1995). The observed neuronal and glial cell loss of the subgenual cingulate region could be a result in both loss of these growth factors and cholinergic dysregulation as revealed by altered functional connectivity between the basal forebrain cholinergic and the subgenual cingulate observed in this study.

The anterior insula was one of the few regions in which the CBF correlations changed in opposite directions with procaine in the two subject groups, rather than moving in the same direction. In the healthy controls, the CBF correlations became significantly more positive, while in patients, CBF correlations with the anterior insula moved in a negative direction, although not always significantly. These often non-significant changes in opposite directions in the two groups resulted in a significant interaction between all forebrain ROIs and bilateral anterior insula. This region has been associated with differential treatment response in patients with bipolar disorder (Ketter et al., 1999). Baseline hyper-metabolism especially in the left insula was predictive of eventual response to the anti-convulsant carbamazepine, while baseline hypo-metabolism was predictive of the response to the calcium-channel blocker nimodipine. Although neither of these drugs has a primary cholinergic mechanism action, cholinergic innervation may well influence the activity of this region.

4.4.3 Emotion and CBF Relationships

The most noteworthy finding of the examination of blood flow and emotional-sensory experiences induced by procaine was that CBF changes in regions containing cholinergic cell bodies and their targets explained a substantial portion of the variance of anxiety and euphoria ratings, although in different ways in patients and controls. As such, these changes in associations between emotional experiences and rCBF may indicate a abnormalities in the neural networks subserving emotion in patients compared to controls.

The procaine-induced anxiety ratings were explained to a fair degree in both groups by the same (left) or similar (right) cholinergic model, but in a different manner. For example, while changes in the same regions on the left (BF, subgenual AC, DLPFC) were associated with changes in anxiety ratings in both groups, the relationships of BF and subgenual AC CBF changes to changes in anxiety were in opposite directions in patients and controls. This was not the case, however, with DLPFC, where changes in rCBF had similar relationship to changes in anxiety ratings in both groups. In addition, while CBF changes in identical ROIs on the right (BF, subgenual AC, DLPFC) explained the most variance in anxiety ratings in controls, the patients differed in that changes in the right pregenual AC and Drevets CBF were associated with changes in anxiety.

Although similar degrees of euphoria ratings were endorsed in both groups, only changes in AChNet CBF in patients were associated with changes in euphoria ratings. In the controls, changes in caudate CBF were significantly correlated with changes in euphoria ratings. This is expected, as changes in caudate and nucleus accumbens are integral to pleasure-reward systems (Koob and Nestler, 1997; Salamone et al., 1997).

These findings suggest there may be alterations in the neural networks subserving the functional response of euphoria in patients. There is considerable evidence indicating the dopamine activity influences cholinergic activity and vice versa in the dorsal (Graybiel, 1990, 2000) and ventral striatum (Holt et al., 1997; Záborszky and Cullinan, 1992). Procaine can directly modulate cholinergic activity (Benson, 2004), thus could yield indirect modulation of dopamine systems in ventral

regions. Moreover, dopamine may regulate cortically projecting basal forebrain neurons (Day and Fibiger, 1992, 1993), as well as septohippocampal projections (Day and Fibiger, 1994). Taken together, if the cholinergic system is altered in the patients, dopamine function may be affected such that shifts in the neural networks subserving the pleasure-reward functions.

On a different level, changes in euphoria ratings were associated with CBF changes in the right DPLFC CBF changes, while anxiety was associated with the left DLPFC CBF changes in the patients. This data follows what has been accepted in lesion data from human prefrontal cortex lesions, where stroke occurring on the left prefrontal cortex usually results in depression, while right-side damage is associated with mania (Robinson, 2003). Davidson and colleagues (2002) hypothesize that left-sided prefrontal cortex functions to regulate "approach-related appetitive goals" and dysfunction of DLPFC may result in "deficits in pre-goal attainment forms of positive affect," i.e., depressive symptoms. Right-sided functions may modulate inhibition, and disruption would result in excessive behavioral inhibition (Davidson, et al., 2002). The data in this study suggest that right DLPFC hypo-activity could cause loss of inhibition in the form of mania.

4.4.4 Consolidation of Findings

Consolidating the findings from the three different methodological approaches gives support to the hypothesis that the cholinergic system is contributing to the emotional-sensory response induced by procaine. Patients compared to controls had BF hypo-perfusion at baseline, positive correlations between HDRS and procaine-

induced change in BF CBF, and increased strength of correlations between BF and the CBF in multiple ROIs at baseline and with procaine. In conjunction with the direct relationship between increased BF blood flow and increased anxiety ratings in patients, these results suggest increased activity in the BF region in patients may be dysfunctional at rest and that depending on the cholinergic target, during emotional situations the influence may become over- (DLPFC, MOFC, supragenual AC, hippocampus) and under-driven (anterior insula).

The relationship of DLPFC blood flow and experiential effects of procaine appears to be complex. In patients, procaine induced hyper-perfusion ion the DLPFC and hyper-association to CBF in cholinergic cell body regions compared to healthy controls, while the associations between change in DLPFC CBF and anxiety ratings was fairly similar in patients and controls. A possible explanation could be that cholinergic influence on the DLPFC may be enhanced (functional connectivity) in patients compared to controls resulting in increased perfusion (activation response), and an equivalent anxiety response.

Combining the results across the three methods employed in the study, the association of changes in emotion ratings with differential CBF changes in BF and subgenual AC (or Drevets region) extends the understanding of the regional neurochemistry and physiology of bipolar illness. Although serotonin, norepinephrine and dopamine systems have been most commonly implicated in affective disorders, an enhanced ratio of cholinergic to adrenergic function has also been hypothesized to play a role in mania and depression (Fritze 1993; Janowsky et al. 1972b). In this theory, when the balance shifts to favor noradrenergic

predominance manic symptoms emerge, and cholinergic dominance is associated with depressive symptoms. While, depression ratings did not change significantly during the study, anxiety is commonly present during and often covaries with depression. Cholinergic overdrive in patients is supported by the direct relationship between BF rCBF and anxiety ratings.

Cholinergic challenge studies lend support to Janowsky's theory. For example, physostigmine can reduce manic symptoms and exacerbate depressive symptoms in bipolar patients (Janowsky et al. 1972a). Furthermore, physostigmine administration results in relapse of depressive symptoms in bipolar patients successfully treated with lithium, while healthy controls do not develop depressed mood. These patients have concomitant hormonal disturbances and emotional arousal expressed as dysphoria (Janowsky et al. 1986) that overlap those seen with procaine.

Arecoline, a nonselective muscarinic agonist, has also induced dysphoria in mood disorder patients whether or not they were currently depressed and in individuals with a family history of depression compared to those without such history (Gillin et al. 1991; Nurnberger et al. 1989). Decreased REM latency in patients with major depression, but not in healthy controls, was observed with i.v. arecoline (Sitaram et al. 1980) and donepezil, a cholinesterase inhibitor (Perlis et al. 2002).

While most tricyclic antidepressants are known to have anti-cholinergic adverse effects (such as dry mouth and urinary retention), it is also possible that relative cholinergic blockade could contribute to their efficacy and increased

proclivity to cause switches in mania compared with less anticholinergic antidepressants (Guille et al. 1999; Peet 1994). On the other hand, donepezil has been reported to be efficacious in treating bipolar disorder (Burt et al. 1999) and relieves some of the anti-cholinergic adverse effects with some risk of mania induction (Jacobsen and Comas-Diaz 1999), suggesting that maintaining cholinergic balance may be important to mood disorders.

The predominance of positive CBF correlations between regions with cholinergic neurons and other areas at baseline and with procaine in both patients and controls suggest that, in general, cholinergic neurotransmission may be associated with cortical activation. However, an interesting dichotomy in how the relationships changed from baseline to procaine between CBF in the cholinergic and other brain ROIs was revealed for the brainstem and forebrain cholinergic ROIs. Significant changes from baseline to procaine in brainstem cholinergic (tegmentum) CBF relationships moved from positive to negative associations. This could reflect differences in procaine's effects in cholinergic cell body and terminal regions.

In contrast, the forebrain cholinergic ROI relationships with other brain regions were more variable after procaine. The ROI differential relationships in brainstem vs forebrain cholinergic areas may indicate a basic difference in the functional connectivity of these two cholinergic systems. The cholinergic brainstem system has been associated with the ascending reticular activating system (ARAS). These tegmental pathways are part of the ARAS. The function of this system is hypothesized to activate the basal forebrain nuclei involved in mediating arousal level. The direct relationships observed in this study are consistent with this view. In

contrast, the basal forebrain nuclei may play a crucial role in attention, emotional learning, and memory given their strategic amygdala, hippocampal and paralimbic connections.

4.4.5 Limitations

This study has several limitations. Most importantly, it depends on post-hoc data analyses. Although the hypothesis were, in part, developed by some of the preliminary data incorporated in this analysis, the most significant aspect of the study, the alteration of cholinergic functional connectivity in the patient group, had not been previously explored in this manner. These findings need to be replicated in a study where procaine and/or other cholinergic agents are used to modulate brain activity to test the apriori hypothesis that the cholinergic regions contribute to the processing of emotional experiences.

The sample size in this study was relatively small for the statistical methods employed. Functional connectivity and regression analyses require large sample sizes to minimize Type II error that can potentially occur from random or atypical sampling. The potential for Type II error, in this case falsely rejecting the null hypothesis of equivalent correlations, is noteworthy when the sample size falls under 20 (Stevens, 1996), thus reaching significance in this smaller group may be more a function of power than true differences between the groups. Fluctuations in correlations are routine with insufficient sample sizes. Also, these methods do not address issues of multiple comparisons. The functional connectivity in the patients with bipolar disorder often displayed a similar pattern as seen in the healthy controls

at baseline, providing some internal replication, but not always. Therefore, these results in this exploratory study should be viewed with caution and require replication.

The demographics of the sample limit the extrapolation to bipolar illness, in general. These patients were predominately rapid-cycling, treatment-refractory BPII sub-type. They also had previous medication trials and were significantly older than the control group (which was statistically controlled when appropriate). Thus, the possibility of medication effects or the existence of significant alterations in brain neurochemistry due to the aging process in this disease versus healthy controls remains to be ruled out.

A third limitation is the inability to segment regions that are solely cholinergic, given the lack of cholinergic specificity of the basal forebrain neurons. It is well known that the cholinergic neurons are interspersed with other non-cholinergic neurons, most notably GABAergic projections, thus regional CBF is related to diverse neuronal influences beyond cholinergic activity. Despite this, the cholinergic functional attributes of the basal forebrain are of major significance and deserve further more specific dissection in the study of emotion and memory.

Lastly, with respect to amygdala findings, results reported here may differ slightly from the work of Ketter et al. (1996), due to different methodology. Whereas Ketter et al. (1996) explored small regions of interest in the center of the amygdala (determined by PET images co-registered MRIs) on the unfiltered original PET images not processed, the current study used highly processed PET CBF images that had undergone reorientation, stereotactic normalization and substantial filtering for

signal-to-noise enhancement purposes. The amygdala as whole was markedly activated by procaine and the lack of significant correlations of its global blood flow could reflect a failure to distinguish among its many clusters of small nuclei.

4.4.6 Conclusion

In conclusion, procaine appeared to modify cholinergic pathways as evidenced by alterations in the functional connectivity between cholinergic regions and their targets in healthy controls. The functional connectivity of cholinergic brain regions in patients with bipolar disorder revealed alterations from the pattern observed in healthy controls with procaine administration. These findings supplement those in the literature regarding absolute or relative CBF changes from controls in prefrontal cortex, anterior cingulate, insular cortex, temporal cortex and basal forebrain regions in patients. Moreover, cholinergic regions and their primary targets were differentially associated with emotional responses in patients and healthy controls, suggesting abnormalities in the function of cholinergic networks in patients. This study provides further support for cholinergic involvement in mood disorders as summarized decades ago by Janowsky and coworkers (1972) and more recently by Overstreet and colleagues (1998). Further studies are required to replicate this finding as well as to examine cholinergic function in mood disorders with other cholinergic agents that modify brain activity.

Chapter 5: Conclusions and Implications

5.1 Overall Summary

The major findings in both clinical and preclinical studies support the notion of cholinergic involvement in emotion regulation. This is suggested by the binding of procaine to muscarinic M_2 receptors *in vivo* in the preclinical experiment and the association of blood flow changes in cholinergic basal forebrain areas with brief intense emotional responses induced by procaine in the clinical experiment. Procaine blocked the binding of a muscarinic ligand in a dose-related manner (IC_{50} = 1.31 μM), globally and uniformly across the primate brain, suggesting direct binding with M_2 muscarinic receptors. In addition to the muscarinic binding, the cerebral blood flow, as measured by K_1, significantly increased globally and regionally in the anterior paralimbic regions on all doses. The largest K_1 changes occurred at plasma levels near the IC_{50}. This suggests that the K_1 effects associated with the lower doses could possibly be related to M_2 muscarinic blockade. Moreover, the pattern of K_1 increases in this study is similar to the blood flow increases seen globally and regionally in anterior paralimbic areas observed in humans (Ketter et al. 1996). If comparable occupancy occurs in humans as in this study with monkeys, a cholinergic mechanism of procaine's limbic activation is viable.

It cannot be determined from this study whether procaine is acting as an agonist or antagonist on the muscarinic receptors. Agonist activity is supported by the following results: cholinomimetics induce kindling similar to procaine (Wasterlain et al. 1981); physostigmine weakly facilitates procaine-induced kindling

while atropine slows this process (Heynen et al. 1995); and procaine induces membrane voltage changes that are short acting and reversible (Isreal, 1979). Moreover, the clinical effects of physostigmine share similarities to procaine (Janowsky et al. 1986).

Conversely, antagonist activity is suggested by procaine competitively inhibiting acetylcholine-induced contraction (Ishii and Shimo, 1984) of guinea pig cecum. Furthermore, procaine inhibits opening of cation channels on guinea pig ileal smooth muscle cells thus affecting the acetylcholine induced cationic currents; GTPγS currents are inhibited in a similar manner (Chen et al. 1993). Lastly, procaine is known to have a biphasic effect on neuronal excitation with anticonvulsant activity at lower concentrations and pro-convulsant activity at higher concentrations (De Jong 1994; Foldes et al. 1965; Foldes et al. 1960). It is possible that procaine could also have biphasic effects on cholinergic neurotransmission yielding both agonist and antagonist activity.

The biphasic actions could stem from numerous sources. Procaine may initiate its effects by preferential affinity to cholinergic, sigma, and serotonergic receptors, but their interactions among these and with other neurotransmitter systems, could yield opposing effects. In addition, with regard to cholinergic receptors, activation may be coupled to inhibitory (M_2) or excitatory (M_1) neuronal effects.

Procaine, as a limbic selective probe that binds to cholinergic M_2 receptors, appears to be a useful way of assessing emotion regulation in healthy individuals and patients with psychiatric disease. The second experiment examined emotion regulation using several approaches based on a cholinergic model that resulted in

three principle findings: 1) with traditional brain imaging activation methodology, treatment-refractory patients with bipolar illness exhibited equivalent emotional responses, but blunted anterior paralimbic rCBF increases to procaine administration; 2) analysis of the functional associatively of cholinergic forebrain regions with and without procaine administration demonstrated cholinergic pathway modulation as evidenced by enhanced associative relationships on procaine between regions believed to be connected via cholinergic neuronal pathways in patients and controls; and 3) blood flow changes in core regions of cholinergic cell bodies and their targets were correlated with changes in ratings of anxiety and euphoria. However, these associations differed in patients and controls. Cholinergic basal forebrain (NBM and septal area) ROI was directly related to anxiety in controls and inversely related in patients. Only the bipolar patients showed any associative relationships between euphoria ratings and the cholinergic regions in the model.

With regard to the second main clinical finding, in the healthy control group procaine increased the positive associations of cholinergic regions with the anterior cingulate, anterior insula, and striatum, and negative relationships of the hippocampus, temporal pole, and cerebellum. These changes could reflect known cholinergic pathways, including the substantia inominata to prefrontal area, septo-hippocampal and tegmental-midbrain pathways. These results suggest a minor alteration in the proposed AChNet model and are depicted in Figure 32. The MOFC did not show increased associativity with procaine compared to baseline in the controls. This may be a function of the complexity of the neuromodulatory influences on this cortical region.

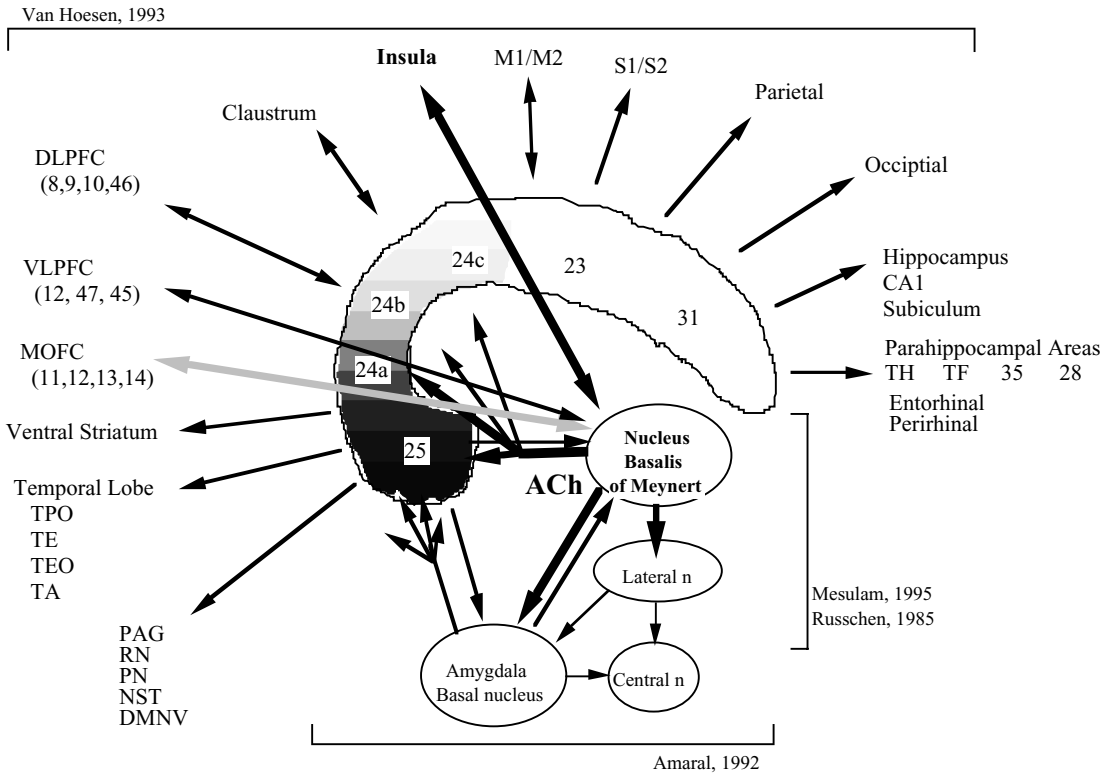

Figure 32. Cholinergic Model in Healthy Controls
The functional connectivity data suggest a modification of the proposed model in Figure 9 in healthy controls. The contribution of the cholinergic innervation of the MOFC appears to be less substantial than the implied by cholinergic pathway analysis in this procaine-infusion paradigm. Shading represents density of dual innervation, direct cholinergic and indirect via the amygdala. Numbers in the anterior cingulate indicate Brodmann areas (1909). Abbreviations: AC, anterior cingulate; ACh, acetylcholine; DLPFC. Dorsolateral prefrontal cortex; DMNV, dorsal motor nucleus of the vagus; DR, dorsal raphe; LC, locus coeruleus; MOFC, medial orbitofrontal cortex; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; NST, n. solitary tract; M1/M2, primary and secondary motor cortex; PAG, periaquaductal grey; PN, pontine nuclei; RN, red n.; S1/S2, primary and secondary sensory cortex; CA1, hippocampus; TF, TH, TE, TEO, TA, TPO, temporal regions designated by Bonin and Bailey (1947); TP, temporal pole; VLPFC, ventrolateral prefrontal cortex; VTA, ventral tegmental area.

Patients with mood disorders compared to healthy controls show significantly weaker positive relationships amongst the cholinergic forebrain regions at baseline, which became stronger and similar to controls with procaine. Procaine increased the strength of positive relationships of these cholinergic regions with anterior cingulate, amygdala, MOFC, DLPFC, posterior temporal cortex, thalamus, and striatum compared to baseline in patients. This pattern in the patients reflects most of the cholinergic pathways, but some discrepancies appear. Specifically, the anterior insula, hippocampus, and temporal pole changes in correlative relationships with cholinergic regions were not observed in the patients. Also, some additional changes in the cholinergic correlative relationships, the amygdala, MOFC, DLPFC, posterior temporal cortex, and thalamus were observed that were not present in healthy controls. These changes form the AChNet are depicted in Figure 33.

These findings are of interest because many of the regions with altered functional connectivity between patients with bipolar illness and controls are also regions found to be hyper-metabolic or hypo-metabolic at baseline in patients compared to controls (Ketter et al., 2001). For example, the anterior cingulate and prefrontal areas are hyper-metabolic in patients in the depressed phase of their illness compared to controls, while the cerebellum and basal forebrain structures, such as the striatum and thalamus, are hyper-metabolic irrespective of mood state in patients compared to controls. Thus, commonly reported alterations in activity in prefrontal, insular, temporal, striatal, thalamic, and cerebellar brain regions in mood disorders (see reviews; Dougherty and Rauch, 1997; Ketter et al., 1997; Drevets, 1998) may have a cholinergic component to their dysregulation.

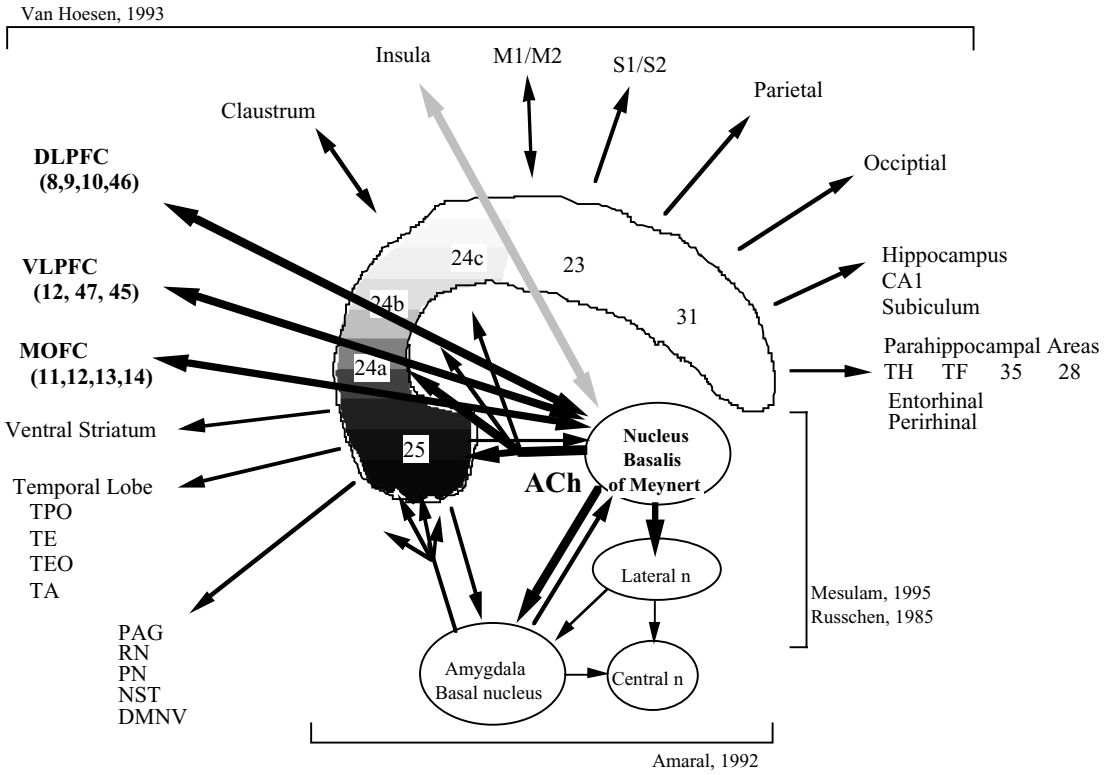

Figure 33. Cholinergic Model in Patients with Bipolar Disorder
The functional connectivity data suggest a different modification of the proposed model in Figure 9 for the patients with bipolar disorder than in the healthy controls. The contribution of the cholinergic innervation of the DLPFC, and VLPFC appears to be more substantial than the implied by cholinergic pathway analysis in this procaine-infusion paradigm. In contrast, the cholinergic innervation of the insula may be less influential than thought based on neuroanatomy. Shading represents density of dual innervation, direct cholinergic and indirect via the amygdala. Numbers in the anterior cingulate indicate Brodmann areas (1909). Abbreviations: AC, anterior cingulate; ACh, acetylcholine; DLPFC. Dorsolateral prefrontal cortex; DMNV, dorsal motor nucleus of the vagus; DR, dorsal raphe; LC, locus coeruleus; MOFC, medial orbitofrontal cortex; Ch4-NBM, cholinergic portion of the nucleus basalis of Meynert; NST, n. solitary tract; M1/M2, primary and secondary motor cortex; PAG, periaquaductal grey; PN, pontine nuclei; RN, red n.; S1/S2, primary and secondary sensory cortex; CA1, hippocampus; TF, TH, TE, TEO, TA, TPO, temporal regions designated by Bonin and Bailey (1947); TP, temporal pole; VLPFC, ventrolateral prefrontal cortex; VTA, ventral tegmental area.

The blunted blood flow response in the anterior cingulate and the increased functional connectivity of the prefrontal cortex could be directly related to M_2 receptors. Recently, significant reductions in M_2 receptor levels, as measured with [^{18}F]FP-TZTP, was observed in the anterior cingulate, as well as in whole brain, striatum and occipital cortex at rest in medication-free patients with bipolar disorder (D. Cannon, personal communication). Notably, blood flow in the hippocampus and posterior cingulate were not reduced. These changes were not observed in patients with major depression. Exacerbated functional connectivity could be a direct result of loss of muscarinic inhibition of excitatory processes. This is further supported by increased functional connectivity of other brain regions, such as the thalamus and the insula, with widespread brain regions (B. Benson, unpublished data). An emerging hypothesis could be that the M_2 receptors play a critical role in the inhibition of excitatory processes in normal cholinergic function, supporting a system of balance in emotion and mood regulation. M_2 receptor loss in patients with bipolar disorder may alter the cholinergic networks such that the normal cholinergic inhibition is no longer in place, and thus creates an imbalance between the cholinergic and excitatory systems. An example at the cellular would be in amygdalar neurons, where a complement of muscarinic receptors (M_1-M_4) mediate the excitatory actions of glutamate (Yajeya et al., 2000), and may contribute to the spontaneous firing of fast-firing neurons that may belong to a population of intrinsic inhibitory neurons (Washburn and Moises, 1993). Loss of M_2 receptors would alter this inhibitory balance. The ideas presented here suggest another mechanism of cholinergic imbalance in mood disorders as proposed by others, e.g., cholinergic / noradrenergic

(Janowsky et al., 1972a, 1972b, 1986), and cholinergic / serotonergic systems (Overstreet et al., 1998), that may be contributing to the emotion and mood dysregulation these patients experience.

The neuroanatomical distribution of the cholinergic system in limbic structures overlaps with the pattern of selective limbic activation induced by procaine (Ketter et al., 1996). Telencephalic cholinergic projections originate in the basal forebrain nuclei, such as the substantia inominata and nucleus basalis, and send efferents to most of the cortex as well as to the basolateral amygdala and anterior cingulate (Mesulam 1995; Mesulam et al. 1983). Both the amygdala and the anterior cingulate send efferents to the basal forebrain cholinergic nuclei, suggesting feedback modulation of their own afferents (Russchen et al., 1985a, 1985b; Amaral et al., 1984, 1989, 1992). These reciprocal connections create an opportunity for enhancement, diminution, or refinement of neuronal activity related to the valence and saliency of the environment stimuli, thus affecting potential behavioral outcome (Mesulam, 1995; Heimer and Alheid, 1991, Záborszky, 1993). The cholinergic drive to the amygdala has been hypothesized to an essential component of the "general emotion system" (LeDoux, 1996).

The anterior cingulate may well be another structure integral to emotion regulation. The pregenual and subgenual regions of the anterior cingulate activate less so in the patients compared to controls with procaine compared to baseline. In addition, the subgenual / Drevets region of the anterior cingulate was one of the three regions of the core cholinergic model were associated with anxiety ratings in both patients and controls. This region is reported to have reduced glial and neuronal cell

numbers in bipolar and unipolar illness (Drevets et al., 1997, Öngur et al., 1998; Rajkowska, et al., 1999; Rajkowska, 2000) that may be a function of altered cholinergic and NGF influences from the basal forebrain. In addition, the pregenual anterior cingulate was associated with euphoria ratings in patients only. These relationships are in agreement with the literature of emotion induction studies, where the anterior cingulate was one of the regions most often activated (Ketter et al., 2003).

In addition to the anterior cingulate, the functional response of the DLPFC in patients with bipolar illness compared to healthy controls exhibited differential responses: 1) DLPFC is hypo-perfusional at baseline in patients compared to controls; 2) DLPFC activity increases more so in patients compared to controls with procaine (interaction results); and 3) there is increased correlative strength between DLPFC and BF cholinergic ROIs with procaine in patients compared to controls, yet similar relationship of change in DLPFC activity with anxiety response in both patients and controls. Combined, these differences suggest that the DLPFC of the patients maybe be under-functioning at rest, and with procaine activity is over-driven possibly via cholinergic mechanisms to yield equivalent (to controls) emotional response of anxiety. The DLPFC is one of the components mediating working memory as part of the general emotion system put forth by LeDoux (1996). Thus, all three regions of the final model explaining the variance in anxiety ratings generated by the multivariate multiple regression were integral components to his theory.

The basal forebrain ROI exhibited differential responses in the patients and controls with respect to the associations with anxiety and euphoria ratings induced by procaine. The subcortical region (BF) exhibited inverse relationships with emotion

ratings of anxiety, while DLPFC remained similar in patients and controls according to the multivariate multiple regression model. This is suggestive of alterations in the neural networks subserving emotion in patients with bipolar illness. The amygdala-triggered arousal-attentional mechanisms are sustained by basal forebrain ACh release that influences the anterior cingulate and DLPFC (LeDoux, 1996). Thus, in patients the basal forebrain portion of the system seems to be under-active with anxiety responses and over-active with euphoria responses to procaine compared to controls.

The results of the two experiments combined is consistent with the notion that procaine acting preferentially on M_2 muscarinic receptors may participate in the modulation of anxiety in patients and controls, and in euphoria in patients. Since the majority of the patients were moderately depressed in this study, the expectation is the cholinergic-noradrenergic balance would favor cholinergic over-activity in patients according to Janowsky and colleagues (1972). From this perspective, the differential relationship of the cholinergic BF region with emotion ratings in patients compared to controls is consistent with their work. The enhanced strength of the euphoria – BF strong relationship in patients supports these ideas well. However, the opposite relationship of anxiety ratings with BF rCBF in patients and controls is only indirectly supportive in that differences exist from controls. Additional support comes from the correlation of increased blood flow in the amygdala, MOFC, and a small region in the cholinergic forebrain area with severity of mood (HDRS).

For the most part the emotional responses were predominately dichotomous – categorized as euphoria or dysphoria. This was true for both patients and controls.

Although changes in basal forebrain blood flow was associated with a change in anxiety ratings in an opposite manner in patients and controls, eventual procaine-induced emotional response was not predicted by any baseline clinician or self-ratings in the patients. For example, in the patients HDRS scores were correlated with SSAS, baseline depression and anxiety ratings, and depression ratings with procaine. SSAS scores correlated with baseline depression, fear and anxiety ratings, but not with ratings collected during procaine administration. Spielberger self-ratings correlated with baseline depression, fear and anxiety ratings, and depression ratings with procaine. Thus, current mood state did not "predict" a euphoric or dysphoric response. These results were similar in the controls. While the controls would not have sufficient range in the clinical and self-ratings collected at baseline for predictive power, the baseline likert-based ratings of emotions were not predictive of eventual procaine-induced emotion response (T. Ketter, personal communication).

The basis for this apparent lack of prediction remains to be determined. Most likely, the procaine-induced emotional and sensory experiences are a result of a combination of factors that could not measured during this study. Possible explanations could include the following. Analogous to context-dependent (either environmental or conditioning) differential drug response, the relative neurochemical equilibrium at the time of infusion, as well as how procaine affects these neurochemical systems may provide different "starting points" that would result in either euphoria or dysphoria. In a related manner, it might be feasible that the recent history of these systems may be influencing responses in the near future. A second possibility is the relative complement of muscarinic receptor subtypes and/or balance

of muscarinic receptors with other receptors, such as GABA, may influence a response in either direction. Additionally, the respective complements of other receptors with similar high affinity to procaine, such as sigma-1, 5-HT_3, and 5-HT_{1A} receptors, could produce these differential effects. However, in the controls, repeated administrations of procaine with subsequently higher doses show that within minutes on the same day in an individual can have either response (R. Post, personal communication). This would argue in favor of the relative equilibrium of the affected neurochemistry shifts over time.

5.2 Implications

5.2.1 Basal Forebrain Arousal Mechanisms

The findings in this study suggest that procaine-induced emotional experiences are associated with blood flow increases in basal forebrain, the amygdala and prefrontal cortical regions, including the anterior cingulate and DLPFC. Exactly how procaine, which binds to muscarinic receptors, could produce blood flow activation may be explained by procaine acting as an agonist on both M_1 and M_2 receptors. Procaine was shown to bind to M_2 receptors i*n vivo* in the first experiment presented herein. However, procaine has been shown to bind to M_1 receptors *in vitro* (Sharkey et al., 1988).

First, as would be expected, agonist activity on M_1 receptors would increase cortical excitability. The binding of procaine to M_1 receptors results in an intracellular response to increase PI turnover and phosphorlylation of Ca^{++} and K^+ channels to have an overall effect of neuronal excitation. Activation of M_1 receptors

on cortical pyramidal cells would initiate the release of the excitatory amino acid glutamate, and subsequent downstream arousal. This would occur in conjunction with M_1 activation on the amygdala, as well, which may cause the release of ACh from the basal forebrain.

The basal forebrain, including the NBM and the amygdala, has been implicated in cortical arousal mechanisms. NBM activity and the associated release of ACh correlated with elevated behavioral arousal (Buzasáki and Gage, 1989), with anticipation of highly predictable reward (Inglis et al., 1994), with induction of slow-wave sleep (Nuñez, 1996) and during production of low-voltage fast EEG activity (Celesia and Jasper, 1966). Ch4-NBM lesions may inhibit the cortex to attend and process brief, highly salient sensory stimuli (Robbins and Everitt, 1995). Thus, for the most part, acetylcholine release appears to stimulate the brain.

A second route to cortical arousal from muscarinic receptor activation may occur via agonist activity on M_2 receptors. M_2 receptor activation results in a decreases in cAMP and a subsequent decrease in the phosphorlylation of Ca^{++} and K^+ channels; the overall effect of M_2 activation is that of inhibition. Activation of M_2 receptors on GABAergic neurons could inhibit the inhibitory effects of GABA on the cortical pyramidal cells, i.e., a release of inhibition would occur. Thus, activation of M_2 receptors could also result in glutamatergic release from cortical pyramidal cells and amygdalar-basal forebrain activation as described with M_1 binding.

Stimulation of the NBM *in vivo* results in EEG activation originating from the cortex, depolarizes cortical neurons, and changes large-amplitude, slow oscillations into low-amplitude, fast oscillations (Metherate et al., 1992). Furthermore,

endogenous ACh applied to the cortex *in vitro* was associated with the replacement of slow, rhythmic discharges to higher-frequency, single spike discharges, and facilitates the appearance and magnitude of intrinsic membrane potential oscillations. Modulation of these cholinergically mediated cortical oscillations may be counter-balanced by GABA (Jones, 2004).

Záborszky hypothesizes that these fast cortical oscillations (20-40 Hz) are part of a basal forebrain-prefrontal cortical loop. The basal forebrain exhibits topographical organization of prefrontal cortex efferents that are positioned to coordinate cortical oscillations with other cortical loops, thus building larger functional networks. Moreover, they may work concurrently with cortico-thalami-cortical or brain stem-thalamic oscillators (Steriade, 1990). Oscillations in the basal forebrain regions maintained by cholinergic cells (Metherate et al., 1992), which rely on NGF to sustain their viability, but also have intrinsic pacemaker qualities (Khateb et al., 1992). Muscarinic cholinergic synapses on modules of pyramidal cells in the cortex may serve as an integral component.

Cholinergic mechanisms in mediating amygdalar neuronal activity (Yajeya et al. 1997) have been identified postsynaptically (Washburn and Moises 1992) and presynaptically (Sugita et al. 1991). Moreover, similar neuronal depolarization responses due to cholinergic mechanisms have been implicated in other paralimbic structures such as hippocampus, entorhinal and piriform cortices and anterior cingulate (Benson et al. 1988; Colino and Halliwell 1993; Hasselmo and Bower 1992; Klink and Alonso 1997; McCormick and Prince 1986), as well as septum, basal forebrain, striatum, thalamus and neocortical structures (Hasuo et al. 1988; Hsu et al.

1995; McCormick and Prince 1987; Szerb et al. 1994). Thus, it is possible that procaine acting on M_2 receptors could initiate bursting activity and enhance firing in limbic regions. These results suggest that the interaction of muscarinic receptors with the local neuronal environment could contribute to procaine's limbic effects.

The amygdala may also participate in oscillation systems. A majority of amygdala projections are reciprocal, suggesting an anatomical basis for affective processing phenomena described over 40 years ago. The amygdala has intrinsic 40 Hz oscillations that are hypothesized to be a source of binding emotional memory (Gault and Coustan, 1965). The broad rostral cortical region innervated by the basal nucleus suggests it may have a significant role in attention, i.e., it may be acting as a general amygdalar switch that brings all the cortical regions "on-line" (activated online) when emotional processing is required to modulate executive functions. The other amygdalar nuclei may contribute more specifics about the relevance of the ongoing experience. The amygdala is considered an integral component of the neural network subserving emotion, encompassing the assessment of emotional valence and the expression of emotion.

Cholinergic innervation from the basal forebrain of the amygdala and prefrontal cortex may contribute to oscillations associated with emotional memories as well as the conscious experience of emotions. Pelletier and Pare (2004) outline a detailed method how oscillations in the amygdala may contribute to the consolidation of memory with emotional contexts. The basolateral amygdala increases its discharge rate in fear conditioning paradigms and directly affects cortical activity. The short recurring time windows between these phase-locked oscillations may facilitate of

synaptic plasticity. As the basolateral amygdala recruits the cholinergic basal forebrain to release ACh to cortical structures, the anterior cingulate and DLPFC, to initiate and maintain attention during emotional situations (LeDoux, 1996), a scenario involving muscarinic and glutamatergic receptors could be envisioned. Cortical synaptic plasticity could involve muscarinic and glutamatergic activation of cortical pyramidal cells, which stimulate glutamate release onto NMDA receptors to encode the new memories. This could serve as a basis for working memory in the DLPFC. These associations may be enhanced via a second phase of "replay" in sleep or through continued oscillations resulting in long-term synaptic changes of prefrontal networks.

The findings in this study suggest that the basal forebrain, the amygdala and prefrontal cortical regions, including the anterior cingulate and DLPFC, are associated with the emotional response induced by procaine. These same regions are integral to LeDoux's theory of a "general emotion system." Cholinergic modulation of prefrontal cortex is essential to modulate the attentional systems required to maintain emotional processing in working memory. An emerging hypothesis regarding emotion could be as follows. Utilizing the circuitry outlined by Záborszky, the loops through the amygdala and prefrontal cortex are modulated by basal forebrain activity. Oscillations in the basal forebrain regions maintained by cholinergic cells (Metherate et al., 1992), which rely on NGF to sustain their viability, also have intrinsic pacemaker qualities (Khateb et al., 1992). Muscarinic cholinergic synapses on modules of pyramidal cells in the cortex may serve as an integral component. This muscarinic modulation may build intracellular coherence and

collapse of microtubules consisting of MAP-2 proteins that are regulated by cholinergic mechanisms (Woolf, 1997, 1999). These processes, occurring in a timeframe of 250 msec, are hypothesized to be a basis of consciousness. Moreover, Robbins and colleagues (1995, 2002) hypothesize that cholinergic mechanisms are essential to the maintenance of selective attention. It is an easy progression to extend these ideas to emotion regulation. Indeed, MAP-2 levels in the subgenual anterior cingulate have been shown to be decreased (29.5%) in bipolar disorder (Bouras et al., 2001). The muscarinic modulation of oscillations in the specific prefrontal, amygdalar, basal forebrain loops may support the coherence and self-collapse of the microtubules. Thus, it is the ebb and flow of these intracellular infrastructures in these limbic areas that may sustain attention on emotional experiences. The influence of cholinergic receptor activation on synaptic plasticity leading to the formation of emotional memories and intracellular mechanisms linked to the timing of conscious thoughts suggest that the muscarinic cholinergic system may be tied to emotional processing more intimately than previously believed.

5.2.2 Clinical Applications

Historically, procaine has been used in geriatric depression (De Jong, 1994), but has fallen out of favor in preference to tricyclic antidepressants and SSRIs. A case study showed that procaine can reduce depressive symptoms (Ketter, personal communication). However, tolerance develops quickly and successively higher doses are required to achieve the same benefit. This, as well as the potential for developing seizures at higher doses, makes procaine an unlikely candidate for the treatment of

major depression or bipolar disorder. However, these limited results suggest that the cholinergic system may be another avenue for the treatment of depression to be modulated in conjunction with the serotonergic and noradrenergic mediation.

SSRIs have recently been identified as having cholinergic effects in addition to their primary serotonergic mechanisms of action. The anticholinergic effects of tricyclic antidepressants and SSRIs have been traditionally thought as side effects, such as dry mouth and difficulty in urination. However, recent data have been showing that cholinergic effects may be contributing to alleviating symptoms of depression (Burt et al., 1999). In a small cohort of patients with bipolar disorder, 6 of 11 (54.5%) showed marked improvement with an open trial of donepezil. Others have shown there is an increased proclivity to cause mood switches into mania compared with less anticholinergic antidepressants (Guille et al. 1999; Peet 1994). On the other hand, donepezil relieves some of the anti-cholinergic adverse effects with some risk of mania induction (Jacobsen and Comas-Diaz 1999), indicating that maintaining cholinergic balance may be important to mood disorders. These studies suggest that controlled clinical trials are indicated to explore the efficacy of cholinergic agents in mood disorders.

The cholinergic system most likely works in concert with many other neurotransmitters and neuromodulators to produce its effects on emotion regulation and the brain. Presumably these influences work in an integrated fashion such that a change in one system modifies other systems in turn. In this regard, the cholinergic system may be just one avenue for modulating other systems, for instance

serotonergic, noradrenergic, or dopaminergic, that together contribute to emotion regulation.

5.2.3 The Cholinergic System from an Evolutionary Perspective.

If one accepts the view that the basal forebrain cholinergic system is involved in emotion regulation, then the question emerges of how and when this connection may have occurred with respect to phylogenetic development. From a behavioral perspective, basic survival needs underlie the motivation and emotions, which are inextricably related. The clustering of cell bodies in the basal forebrain and brainstem, regions that are relatively newer and older phylogenetically, indicate that this system has been developing over time. The cholinergic basal forebrain system is considered to be a conserved system, emerging with tetrapods, but also may have originated independently in teleosts (Semba, 2004).

Moreover, the phylogenetic development in terms of size and complexity of the BF region and it projections parallel the evolution of the brain. The number of neurons and complexity of the innervation is relatively small in fishes, with the exception of teleosts, compared to humans and cetaceans (Semba, 2004). The increase in the size of the cortical mantle from fishes to humans predicates neuronal systems for integration and support of the neural processing therein. At some point in evolution, presumably with the emergence of bony fishes, the creation of a second locus of cholinergic cell bodies in the basal forebrain was apparently adaptive. Semba (2004) hypothesizes that this increase in size and complexity may be an

evolutionary adaption to support "higher" functions associated with a more complex cortex.

Several outstanding questions remain. 1) Why did this occur? Semba (2004) hypothesizes that this increase in size and complexity may be an evolutionary adaption to support "higher functions" associated with a more complex cortex. 2) Why was it the cholinergic system, and not some other system? 3) Why was it only the cholinergic system? 4) What advantage does the cholinergic system have that made it the candidate to emerge with a forebrain system? One answer to all these questions may be that the cholinergic system is considered to be activating, a behavioral response that is in agreement with the notion of survival; when challenged with a threatening or an approachable situation, appropriate activation may serve the individual well. For example, a threatening situation requires a fear response and the appropriate behavioral reaction to ensure safety. An approachable situation may be the presentation of another individual to engage in bonding. In both situations, successful emotion regulation serves to engender social and personal success of the individual while maintaining safety and integrity.

While the focus has been on the cholinergic system, it most likely works in concert with many other neurotransmitters and neuromodulators to produce its effects on emotion regulation and the brain. The brainstem monaminergic and cholinergic nuclei emerged much earlier than the cholinergic forebrain nuclei. Presumably these influences work in an integrated fashion such that a change in the cholinergic system modifies these other systems in a complementary manner.

5.3 Explanation of Anomalous Results

The first experiment demonstrated, as hypothesized, that procaine blocks the muscarinic receptors *in vivo* while increasing blood flow in limbic areas. The second experiment showed that procaine increased the functional connectivity between core cholinergic forebrain regions and its primary targets in healthy controls and patients with mood disorders. In addition, the core cholinergic regions were associated with anxiety in healthy controls and patients with mood disorders, and varied with euphoria only in the patients. There was a lack of the expected strong relationship of anxiety ratings with rCBF in MOFC in healthy controls. Sarter and Bruno (2002) argue that the cholinergic and GABAergic inputs to the cortex give rise to a complex mixture of excitatory and inhibitory influences. Instead, rCBF in DLPFC in conjunction with the BF and amygdala increased with increasing anxiety ratings. These results are not entirely unexpected, as abnormal activity in DLPFC has been implicated in mood disorders. However, it is primarily the amygdala and anterior cingulate that are most commonly activated in emotion induction studies (Ketter et al., 2003). Kimbrell and colleagues reported increased MOFC CBF with induction of anxiety (1999). The differences between their work and this study may lie in the different method of emotion induction (self-induced vs pharmacological), but also in the mixture of emotions generated by procaine.

Correlations between the amygdala and the cholinergic ROIs did not increase with procaine as one might have expected based on the heavy cholinergic innervation of the amygdala. In addition, when examining relationships between procaine-induced emotion ratings and the cholinergic model, changes in the amygdala were not

implicated. Although initially associated with anxiety ratings in both patients and controls, the amygdala was not part of the final model. The methodology employed for this study may have occluded any potential relationships. The utilization of stereo normalization may have over-processed the data such that smaller regions like the amygdala may have lost their "signal." Co-registration of subjects's MRI to their PET data would significantly improve the ability to measure amygdala activity. This method was not chosen because of the number of regions and subjects included in the study. Each measurement is time-intensive; with 47 subjects and 63 regions, the amount of time required to complete the measurements was prohibitive. The alternative of using MRI-directed measurement of non-stereo normalized amygdala in conjunction with the remainder of the regions measured with stereo normalized data was considered and rejected as this type of mixing methodologies is generally not recommended.

With the stated goal of relating the emotional response of procaine to specific brain regions, the multivariate multiple regression deliberately limited the regions assessed to test a cholinergic hypothesis. This may have resulted in altered associative relationships that might be incomplete, because of masked or missed important relationships with other brain regions. Future studies designed to test other hypotheses regarding functional connectivity with other ligands will help provide a more complete picture of how the brain processes emotion.

5.4 Study Refinement and Future Directions

This work could be improved in several ways. The first experiment for the most part yielded the desired outcome, however, there are three areas in particular that could be enhanced. First, the data showed significant blockade at the lowest dose tested that had a sight hint of evidence of differential binding for low and high receptor affinity states. Additional procaine doses below the lowest tested could tease out the likelihood of this case, beyond just completing the binding curve. There is evidence that suggests the muscarinic receptors have high and low affinity states mediated by allosteric binding (Krejci et al., 2004).

Second, the cerebral blood flow (CBF) measures were taken from a non-traditional method. Although, the flow of any radiotracer can be related to the true cerebral blood flow (best measured with $H_2{}^{15}O$ studies), how highly they correlate can range from poor to quite well. In the case of [^{18}F]FP-TZTP, the correlation between $H_2{}^{15}O$ CBF studies and flow measured from [^{18}F]FP-TZTP proved to be sufficiently high ($r=0.85$; Carson et al., 1998), yielding good confidence in the [^{18}F]FP-TZTP flow measures. However, utilizing the standard method of measuring CBF would leave less to question. On each study day, the performance of $H_2{}^{15}O$ studies with each dose before the [^{18}F]FP-TZTP study would have given the additional information with respect to the effects of procaine on cerebral blood flow (the CBF studies would have to be before the binding studies because the half-life of the [^{18}F]FP-TZTP ligand is fairly long (180 minutes), while $H_2{}^{15}O$ ($t_{1/2}$ = 2 minutes) could be cleared well before the onset of the binding study). However, since the primary goal was to measure muscarinic receptor occupancy, the experiment was

designed and executed as reported in the first experiment. Altering the study to include CBF studies could significantly weaken the strength of the binding experiment. It is very possible that the first application of procaine for CBF measurement could alter the binding profile attained during the [^{18}F]FP-TZTP experiment.

Third, another way to improve the first experiment would have been to add more subjects. Although the three subjects were very close in dose response, additional subjects would result in greater confidence in the results. The number of subjects was limited due to pilot work indicating strong effect size, to reduce the cost of the study, and the desire to limit the unnecessary use of animals in research.

The second experiment also could be improved in several ways. First, the most serious problem was the retrospective and exploratory nature of the study. Utilizing data already collected to test the cholinergic hypothesis of emotion regulation limits the potential questions that can be asked and thus in turn the conclusions and implications that can be reliably drawn. Specifically, hypotheses related to potential cholinergic or anticholinergic effects of procaine would be beneficial in explaining the role of cholinergic modulation of emotion and mood.

An improved study design would have been to examine the psychological effects of procaine with and without procaine administration while measuring muscarinic receptor occupancy with FP-TZTP. The degree of receptor occupancy would be correlated with degree of psychological effects induced with procaine. A prospective study was not possible because of the provocative nature of a procaine challenge. Studies of agents that potentially induce strong psychological effects

require a very strong scientific rationale and receive high scrutiny by Institutional Review Board (IRB) for ethical and scientific soundness. This type of study is also is time-intensive, taking years to enroll subjects and collect the data.

The ability to extrapolate these findings extrapolate to bipolar illness, in general, is another area for improvement. The patients in this study were primarily treatment-refractory and rapid-cycling BPII subtype. Therefore, the findings in this study apply only to this sub-group. The demographics of the sample limit the extrapolation to bipolar illness, in general. These patients were predominately rapid-cycling, treatment-refractory BPII sub-type. They also had previous medication trials and were significantly older than the control group (which was statistically controlled when appropriate). Thus, the possibility remains that medication effects or the aging process on the brain in this disease is different than normal has significantly altered brain neurochemistry. Including a broader range of patients with bipolar disorder would benefit the understanding of this heterogeneous illness.

Several studies would be useful to further explore the role of the cholinergic system in emotion regulation. 1) Increase the sample size and sub-types of patients and controls to apply more sophisticated analysis methods, such as path analysis. 2) Using PET and FP-TZTP, measure simultaneously muscarinic receptor occupancy and rCBF responses to procaine in healthy controls and patients with mood disorders with the goal of directly correlating the degree of occupancy with emotional responses, as mentioned above. 3) Compare the muscarinic receptor occupancy with other known muscarinic agents, such as arecoline, in healthy controls and patients with mood disorders. This would expand the understanding of cholinergic agents

effects on emotions. 4) Examine receptor occupancy with other radioligands, such as WAY10035 (binds to 5-HT_{1A} receptors) or raclopride (binds to D_2 receptors), during procaine administration. This could yield comparative data of how the potential binding of procaine to cholinergic, serotonergic and dopaminergic systems may contribute to its effects on cerebral blood flow and emotional and sensory experiences.

5.5 Conclusion

Intravenous procaine binds to muscarinic M_2 receptors *in vivo* while selectively increasing blood flow in anterior paralimbic brain regions in anesthetized monkeys. Procaine selectively increased anterior paralimbic blood flow in conjunction with strong emotional and sensory experiences to a lesser degree in patients with bipolar disorder compared to healthy controls. Procaine increased functional connectivity between core cholinergic forebrain regions and their targets in healthy controls and patients. Group differences in associativity between core cholinergic regions and prefrontal cortex implicate cholinergic involvement in the dysfunction of prefrontal regions that are commonly reported to be abnormal in patients compared to controls. Procaine-induced anxiety was associated with increased blood flow in cholinergic forebrain region in controls and decreased blood flow in the same region in patients. Moreover, procaine-induced euphoria was directly related to core cholinergic regions in patients, but not in controls. These findings are consistent with the notion that the cholinergic system is intimately involved in emotion regulation, and moreover, its modulatory effects are altered in patients compared to controls.

Appendices

A. O-15 Mood Questionnaire

B. Extended Hamilton Psychiatric Rating for Depression

C. Young Mania Rating Scale

D. Beck Depression Inventory

E. Spielberger State Anxiety Scale

F. Correlative Relationships Between Cholinergic Brain regions and ROIs Across the Brain in 32 Healthy Controls Using Comparative methods of Steiger (1980)

G. Correlative Relationships Between Cholinergic Brain regions and ROIs Across the Brain in 15 Patients with Bipolar Disorder Using Comparative methods of Steiger (1980)

H. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls at Baseline

I. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls with Procaine Administration

J. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls - Delta CBF / Delta Rating

K. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder at Baseline

L. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder with Procaine

M. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder - Delta CBF / Delta Rating

N. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 32 Healthy Controls – Negative Emotions

O. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 32 Healthy Controls – Positive Emotions

P. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 15 Patients with Bipolar Disorder – Negative Emotions

Q. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 15 Patients with Bipolar Disorder – Positive Emotions

Appendix A. O-15 Emotion and Mood Questionnaire

Name: IV: L R
Date: Art Line: L R
Hospital #:
Date:

Rating Scale: 0 1 2 3 4 5 6
None v. slight slight mild mod severe v. severe

1.) What is your mood? How do you feel? (0 – 100): _____

2.) Please rate each of the following:

a. depression	0	1	2	3	4	5	6
b. euphoria	0	1	2	3	4	5	6
c. fear	0	1	2	3	4	5	6
d. anxiety	0	1	2	3	4	5	6
e. anger	0	1	2	3	4	5	6
f. calmness	0	1	2	3	4	5	6
g. tiredness	0	1	2	3	4	5	6

3.) Overall, please rate how "good" or "bad" you feel using 0 = worst ever; 6 = best ever.

0 1 2 3 4 5 6

4.) Have you noticed any unusual physical sensations in the following body areas? Please describe and rate.

a. Head:	0	1	2	3	4	5	6
b.Chest, Back or Abdomen:	0	1	2	3	4	5	6
c. Arms or Legs:	0	1	2	3	4	5	6
d. Groin	0	1	2	3	4	5	6

5.) Have you noticed anything unusual with your hearing? If so, please describe and rate.

0 1 2 3 4 5 6

6.) Have you noticed anything unusual with your vision? Describe and rate.

0 1 2 3 4 5 6

7.) Have you noticed anything unusual with your sense of taste? Describe and rate.

0 1 2 3 4 5 6

8.) Have you noticed anything unusual with your sense of smell? Describe and rate.

0 1 2 3 4 5 6

9.) Have you noticed anything unusual about your thoughts or thinking? Describe and rate.

a. Racing thoughts? Yes / No

0 1 2 3 4 5 6

b. Slowed thoughts? Yes /No

0 1 2 3 4 5 6

c. Preoccupation with a particular thought or type of thought? No/ Yes _____

0 1 2 3 4 5 6

10.) Do you have other comments about what you have been experiencing or thinking?

Appendix B. Extended Hamilton Psychiatric Rating for Depression

Name: _____

Date:_____

For each item, select the "cue" which best characterizes the patient. Record the answer in the space provided.

_____**1. DEPRESSED MOOD** (Sadness, hopeless, helpless, worthless)

0. Absent.
1. These feeling states indicated only on questioning.
2. These feelings spontaneously reported verbally.
3. Communicates feeling states non-verbally, i.e., through facial expression, posture, voice, and tendency to weep.
4. Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and non-verbal communications.

_____**2. FEELINGS OF GUILT**

0. Absent.
1. Self reproach; feels he has let people down.
2. Ideas of guilt or rumination over past errors or sinful deeds.
3. Present illness is a punishment. Delusions of guilt.
4. Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations.

_____**3. SUICIDE**

0. Absent.
1. Feels life is not worth living.
2. Wishes he were dead or any thought of possible death to self.
3. Suicidal ideas or gesture.
4. Attempts of suicide (any serious attempt rates 4).

_____**4. INSOMNIA, EARLY**

0. No difficulty falling asleep.
1. Complains of occasional difficulty falling asleep i.e., more than 1/2 hour.
2. Complains of nightly difficulty falling asleep.

_____**5. INSOMNIA, MIDDLE**

0. No difficulty.
1. Patient complains of being restless and disturbed during the night.
2. Waking during the night- any getting out of bed rates 2 (except for purposes of voiding).

_____**6. INSOMNIA, LATE**

0. No difficulty.
1. Waking in early hours of the morning but goes back to sleep.
2. Unable to fall asleep again if gets out of bed.

_____**7. WORK AND ACTIVITIES**

0. No difficulty.
1. Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies.
2. Loss of interest in activity, hobbies or work- either directly reported by patient or indirect in listlessness, indecision and vacillation (feels he has to push self to work or activities).
3. Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores.
4. Stopped working because of present illness. In hospital, rate 4 if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted.

Appendix B. Cont'd.

_____**8. <u>RETARDATION</u>** (Slowness of thought and speech; impaired ability to concentrate; decreased motor activity)

0. Normal speech and thought.
1. Slight retardation at interview.
2. Obvious retardation at interview.
3. Interview difficult.
4. Complete stupor.

_____**9. <u>AGITATION</u>**

0. None.
1. "Playing with" hands, hair, etc.
2. Hand-wringing, nail-biting, hair-pulling, biting of lips**.**

_____**10. <u>ANXIETY, PSYCHIC</u>**

0. No difficulty.
1. Subjective tensions and irritability.
2. Worrying about minor matters.
3. Apprehensive attitude apparent in face or speech.
4. Fears expressed without questioning.

_____**11. <u>ANXIETY, SOMATIC</u>** (Physiological concomitants of anxiety, such as: Urinary frequency; sweating; Gastro-intestinal-dry mouth, wind, indigestion, diarrhea, cramps, belching; Cardio-vascular-palpitations, headaches; Respiratory-hyperventilation, sighing)

0. Absent.
1. Mild.
2. Moderate.
3. Severe.
4. Incapacitating.

_____**12. <u>SOMATIC SYMPTOMS, GASTROINTESTINAL</u>**

0. None.
1. Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
2. Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for G.I. symptoms.

_____**13. <u>SOMATIC SYMPTOMS, GENERAL</u>**

0. None.
1. Heaviness in limbs, back, or head. Backaches, headaches, muscle aches. Loss of energy and fatigability.
2. Any clear cut symptom rates 2.

_____**14. <u>GENITAL SYMPTOMS</u>** (Symptoms such as loss of libido, menstrual disturbances)

0. Absent.
1. Mild.
2. Severe.

_____**15. <u>HYPOCHONDRIASIS</u>**

0. Not present.
1. Self-absorption (bodily).
2. Preoccupation with health.
3. Frequent complaints, requests for help, etc.
4. Hypochondriacal delusions.

REMINDER: PLEASE COMPLETE ONLY PART ONE OR PART TWO OF QUESTION #16.

_____**16. LOSS OF WEIGHT**

When rating by history:

0. No weight loss.
1. Probable weight loss associated with present illness.
2. Definite (according to patient) weight loss.

On weekly ratings by ward psychiatrist, when actual weight changes are measured:

0. Less than 1 lb. weight loss in week.
1. Greater than 1 lb. weight loss in week.
2. Greater than 2 lb. weight loss in week.

_____**17. INSIGHT**

0. Acknowledges being depressed and ill.
1. Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2. Denies being depressed and ill.

_____**18. DIURNAL VARIATION**

A. Note whether symptoms are worse in morning or evening. If no diurnal variation, mark none.

0. No variation.
1. Worse in A.M.
2. Worse in P.M.

_____ B. When present, mark the severity of the variation. Mark "none" if no variation.

0. None.
1. Mild.
2. Severe.

_____**19. DEPERSONALIZATION AND DEREALIZATION:** Such as feelings of unreality, nihilistic ideas.

0. Absent.
1. Mild.
2. Moderate.
3. Severe.
4. Incapacitating.

_____**20. PARANOID SYMPTOMS**

0. None.
1. Suspicious.
2. Ideas of reference.
3. Delusions of reference and persecution.
4. Incapacitating.

_____**21. OBSESSIONAL AND COMPULSIVE**

0. Absent.
1. Mild.
2. Severe.

_____**22. FATIGABILITY** (Low energy level, feeling heavy, leaden, weighed down)

0. Does not feel more fatigued than usual.
1. Feels more fatigued than usual, but this has not impaired function significantly; less frequently than in (2).
2. More fatigued than usual; at least one hour a day; at least three days a week.
3. Fatigued much of the time most days.
4. Fatigued almost all the time.

_____**23. SOCIAL WITHDRAWAL**

0. Interacts with other people as usual.
1. Less interested in socializing with others but continues to do so.
2. Interacting less with other people in social (optional) situations.
3. Interacting less with other people in work or family (necessary) situations.
4. Marked withdrawal from others in work or family (necessary) situations.

Appendix B. Cont'd.

_____**24. APPETITE INCREASE**

0. No increase in appetite.
1. Wants to eat a little more than usual.
2. Wants to eat somewhat more than usual.
3. Wants to eat much more than usual.

_____**25. INCREASED EATING**

0. Is not eating more than usual.
1. Is eating a little more than usual.
2. Is eating somewhat more than usual.
3. Is eating much more than usual.

_____**26. CARBOHYDRATE CRAVING** (In relation to total amount of food desired or eaten)

0. No change in food preference.
1. Eating more carbohydrates (starches or sugars) than before.
2. Eating much more carbohydrates (starches or sugars) than before.
3. Irresistible craving for sweets or starches.

_____**27. WEIGHT GAIN**

0. No weight gain.
1. Probable weight gain associated with present illness.
2. Definite (according to patient) weight gain.

_____**28. HYPERSOMNIA**

Compare sleep length to euthymic and not to hypomanic sleep length. If this cannot be established, use 8 hours.

0. No increase in sleep length.
1. At least 1 hour increase in sleep length.
2. Greater than 2 hour increase in sleep length.
3. Greater than 3 hour increase in sleep length.
4. Greater than 4 hour increase in sleep length.

Was euthymic sleep length used? YES____ NO____
Was 8 hour sleep length used? YES____ NO____

Appendix C. Young Mania Rating Scale

Patient Name: _____

Date:_____

_____ 1. ELEVATED MOOD

0 = Absent
1 = Mildly or possibly increased on questioning
2 = Definite subjective elevation; optimistic, self-confident; cheerful; appropriate to content
3 = Elevated, inappropriate to content; humorous
4 = Euphoric; inappropriate laughter; singing

_____ 2. INCREASED MOTOR ACTIVITY-ENERGY

0 = Absent
1 = Subjectively increased
2 = Animated; gestures increased
3 = Excessive energy; hyperactive at times; restless (can be calmed)
4 = Motor excitement; continuous hyperactivity (cannot be calmed)

_____ 3. SEXUAL INTEREST

0 = Normal; not increased
1 = Mildly or possibly increased
2 = Definite subjective increase on questioning
3 = Spontaneous sexual content; elaborates on sexual matters; hyper-sexual by self-report.
4 = Overt sexual acts (towards patients, staff, or interviewer)

_____ 4. SLEEP

0 = Reports no decrease in sleep
1 = Sleeping less than normal amount by up to one hour
2 = Sleeping less than normal by more than one hour
3 = Reports decreased need for sleep
4 = Denies need for sleep

_____ 5. IRRITABILITY

0 = Absent
2 = Subjectively increased
4 = Irritable at times during interview; recent episodes of anger or annoyance on ward
6 = Frequently irritable during interview; short, curt throughout
8 = Hostile, uncooperative; interview impossible

_____ 6. SPEECH (RATE AND AMOUNT)

0 = No increase
2 = Feels talkative
4 = Increased rate or amount at times, verbose at times
6 = Push; consistently increased rate and amount; difficult to interrupt
8 = Pressured; un-interruptible, continuous speech

Appendix C. Cont'd

_____ 7. LANGUAGE-THOUGHT DISORDER

0 = Absent
1 = Circumstantial; mild distractibility; quick thoughts
2 = Distractible; loses goal of thought; changes topics frequently; racing thoughts
3 = Flight of ideas; tangentiality; difficult to follow; rhyming, echolalia
4 = Incoherent; communication impossible

_____ 8. CONTENT

0 = Normal
2 = Questionable plans, new interests
4 = Special project(s); hyper-religious
6 = Grandiose or paranoid ideas; ideas of reference
8 = Delusions; hallucinations

_____ 9. DISRUPTIVE-AGGRESSIVE BEHAVIOR

0 = Absent; cooperative
2 = Sarcastic; loud at times, guarded
4 = Demanding; threats on ward, etc
6 = Threatens interviewer; shouting; interview difficult
8 = Assaultive; destructive; interview impossible

_____10. APPEARANCE

0 = Appropriate dress and grooming
1 = Minimally unkempt
2 = Poorly groomed; moderately disheveled; overdressed
3 = Disheveled; partly clothed; garish make-up
4 = Completely unkempt; decorated; bizarre garb

_____11. INSIGHT

0 = Present; admits illness; agree with need for treatment
1 = Possibly ill
2 = Admits behavior change, but denies illness
3 = Admits possible change in behavior, but denies illness
4 = Denies any behavior change

OTHER ITEMS

1. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli)

2. Increase in goal-directed activity (either socially, at work or school, or sexually).

3. Excessive involvement in pleasurable activities which have a high potential for painful consequences (i.e., the person engages in unrestrained buying sprees, sexual indiscretions, or foolish business investments)

Appendix D. Beck Depression Inventory

Name:_____ Date: _____

On this questionnaire are groups of statements. Please read each group of statements carefully. Then pick out the one statement in each group which best describes the way you have been feeling the PAST WEEK, INCLUDING TODAY. Circle the number beside the statement you picked. If several statements in the group seem to apply equally well, circle each one. Be sure to read all the statements in each group before making your choice.

1. 0 I do not feel sad.
   1 I feel sad.
   2 I am sad all the time and I can't snap out of it.
   3 I am so sad or unhappy that I can't stand it.

2. 0 I am not particularly discouraged about the future.
   1 I feel discouraged about the future.
   2 I feel I have nothing to look forward to.
   3 I feel that the future is hopeless and that things cannot improve.

3. 0 I do not feel like a failure.
   1 I feel I have failed more than the average person.
   2 As I look back on my life, all I can see is a lot of failures.
   3 I feel I am a complete failure as a person.

4. 0 I get as much satisfaction out of things as I used to.
   1 I don't enjoy things the way I used to.
   2 I don't get real satisfaction out of anything anymore.
   3 I am dissatisfied or bored with everything.

5. 0 I don't feel particularly guilty.
   1 I feel guilty a good part of the time.
   2 I feel quite guilty most of the time.
   3 I feel guilty all of the time.

6. 0 I don't feel I am being punished.
   1 I feel I may be punished.
   2 I expect to be punished.
   3 I feel I am being punished.

7. 0 I don't feel disappointed in myself.
   1 I am disappointed in myself.
   2 I am disgusted with myself.
   3 I hate myself.

8. 0 I don't feel I am worse than anybody else.
   1 I am critical of myself for my weaknesses or mistakes.
   2 I blame myself all the time for my faults.
   3 I blame myself for everything bad that happens.

9. 0 I don't have any thoughts of killing myself.
   1 I have thoughts of killing myself, but I would not carry them out.
   2 I would like to kill myself.
   3 I would kill myself if I had the chance.

Appendix D. Cont'd.

10. 0 I don't cry any more than usual.
    1 I cry more now than I used to.
    2 I cry all the time now.
    3 I used to be able to cry, but now I can't cry even though I want to.

11. 0 I am no more irritated than I ever am.
    1 I get annoyed or irritated more easily than I used to.
    2 I feel irritated all the time now.
    3 I don't get irritated at all the things that used to irritate me.

12. 0 I have not lost interest in other people.
    1 I am less interested in other people than I used to be.
    2 I have lost most of my interest in other people.
    3 I have lost all of my interest in other people.

13. 0 I make decisions about as well as I ever could.
    1 I put off making decisions more than I used to.
    2 I have greater difficulty in making decisions than before.
    3 I can't make decisions at all anymore.

14. 0 I don't feel I look any worse than I used to.
    1 I am worried that I am looking old or unattractive.
    2 I feel that there are permanent changes in my appearance that make me look unattractive.
    3 I believe that I look ugly.

15. 0 I can work about as well as before.
    1 It takes an extra effort to get started at doing something.
    2 I have to push myself very hard to do anything.
    3 I can't do any work at all.

16. 0 I can sleep as well as usual.
    1 I don't sleep as well as I used to.
    2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
    3 I wake up several hours earlier than I used to and cannot get back to sleep.

17. 0 I don't get more tired than usual.
    1 I get tired more easily than usual.
    2 I get tired from doing almost anything.
    3 I am too tired to do anything.

18. 0 My appetite is no worse than usual.
    1 My appetite is not as good as it used to be.
    2 My appetite is much worse now.
    3 I have no appetite at all anymore.

19. 0 I haven't lost much weight, if any, lately.
    1 I have lost more than 5 pounds.
    2 I have lost more than 10 pounds.
    3 I have lost more than 15 pounds.
    I am purposely trying to lose weight by eating less.
    .yes_____ no_____

20. 0 I am no more worried about my health than usual.
    1 I am worried about physical problems such as aches and pains; or upset stomach, or constipation.
    2 I am very worried about physical problems and it's hard to think of much else.
    3 I am so worried about my physical problems that I cannot think of anything else.

21. 0 I have not noticed any recent change in my interest in sex.
    1 I am less interested in sex than I used to be.
    2 I am much less interested in sex now.
    3 I have lost interest in sex completely.

Appendix E. Spielberger State-Anxiety

Name: _____

Date: _____

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you <u>feel right now</u>, that is, <u>at this moment</u>. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present mood best.

		Not at all	Some what	Mode rately so	Very much so
1.	I feel calm	1	2	3	4
2.	I feel secure	1	2	3	4
3.	I am tense	1	2	3	4
4.	I am regretful	1	2	3	4
5.	I feel at ease	1	2	3	4
6.	I feel upset	1	2	3	4
7.	I am presently worrying over possible misfortunes	1	2	3	4
8.	I feel rested	1	2	3	4
9.	I feel anxious	1	2	3	4
10.	I feel comfortable	1	2	3	4
11.	I feel self-confident	1	2	3	4
12.	I feel nervous	1	2	3	4
13.	I am jittery	1	2	3	4
14.	I feel high-strung	1	2	3	4
15.	I am relaxed	1	2	3	4
16.	I am content	1	2	3	4
17.	I am worried	1	2	3	4

Appendix E. Cont'd

18.	I feel overexcited and rattled	1	2	3	4
19.	I feel joyful	1	2	3	4
20.	I feel pleasant	1	2	3	4

Appendix F. Correlative Relationships Between Cholinergic Brain Regions and ROIs Across the Brain in 32 Healthy Controls Using Comparative Methods of Steiger (1980).

	Saline									Procaine									Procaine vs Saline								
	Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean		
ROI	L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegementum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF	
L Tegementum		0.51	0.31	0.27	0.56	0.57	0.52	0.50			0.67	-0.34	-0.23	-0.21	-0.18	-0.21	-0.16			0	↓	0	↓	↓	↓	↓	
R Tegmentum	0.51		0.05	0.03	0.44	0.37	0.31	0.30		0.67		-0.28	-0.04	-0.04	0.02	-0.12	-0.01		0		0	0	0	0	0	0	
L SI	0.31	0.05		0.61	0.64	0.59	0.81	0.60		-0.34	-0.28		0.80	0.87	0.71	0.95	0.79		↓	0		0	↑	0	↑	0	
R SI	0.27	0.03	0.61		0.43	0.67	0.57	0.76		-0.23	-0.04	0.80		0.74	0.81	0.81	0.94		0	0	0		0	0	0	↑	
L Septal n	0.56	0.44	0.64	0.43		0.85	0.88	0.81		-0.21	-0.04	0.87	0.74		0.86	0.91	0.81		↓	0	↑	0		0	0	0	
R Septal n	0.57	0.37	0.59	0.67	0.85		0.77	0.91		-0.18	0.02	0.71	0.81	0.86		0.76	0.90		↓	0	0	0	0		0	0	
L BF	0.52	0.31	0.81	0.57	0.88	0.77		0.83		-0.21	-0.12	0.95	0.81	0.91	0.76		0.84		↓	0	↑	0	0	0		0	
R BF	0.50	0.30	0.60	0.76	0.81	0.91	0.83			-0.16	-0.01	0.79	0.94	0.81	0.90	0.84			↓	0	0	↑	0	0	0		
Medulla	0.56	0.67	0.39	0.34	0.64	0.61	0.59	0.56		0.78	0.57	-0.15	-0.10	-0.02	-0.13	0.00	-0.02		0	0	↓	0	↓	↓	↓	↓	
Pons	0.75	0.77	0.29	0.01	0.63	0.53	0.53	0.42		0.67	0.85	0.00	0.18	0.21	0.24	0.15	0.24		0	0	0	0	↓	0	0	0	
Midbrain	0.80	0.49	0.41	0.20	0.72	0.62	0.73	0.62		0.31	0.40	0.47	0.50	0.53	0.56	0.61	0.59		↓	0	0	0	0	0	0	0	
L Cerebellum	0.58	0.63	0.18	0.03	0.45	0.33	0.30	0.30		0.77	0.61	-0.40	-0.28	-0.38	-0.41	-0.29	-0.28		0	0	↓	0	↓	↓	↓	↓	
R Cerebellum	0.61	0.69	0.23	0.00	0.43	0.30	0.31	0.25		0.72	0.56	-0.32	-0.24	-0.33	-0.40	-0.22	-0.23		0	0	↓	0	↓	↓	↓	0	
L Cerebellum n	0.54	0.59	0.29	0.14	0.46	0.32	0.42	0.36		0.75	0.52	-0.12	-0.02	-0.17	-0.22	-0.03	-0.03		0	0	0	0	↓	↓	0	0	
R Cerebellum n	0.49	0.62	0.22	-0.01	0.40	0.23	0.34	0.27		0.62	0.40	0.25	0.22	0.19	0.05	0.35	0.23		0	0	0	0	0	0	0	0	
L m Thalamus	0.52	0.44	0.46	0.28	0.69	0.49	0.74	0.59		0.07	0.08	0.58	0.59	0.61	0.60	0.67	0.65		0	0	0	0	0	0	0	0	
R m Thalamus	0.42	0.40	0.29	0.24	0.66	0.56	0.62	0.63		0.12	0.09	0.50	0.58	0.51	0.60	0.53	0.66		0	0	0	0	0	0	0	0	
L Caudate	0.46	0.03	0.60	0.49	0.60	0.56	0.81	0.67		-0.22	-0.11	0.77	0.75	0.75	0.72	0.78	0.77		↓	0	0	0	0	0	0	0	
R Caudate	0.32	0.19	0.32	0.57	0.52	0.57	0.61	0.72		0.01	0.06	0.54	0.70	0.63	0.70	0.60	0.74		0	0	0	0	0	0	0	0	
L Putamen	0.06	-0.17	0.31	0.57	0.26	0.37	0.38	0.50		-0.20	-0.18	0.85	0.77	0.75	0.67	0.82	0.77		0	0	↑	0	↑	0	↑	0	
R Putamen	0.04	-0.03	0.45	0.59	0.17	0.28	0.39	0.50		-0.19	-0.13	0.75	0.83	0.66	0.65	0.73	0.83		0	0	0	↑	↑	0	0	↑	
L Globus Pallidus	0.27	0.21	0.18	0.08	0.47	0.38	0.49	0.44		-0.46	-0.42	0.67	0.48	0.58	0.43	0.65	0.47		↓	↓	↑	0	0	0	0	0	
R Globus Pallidus	0.15	0.21	0.37	0.36	0.59	0.50	0.58	0.62		-0.25	-0.20	0.64	0.66	0.62	0.65	0.65	0.74		0	0	0	0	0	0	0	0	
L Hippocampus	0.36	0.39	0.38	0.45	0.57	0.52	0.57	0.55		0.24	0.24	0.02	0.05	0.07	-0.09	0.14	0.07		0	0	0	0	↓	↓	0	↓	
R Hippocampus	0.41	0.34	0.28	0.39	0.53	0.66	0.52	0.62		0.50	0.49	-0.41	-0.34	-0.24	-0.23	-0.30	-0.24		0	0	↓	↓	↓	↓	↓	↓	
L Amygdala	0.54	0.37	0.45	0.30	0.69	0.57	0.67	0.60		-0.05	-0.16	0.50	0.34	0.53	0.41	0.55	0.46		↓	↓	0	0	0	0	0	0	
R Amygdala	0.34	0.19	0.28	0.14	0.55	0.47	0.49	0.54		0.00	0.18	0.35	0.48	0.46	0.54	0.41	0.62		0	0	0	0	0	0	0	0	
L Ant Insula	-0.35	-0.46	0.26	0.08	-0.35	-0.32	-0.15	-0.25		-0.32	-0.28	0.72	0.73	0.63	0.62	0.66	0.67		0	0	↑	↑	↑	↑	↑	↑	
R Ant Insula	-0.04	-0.17	0.24	0.46	0.06	0.17	0.12	0.24		-0.25	-0.14	0.60	0.74	0.49	0.63	0.50	0.71		0	0	0	0	0	↑	0	↑	
L Post Insula	0.03	-0.07	0.25	0.22	0.07	0.09	0.20	0.24		-0.16	-0.17	0.64	0.55	0.57	0.47	0.60	0.56		0	0	0	0	↑	0	0	0	
R Post Insula	0.07	-0.09	0.04	-0.05	0.01	-0.06	0.19	0.08		-0.33	-0.20	0.69	0.76	0.62	0.68	0.67	0.78		0	0	↑	↑	↑	↑	↑	↑	
L Subgenual AC	0.03	-0.12	0.66	0.56	0.36	0.30	0.52	0.37		-0.06	-0.07	0.64	0.54	0.58	0.49	0.69	0.51		0	0	0	0	0	0	0	0	
R Subgenual AC	0.20	0.06	0.48	0.66	0.54	0.62	0.50	0.67		0.09	0.14	0.35	0.45	0.43	0.42	0.43	0.45		0	0	0	0	0	0	0	0	
L Drevet	-0.03	-0.31	0.42	0.51	0.16	0.28	0.19	0.26		-0.31	-0.26	0.69	0.48	0.49	0.30	0.63	0.40		0	0	0	0	0	0	↑	0	
R Drevet	0.00	-0.18	0.36	0.61	0.19	0.36	0.19	0.40		-0.03	-0.07	0.53	0.61	0.40	0.42	0.45	0.52		0	0	0	0	0	0	0	0	
L Pregenual AC	-0.07	-0.37	0.42	0.68	0.23	0.40	0.30	0.43		-0.36	-0.28	0.66	0.74	0.60	0.63	0.58	0.73		0	0	0	0	0	0	0	0	
R Pregenual AC	-0.12	-0.41	0.25	0.66	0.03	0.25	0.09	0.33		-0.22	-0.31	0.67	0.64	0.53	0.55	0.57	0.59		0	0	↑	0	↑	0	↑	0	
L Mayberg	-0.01	-0.45	0.48	0.68	0.19	0.36	0.31	0.40		-0.28	-0.20	0.61	0.72	0.57	0.64	0.55	0.74		0	0	0	0	0	0	0	↑	
R Mayberg	-0.11	-0.45	0.22	0.62	-0.02	0.19	0.07	0.27		-0.24	-0.30	0.66	0.66	0.53	0.58	0.56	0.62		0	0	↑	0	↑	0	↑	0	
L Supragenual AC	-0.04	-0.48	0.55	0.62	0.23	0.34	0.31	0.40		-0.45	-0.53	0.71	0.62	0.55	0.54	0.60	0.61		0	0	0	0	0	0	0	0	
R Supragenual AC	0.02	-0.43	0.45	0.69	0.12	0.33	0.27	0.43		-0.46	-0.46	0.76	0.68	0.61	0.60	0.67	0.61		↓	0	0	0	↑	0	↑	0	
L Dorsal AC	-0.02	-0.38	0.26	0.65	0.15	0.27	0.33	0.44		-0.23	-0.31	0.63	0.69	0.58	0.68	0.62	0.72		0	0	0	0	0	↑	0	0	
R Dorsal AC	-0.05	-0.41	0.27	0.56	0.04	0.13	0.26	0.33		-0.22	-0.36	0.62	0.73	0.55	0.69	0.61	0.69		0	0	0	0	↑	↑	0	0	
L Post AC	-0.24	-0.10	0.36	0.19	0.00	0.06	0.20	0.16		0.04	-0.11	0.25	0.25	0.16	0.22	0.32	0.37		0	0	0	0	0	0	0	0	
R Post AC	-0.13	-0.30	0.46	0.33	0.11	0.23	0.26	0.31		-0.02	-0.18	0.16	0.19	0.20	0.33	0.25	0.33		0	0	0	0	0	0	0	0	
L Ant Tenporal	0.00	-0.05	0.30	0.20	0.35	0.40	0.24	0.22		0.02	0.17	-0.14	-0.22	-0.06	-0.14	-0.11	-0.21		0	0	0	0	0	↓	0	0	
R Ant Temporal	-0.03	0.02	0.22	0.40	0.07	0.37	0.03	0.28		0.23	0.16	-0.10	-0.02	-0.08	-0.10	-0.07	0.02		0	0	0	0	0	0	0	0	
L Post Temporal	0.16	0.01	0.03	-0.40	0.04	-0.07	-0.01	-0.18		0.15	0.23	-0.08	-0.26	-0.13	-0.32	-0.10	-0.29		0	0	0	0	0	0	0	0	
R Post Temporal	-0.10	0.06	-0.47	-0.47	-0.31	-0.23	-0.43	-0.29		0.15	0.33	-0.31	-0.28	-0.28	-0.27	-0.31	-0.27		0	0	0	0	0	0	0	0	
L TP	0.09	-0.01	0.69	0.62	0.54	0.55	0.63	0.57		0.18	0.12	0.12	0.21	0.15	0.07	0.23	0.21		0	0	↓	0	0	↓	0	0	
R TP	0.04	0.13	0.36	0.47	0.35	0.50	0.33	0.46		0.23	0.28	-0.02	0.17	-0.01	0.01	0.04	0.20		0	0	0	0	0	↓	0	0	
L Inf Parietal	-0.04	-0.10	0.20	0.19	0.06	0.05	0.13	-0.08		-0.20	-0.23	0.49	0.34	0.34	0.24	0.40	0.21		0	0	0	0	0	0	0	0	
R Inf Parietal	-0.18	-0.06	-0.21	0.05	-0.17	0.00	-0.17	-0.10		-0.26	-0.08	-0.01	0.01	0.02	0.07	-0.05	-0.04		0	0	0	0	0	0	0	0	
L Occipital	-0.12	0.17	0.07	-0.21	-0.12	-0.23	-0.06	-0.25		0.22	0.15	-0.04	-0.13	0.05	0.02	-0.07	-0.08		0	0	0	0	0	0	0	0	
R Occipital	-0.24	-0.29	0.07	-0.30	-0.32	-0.38	-0.16	-0.32		-0.01	-0.14	0.07	-0.04	0.04	0.07	0.00	0.03		0	0	0	0	0	0	0	0	
L DLPFC	-0.25	-0.49	0.17	0.00	-0.06	-0.02	-0.02	-0.06		-0.30	-0.32	0.34	0.18	0.21	0.14	0.16	0.14		0	0	0	0	0	0	0	0	
R DLPFC	-0.35	-0.57	-0.11	-0.16	-0.39	-0.32	-0.33	-0.31		-0.49	-0.38	0.34	0.42	0.22	0.34	0.18	0.34		0	0	0	↑	↑	↑	↑	↑	
L VLPFC	0.12	-0.27	0.45	0.47	0.13	0.24	0.31	0.32		-0.34	-0.35	0.61	0.37	0.41	0.24	0.51	0.31		0	0	0	0	0	0	0	0	
R VLPFC	0.23	0.01	0.29	0.52	0.32	0.57	0.28	0.56		-0.13	-0.07	0.40	0.44	0.33	0.32	0.37	0.41		0	0	0	0	0	0	0	0	
L MOFC	0.08	-0.18	0.43	0.45	0.16	0.25	0.21	0.24		0.11	0.14	0.28	0.32	0.25	0.16	0.28	0.27		0	0	0	0	0	0	0	0	
R MOFC	0.15	-0.03	0.40	0.60	0.25	0.41	0.26	0.44		0.22	0.19	0.20	0.36	0.14	0.14	0.22	0.28		0	0	0	0	0	0	0	0	
L LOFC	0.40	0.29	0.22	0.35	0.50	0.46	0.46	0.60		0.31	0.25	0.10	0.24	0.01	0.00	0.17	0.21		0	0	0	0	0	↓	0	0	
R LOFC	0.18	0.22	-0.12	0.18	0.30	0.30	0.18	0.40	Totals	0.13	0.17	-0.13	0.08	-0.17	-0.09	-0.07	0.05	Totals	0	0	0	0	0	0	0	0	
# r > .45	15	8	17	26	22	23	23	24	158	8	7	32	30	30	27	31	30	195	0	0	0	0	0	0	0	0	
# r < -.45	0	4	1	1	0	0	0	0	6	4	2	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	
Total	15	12	18	27	22	23	23	24	164	12	9	32	30	30	27	31	30	201	0	0	0	0	0	0	0	0	
																			Legend				Cholinergic System				
# Positive corrs	40	33	58	55	54	54	54	53	401	26	27	47	49	49	50	47	50	345	r			Δr	Tegmental-Midbrain				
# Negative corrs	22	29	4	7	8	8	8	9	95	36	35	15	13	13	12	15	12	151	>.50	↑		>.50	Substantia Inominata				
Total	62	62	62	62	62	62	62	62	496	62	62	62	62	62	62	62	62	496	<-.50	↓		<-.50	Septo-Hippocampal				

Appendix G. Correlative Relationships Between Cholinergic Brain Regions and ROIs Across the Brain in 15 Patients with Bipolar Disorder Using Comparative Methods of Steiger (1980).

	Saline									Procaine									Procaine vs Saline								
	Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean			Brainstem		Basal Forebrain				BF Mean		
ROI	L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF		L Tegmentum	R Tegmentum	L SI	R SI	L Septal n	R Septal n	L BF	R BF	
L Tegementum		0.69	0.36	0.16	0.28	0.55	0.35	0.28			0.62	0.05	-0.11	0.28	0.44	0.27	0.10			0	0	0	0	0	0	0	
R Tegmentum	0.69		0.19	0.12	0.27	0.54	0.17	0.33		0.62		-0.18	-0.13	0.06	0.15	0.03	0.16		0		0	0	0	0	0	0	
L SI	0.36	0.19		0.31	0.89	0.46	0.82	0.36		0.05	-0.18		0.75	0.80	0.73	0.82	0.75		0	0		0	0	0	0	0	
R SI	0.16	0.12	0.31		0.19	0.57	0.54	0.95		-0.11	-0.13	0.75		0.76	0.64	0.83	0.93		0	0	0		0	0	0	0	
L Septal n	0.28	0.27	0.89	0.19		0.52	0.82	0.33		0.28	0.06	0.80	0.76		0.87	0.96	0.80		0	0	0	0		0	0	0	
R Septal n	0.55	0.54	0.46	0.57	0.52		0.50	0.73		0.44	0.15	0.73	0.64	0.87		0.83	0.77		0	0	0	0	0		0	0	
L BF	0.35	0.17	0.82	0.54	0.82	0.50		0.59		0.27	0.03	0.82	0.83	0.96	0.83		0.85		0	0	0	0	0	0		0	
R BF	0.28	0.33	0.36	0.95	0.33	0.73	0.59			0.10	0.16	0.75	0.93	0.80	0.77	0.85			0	0	0	0	0	0	0		
Medulla	0.34	0.75	0.02	0.14	0.22	0.37	0.23	0.30		0.27	0.68	-0.34	-0.13	-0.06	0.13	-0.14	0.10		0	0	0	0	0	0	0	0	
Pons	0.80	0.85	0.49	0.20	0.58	0.73	0.50	0.40		0.88	0.64	0.34	0.07	0.40	0.55	0.41	0.30		0	0	0	0	0	0	0	0	
Midbrain	0.60	0.70	0.54	0.23	0.65	0.69	0.62	0.44		0.59	0.23	0.79	0.48	0.72	0.84	0.73	0.63		0	0	0	0	0	0	0	0	
L Cerebellum	0.61	0.79	0.15	0.33	0.27	0.57	0.27	0.48		0.68	0.55	-0.21	-0.30	-0.13	0.03	-0.12	-0.12		0	0	↓	0	↓	0	↓	0	
R Cerebellum	0.67	0.78	0.24	0.14	0.35	0.72	0.23	0.36		0.57	0.61	-0.18	-0.26	-0.15	0.12	-0.17	0.01		↓	↓	0	0	0	0	0	0	
L Cerebellum n	0.66	0.48	0.51	0.41	0.36	0.70	0.41	0.53		0.31	0.36	0.24	0.12	0.24	0.33	0.25	0.33		0	0	0	0	0	0	0	0	
R Cerebellum n	0.43	0.68	0.38	0.36	0.32	0.66	0.25	0.51		0.05	0.35	0.27	0.26	0.16	0.23	0.16	0.46		0	0	0	0	0	0	0	0	
L m Thalamus	0.39	0.50	0.59	0.38	0.66	0.53	0.72	0.48		0.36	0.25	0.64	0.42	0.63	0.80	0.66	0.63		0	0	0	0	0	0	0	0	
R m Thalamus	0.66	0.51	0.64	0.35	0.57	0.69	0.63	0.45		0.36	0.31	0.81	0.63	0.80	0.85	0.80	0.79		0	0	0	0	0	0	0	0	
L Caudate	0.19	-0.14	0.56	0.24	0.61	0.18	0.75	0.27		0.23	-0.25	0.58	0.49	0.66	0.43	0.74	0.42		0	0	0	0	0	0	0	0	
R Caudate	0.43	0.01	-0.04	0.50	-0.14	0.29	0.28	0.44		0.35	-0.31	0.51	0.34	0.57	0.53	0.62	0.29		0	0	0	0	0	0	0	0	
L Putamen	0.50	0.21	0.60	0.44	0.49	0.43	0.69	0.49		0.41	0.13	0.77	0.42	0.67	0.77	0.66	0.58		0	0	0	0	0	0	0	0	
R Putamen	0.02	-0.06	0.10	0.79	-0.11	0.35	0.12	0.72		0.16	-0.04	0.66	0.60	0.55	0.73	0.60	0.71		0	0	0	0	0	0	0	0	
L Globus Pallidus	0.38	0.27	0.16	0.51	0.19	0.40	0.43	0.56		0.29	-0.03	0.56	0.37	0.61	0.44	0.66	0.42		0	0	0	0	0	0	0	0	
R Globus Pallidus	0.54	0.46	-0.14	0.33	-0.10	0.42	0.07	0.43		0.04	-0.08	0.62	0.65	0.73	0.69	0.69	0.71		0	0	0	0	0	0	0	0	
L Hippocampus	-0.15	0.08	-0.01	0.34	0.13	0.22	0.25	0.40		0.34	0.34	0.44	0.26	0.35	0.15	0.33	0.29		0	0	0	0	0	0	0	0	
R Hippocampus	-0.03	-0.46	0.33	0.10	0.18	0.06	0.28	0.07		-0.13	0.10	0.18	0.00	-0.20	-0.02	-0.22	0.06		0	0	0	0	0	0	0	0	
L Amygdala	0.36	0.53	0.46	0.56	0.41	0.75	0.40	0.67		0.38	0.58	0.55	0.55	0.50	0.56	0.53	0.73		0	0	0	0	0	0	0	0	
R Amygdala	-0.01	0.20	0.68	-0.09	0.76	0.28	0.41	0.06		0.01	0.26	0.55	0.40	0.30	0.40	0.28	0.54		0	0	0	0	0	0	0	0	
L Ant Insula	0.21	0.53	0.23	-0.19	0.43	0.19	0.19	-0.01		0.37	0.31	-0.24	-0.42	-0.14	0.01	-0.27	-0.27		0	0	0	0	0	0	0	0	
R Ant Insula	0.17	0.30	0.22	-0.32	0.22	-0.25	0.06	-0.26		0.29	0.22	-0.14	-0.46	-0.10	-0.07	-0.20	-0.31		0	0	0	0	0	0	0	0	
L Post Insula	0.30	0.24	0.65	0.18	0.51	0.43	0.39	0.28		0.25	0.31	0.40	0.28	0.25	0.37	0.23	0.45		0	0	0	0	0	0	0	0	
R Post Insula	0.09	0.08	0.64	0.12	0.47	0.24	0.29	0.16		0.17	0.05	0.70	0.43	0.46	0.57	0.43	0.52		0	0	0	0	0	0	0	0	
L Subgenual AC	0.14	-0.10	0.11	0.17	0.09	-0.01	0.31	0.07		0.36	-0.12	0.01	0.21	0.37	0.46	0.38	0.18		0	0	0	0	0	0	0	0	
R Subgenual AC	0.12	-0.06	-0.16	0.78	-0.22	0.33	0.22	0.68		-0.09	-0.09	0.25	0.63	0.43	0.26	0.56	0.59		0	0	0	0	0	0	0	0	
L Drevet	0.45	0.39	0.56	0.41	0.55	0.47	0.61	0.47		0.00	-0.13	0.82	0.75	0.70	0.78	0.71	0.80		0	0	0	0	0	0	0	0	
R Drevet	0.29	0.20	0.54	0.77	0.49	0.75	0.56	0.81		0.06	0.11	0.76	0.83	0.65	0.58	0.74	0.86		0	0	0	0	0	0	0	0	
L Pregenual AC	0.50	0.14	0.39	0.68	0.23	0.47	0.55	0.68		-0.10	-0.16	0.79	0.90	0.72	0.71	0.75	0.90		0	0	0	0	0	0	0	0	
R Pregenual AC	0.34	0.14	0.55	0.72	0.49	0.53	0.76	0.72		-0.04	0.06	0.74	0.76	0.53	0.60	0.65	0.84		0	0	0	0	0	0	0	0	
L Mayberg	0.48	0.03	0.56	0.58	0.42	0.49	0.69	0.58		-0.02	-0.15	0.80	0.91	0.82	0.79	0.84	0.91		0	0	0	0	0	0	0	0	
R Mayberg	0.33	0.05	0.60	0.54	0.51	0.36	0.76	0.53		0.04	0.04	0.72	0.75	0.59	0.72	0.68	0.85		0	0	0	0	0	0	0	0	
L Supragenual AC	0.14	-0.23	0.24	0.10	0.13	-0.03	0.42	0.06		0.06	-0.09	0.72	0.78	0.78	0.72	0.74	0.78		0	0	0	0	0	0	0	0	
R Supragenual AC	0.41	-0.06	0.51	0.15	0.35	0.30	0.45	0.17		0.27	-0.10	0.64	0.59	0.68	0.70	0.73	0.67		0	0	0	0	0	0	0	0	
L Dorsal AC	0.18	-0.18	0.64	0.25	0.50	0.40	0.48	0.27		0.44	0.18	0.50	0.45	0.48	0.56	0.53	0.57		0	0	0	0	0	0	0	0	
R Dorsal AC	-0.05	-0.17	0.42	0.15	0.40	0.21	0.38	0.20		0.25	0.18	0.53	0.45	0.53	0.61	0.55	0.62		0	0	0	0	0	0	0	0	
L Post AC	0.18	0.24	0.11	0.54	0.03	0.27	0.15	0.54		-0.21	0.08	0.21	0.49	0.01	-0.03	0.14	0.47		0	0	0	0	0	0	0	0	
R Post AC	0.32	0.19	0.09	0.46	-0.04	0.24	0.18	0.40		0.02	0.10	0.06	0.37	0.16	0.07	0.30	0.36		0	0	0	0	0	0	0	0	
L Ant Temporal	-0.26	-0.10	-0.52	-0.41	-0.38	-0.34	-0.38	-0.37		0.09	-0.05	-0.70	-0.36	-0.43	-0.46	-0.42	-0.44		0	0	0	0	0	0	0	0	
R Ant Temporal	-0.18	0.09	-0.38	-0.16	-0.28	-0.20	-0.22	-0.14		0.11	0.17	-0.59	-0.40	-0.43	-0.39	-0.50	-0.39		0	0	0	0	0	0	0	0	
L Post Temporal	-0.52	-0.57	-0.59	-0.48	-0.42	-0.41	-0.54	-0.49		0.06	0.09	-0.88	-0.72	-0.68	-0.60	-0.72	-0.73		0	0	0	0	0	0	0	0	
R Post Temporal	-0.67	-0.32	-0.19	-0.21	0.05	-0.13	-0.30	-0.16		-0.30	-0.04	-0.64	-0.54	-0.69	-0.59	-0.73	-0.54		0	0	0	0	0	0	0	0	
L TP	0.22	0.55	0.26	-0.17	0.43	0.21	0.22	-0.04		0.33	0.42	0.12	0.20	0.22	0.35	0.17	0.34		0	0	0	0	0	0	0	0	
R TP	-0.08	0.36	0.18	-0.29	0.41	0.09	0.16	-0.17		0.33	0.50	0.08	0.17	0.20	0.30	0.15	0.32		0	0	0	0	0	0	0	0	
L Inf Parietal	0.00	-0.14	0.10	-0.16	0.03	-0.02	-0.01	-0.16		0.05	0.06	0.57	0.31	0.39	0.43	0.30	0.37		0	0	0	↓	0	0	0	0	
R Inf Parietal	0.11	-0.31	0.60	0.48	0.29	0.11	0.51	0.35		0.08	0.23	0.71	0.57	0.46	0.48	0.53	0.72		0	↑	0	0	0	0	0	0	
L Occipital	-0.09	0.26	-0.07	-0.44	-0.12	-0.25	-0.44	-0.36		-0.42	-0.29	-0.04	-0.21	-0.47	-0.49	-0.36	-0.25		0	0	0	0	0	0	0	0	
R Occipital	0.36	0.39	0.14	-0.44	0.18	0.02	-0.16	-0.33		0.02	-0.12	0.31	-0.10	-0.13	0.03	-0.04	-0.05		0	0	0	0	0	0	0	0	
L DLPFC	0.22	-0.11	0.28	-0.15	0.23	0.02	0.16	-0.18		-0.03	-0.34	0.55	0.56	0.60	0.53	0.61	0.46		0	0	0	0	0	0	0	0	
R DLPFC	-0.27	-0.22	0.11	-0.05	0.10	0.07	-0.11	-0.11		-0.14	-0.40	0.77	0.66	0.55	0.60	0.55	0.57		0	0	0	0	0	0	0	0	
L VLPFC	0.19	-0.33	-0.01	0.39	-0.12	0.15	0.27	0.30		-0.04	-0.46	0.48	0.57	0.64	0.54	0.66	0.44		0	0	0	↓	0	0	0	0	
R VLPFC	-0.19	-0.38	0.41	0.42	0.34	0.19	0.36	0.31		-0.05	-0.08	0.45	0.25	0.21	0.45	0.20	0.35		0	0	0	0	0	0	0	0	
L MOFC	0.12	0.36	0.09	0.09	0.26	0.22	0.19	0.16		-0.10	-0.24	0.57	0.68	0.64	0.66	0.55	0.61		0	0	0	0	0	0	0	0	
R MOFC	0.09	0.20	0.14	0.66	0.21	0.65	0.25	0.69		-0.18	0.10	0.50	0.85	0.55	0.46	0.58	0.81		0	0	0	0	0	0	0	0	
L LOFC	-0.18	-0.21	-0.40	0.31	-0.38	-0.10	-0.17	0.20		-0.04	0.11	-0.37	0.04	-0.05	-0.09	-0.01	0.01		0	0	0	0	0	0	0	0	
R LOFC	-0.44	-0.58	-0.57	0.09	-0.60	-0.31	-0.44	-0.09	Total	0.06	0.18	-0.56	-0.17	-0.25	-0.28	-0.25	-0.19	Total	0	0	0	0	0	0	0	0	
# r > .45	12	15	22	17	17	22	20	20	145	5	7	35	27	32	33	32	32	203	0	0	0	0	0	0	0	0	
# r < -.45	2	3	3	1	1	0	1	1	12	0	1	5	3	3	4	3	2	21	0	0	0	0	0	0	0	0	
Total	14	18	25	18	18	22	21	21	157	5	8	40	30	35	37	35	34	224	0	0	0	0	0	0	0	0	
																			Legend				Cholinergic System				
# Positive corrs	48	42	50	48	50	51	52	48	389	45	39	49	48	48	52	48	52	381	r			Δr					
# Negative corrs	14	20	12	14	12	11	10	14	107	17	23	13	14	14	10	14	10	115	>.50		↑	>.50	Substantia Inominata				
Total	62	62	62	62	62	62	62	62	496	62	62	62	62	62	62	62	62	496	<-.50		↓	<-.50	Septo-Hippocampal				

Appendix H. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls at Baseline

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	-0.02	0.15	-0.28	.	-0.16	-0.19	.	0.25	-0.18	-0.30
L Tegmentum	0.03	-0.08	-0.08	.	0.14	0.28	.	0.06	0.25	0.03
R Tegmentum	-0.09	0.22	0.10	.	-0.07	0.18	.	0.05	-0.16	-0.22
L SI	0.00	-0.12	0.04	.	0.31	0.40	.	-0.30	0.24	0.24
R SI	-0.03	-0.14	-0.31	.	0.45	0.31	.	0.08	0.36	0.26
L Septal n	-0.03	-0.06	-0.06	.	0.17	0.30	.	-0.26	0.21	0.06
R Septal n	-0.07	-0.10	-0.18	.	0.25	0.18	.	-0.04	0.30	0.06
L BF	0.06	-0.05	-0.06	.	0.28	0.44	.	-0.13	0.20	0.18
R BF	-0.01	-0.12	-0.22	.	0.33	0.25	.	0.05	0.31	0.15
Medulla	-0.10	-0.01	-0.02	.	0.10	0.30	.	-0.14	0.03	-0.13
Caudal Pons	0.06	0.17	0.25	.	-0.08	0.14	.	-0.08	-0.07	-0.14
Rostral Pons	0.20	0.00	0.04	.	0.17	0.33	.	-0.02	0.22	0.04
L Cerebellum	0.04	0.05	0.16	.	0.23	0.22	.	-0.29	0.17	0.03
R Cerebellum	-0.07	0.08	0.22	.	0.20	0.26	.	-0.26	0.18	-0.04
L Cerebellum n	0.14	-0.07	0.07	.	0.27	0.37	.	-0.32	0.17	0.14
R Cerebellum n	-0.09	-0.05	0.17	.	0.31	0.34	.	-0.26	0.21	0.02
L M Thalamus	0.02	0.03	-0.05	.	0.20	0.37	.	-0.25	0.04	0.16
R M Thalamus	-0.01	0.10	-0.02	.	0.26	0.26	.	-0.13	0.18	0.11
L Caudate	0.18	-0.07	-0.09	.	0.21	0.35	.	0.00	0.11	0.23
R Caudate	0.03	-0.18	-0.39	.	0.16	0.27	.	0.04	0.12	0.12
L Putamen	-0.20	-0.15	-0.27	.	0.14	0.06	.	0.27	0.22	0.04
R Putamen	-0.05	-0.07	-0.07	.	0.19	0.09	.	0.01	0.05	0.10
L Globus Pallidus	0.37	-0.04	0.06	.	-0.05	0.11	.	0.18	-0.05	-0.15
R Globus Pallidus	-0.07	-0.05	-0.02	.	-0.06	-0.03	.	0.05	-0.08	-0.10
L Hippocampus	0.14	0.08	-0.05	.	0.06	0.28	.	-0.12	-0.02	-0.06
R Hippocampus	0.06	-0.03	-0.25	.	0.17	0.34	.	0.07	0.10	0.08
L Amygdala	0.03	-0.06	0.08	.	0.23	0.22	.	-0.01	0.27	0.05
R Amygdala	-0.12	-0.11	0.09	.	0.13	0.17	.	-0.21	0.10	0.08
L Ant Insula	-0.12	0.02	0.16	.	0.25	-0.01	.	-0.11	0.25	0.25
R Ant Insula	-0.07	-0.15	-0.24	.	0.49	0.14	.	-0.09	0.51	0.26
L Post Insula	0.00	-0.12	0.26	.	0.20	0.07	.	0.20	0.30	0.16
R Post Insula	0.63	0.04	0.21	.	0.10	0.11	.	0.22	0.07	0.09
L Subgenual AC	0.12	0.04	0.01	.	0.33	0.29	.	-0.19	0.13	0.14
R Subgenual AC	-0.03	-0.16	-0.12	.	0.34	0.18	.	-0.24	0.19	0.32
L Drevets	0.09	0.04	0.01	.	0.29	-0.03	.	-0.26	0.23	0.24
R Drevets	0.02	0.02	-0.07	.	0.28	-0.08	.	-0.29	0.22	0.22
L Pregenual AC	0.00	-0.09	-0.28	.	0.47	0.19	.	-0.17	0.28	0.22
R Pregenual AC	-0.05	-0.16	-0.26	.	0.48	-0.01	.	-0.08	0.50	0.29
L Mayberg	-0.02	-0.11	-0.26	.	0.54	0.24	.	-0.13	0.40	0.30
R Mayberg	-0.06	-0.12	-0.26	.	0.52	0.03	.	-0.02	0.48	0.34
L Supragenual AC	-0.13	-0.22	-0.10	.	0.30	0.06	.	-0.29	0.32	0.24
R Supragenual AC	0.02	-0.30	-0.23	.	0.49	0.16	.	-0.17	0.46	0.46
L Dorsal AC	0.08	-0.22	-0.36	.	0.33	0.26	.	0.08	0.29	0.45
R Dorsal AC	0.09	-0.21	-0.45	.	0.30	0.23	.	0.05	0.22	0.43
L Post AC	-0.03	0.29	0.19	.	-0.20	-0.14	.	0.16	-0.21	-0.18
R Post AC	-0.08	-0.01	0.17	.	0.10	-0.11	.	0.18	0.18	0.02

Appendix H. Cont'd.

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	-0.24	-0.01	0.15	.	-0.10	-0.06	.	-0.09	-0.04	-0.23	
R Ant Temporal	-0.15	0.19	0.03	.	0.16	-0.15	.	0.10	0.01	-0.02	
L Post Temporal	-0.24	0.04	0.43	.	-0.30	-0.17	.	-0.17	-0.21	-0.14	
R Post Temporal	-0.14	0.10	0.11	.	-0.40	-0.37	.	0.15	-0.30	-0.29	
L TP	0.16	-0.01	-0.07	.	0.21	0.19	.	-0.20	0.09	0.03	
R TP	0.05	0.12	-0.03	.	0.05	-0.05	.	-0.11	0.00	0.04	
L Inf Parietal	0.03	0.05	-0.16	.	0.32	0.24	.	-0.15	0.19	0.29	
R Inf Parietal	-0.08	-0.06	-0.38	.	0.11	-0.01	.	0.19	0.00	-0.04	
L Occipital	-0.09	0.21	0.10	.	-0.12	0.22	.	-0.03	-0.16	-0.19	
R Occipital	-0.03	0.14	0.23	.	-0.13	-0.16	.	0.12	-0.08	-0.24	
L DLPFC	-0.23	-0.37	-0.01	.	-0.07	-0.23	.	-0.24	0.18	0.09	
R DLPFC	0.17	-0.25	-0.06	.	0.25	-0.01	.	-0.15	0.27	0.21	
L VLPFC	-0.19	-0.18	0.00	.	0.29	0.07	.	-0.26	0.25	0.33	
R VLPFC	0.04	-0.29	-0.19	.	0.46	0.10	.	-0.16	0.40	0.24	
L MOFC	0.12	0.18	0.07	.	0.23	-0.06	.	-0.22	0.16	0.17	
R MOFC	0.08	0.09	-0.03	.	0.21	-0.06	.	-0.24	0.08	0.16	
L LOFC	0.00	0.01	-0.01	.	0.21	0.05	.	-0.06	0.18	0.14	
R LOFC	0.01	-0.03	-0.11	.	0.21	0.05	.	0.03	0.09	0.01	
# r > .45	1	0	0	0	8	0	0	0	4	1	14
# r < -.45	0	0	0	0	0	0	0	0	0	0	0
Total	1	0	0	0	8	0	0	0	4	1	14
# r > .5	1	0	0	0	2	0	0	0	1	0	4
# r < -.5	0	0	0	0	0	0	0	0	0	0	0
Total	1	0	0	0	2	0	0	0	1	0	4
# Positive corrs	29	24	25	0	52	45	0	21	50	48	294
# Negative corrs	34	39	38	0	11	18	0	42	13	15	210
Total	63	63	63	0	63	63	0	63	63	63	504

Euphoria		0.05	0.24	.	-0.06	0.07	.	0.04	-0.08	0.07
Calmness	0.05		0.33	.	-0.32	-0.37	.	0.30	-0.49	-0.35
Good/Bad	0.24	0.33		.	-0.27	-0.29	.	-0.15	-0.29	-0.08
Depression	.	.	.		.	.	.	.	.	.
Fear	-0.06	-0.32	-0.27	.		0.60	.	-0.04	0.81	0.49
Anxiety	0.07	-0.37	-0.29	.	0.60		.	-0.18	0.40	0.38
Anger	.	.	.	.	.	.		.	.	.
Tiredness	0.04	0.30	-0.15	.	-0.04	-0.18	.		-0.06	-0.31
Auditory	-0.08	-0.49	-0.29	.	0.81	0.40	.	-0.06		0.42
Visual	0.07	-0.35	-0.08	.	0.49	0.38	.	-0.31	0.42	

Appendix I. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls with Procaine Administration

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	0.10	-0.08	-0.10	0.21	-0.01	0.05	0.11	0.16	0.11	0.09
L Tegmentum	-0.22	-0.20	-0.41	-0.10	0.42	0.25	0.48	0.04	0.04	0.16
R Tegmentum	-0.23	-0.13	-0.30	-0.17	0.33	0.29	0.21	0.00	-0.11	0.00
L SI	0.14	-0.07	0.15	-0.20	-0.17	0.17	-0.26	-0.35	0.37	0.16
R SI	0.10	-0.12	0.17	-0.23	-0.17	0.09	-0.24	-0.23	0.26	-0.13
L Septal n	0.11	-0.10	0.05	-0.20	-0.02	0.30	-0.38	-0.40	0.42	0.13
R Septal n	0.01	-0.08	0.08	-0.27	-0.10	0.17	-0.41	-0.31	0.44	0.00
L BF	0.08	-0.16	0.08	-0.23	0.00	0.30	-0.27	-0.26	0.30	0.07
R BF	0.04	-0.07	0.18	-0.32	-0.11	0.10	-0.31	-0.16	0.32	-0.12
Medulla	-0.19	-0.22	-0.36	-0.01	0.47	0.45	0.34	0.16	-0.11	0.11
Caudal Pons	-0.23	-0.19	-0.36	-0.19	0.34	0.32	0.17	-0.15	0.18	0.07
Rostral Pons	-0.17	-0.10	-0.17	-0.28	0.22	0.28	-0.01	-0.14	0.36	0.09
L Cerebellum	-0.18	-0.13	-0.37	0.04	0.43	0.16	0.48	0.17	-0.22	0.08
R Cerebellum	-0.12	-0.07	-0.25	0.00	0.32	0.03	0.42	0.18	-0.28	-0.03
L Cerebellum n	0.02	-0.22	-0.14	-0.26	0.26	0.13	0.49	0.08	-0.10	0.10
R Cerebellum n	-0.05	-0.24	-0.20	-0.17	0.26	0.18	0.27	-0.10	0.03	0.04
L M Thalamus	-0.02	0.00	-0.01	-0.30	-0.03	0.11	-0.12	-0.14	0.36	-0.01
R M Thalamus	-0.19	0.04	-0.09	-0.24	-0.01	-0.08	-0.14	-0.11	0.39	-0.04
L Caudate	0.26	-0.03	0.30	-0.23	-0.21	0.16	-0.32	-0.30	0.36	0.06
R Caudate	0.11	-0.20	0.12	-0.23	0.09	0.25	-0.23	-0.24	0.33	-0.02
L Putamen	0.19	0.02	0.26	-0.31	-0.20	0.11	-0.21	-0.36	0.41	0.08
R Putamen	0.16	0.05	0.33	-0.36	-0.27	-0.07	-0.23	-0.22	0.29	-0.05
L Globus Pallidus	0.37	0.28	0.40	-0.12	-0.39	-0.16	-0.53	-0.29	0.14	-0.16
R Globus Pallidus	0.27	0.27	0.37	-0.35	-0.14	-0.05	-0.25	-0.09	0.41	0.13
L Hippocampus	-0.21	-0.26	-0.17	0.03	0.23	0.34	-0.05	0.12	-0.26	-0.21
R Hippocampus	-0.28	-0.31	-0.38	-0.03	0.40	0.43	0.37	0.34	-0.23	0.02
L Amygdala	-0.14	-0.26	-0.07	-0.25	0.17	0.41	-0.11	-0.25	0.28	0.17
R Amygdala	-0.19	-0.23	-0.07	-0.38	-0.03	0.19	-0.03	0.06	0.15	-0.12
L Ant Insula	0.03	0.00	0.13	-0.26	-0.28	-0.03	-0.18	-0.44	0.44	0.06
R Ant Insula	0.16	0.15	0.30	-0.25	-0.35	-0.23	-0.22	-0.31	0.41	-0.04
L Post Insula	0.10	0.10	0.25	-0.13	-0.20	-0.22	-0.32	-0.22	0.16	-0.14
R Post Insula	0.06	-0.06	0.20	-0.27	-0.32	-0.02	-0.23	-0.08	0.21	-0.10
L Subgenual AC	0.05	-0.26	-0.05	-0.15	-0.02	0.33	-0.04	-0.16	0.20	0.18
R Subgenual AC	0.08	-0.22	0.01	-0.25	0.01	0.23	0.00	-0.09	0.04	0.02
L Drevets	0.17	0.04	0.16	-0.17	-0.25	-0.09	-0.09	-0.25	0.15	0.11
R Drevets	0.05	-0.19	-0.09	-0.21	-0.05	0.05	0.20	-0.32	0.41	0.13
L Pregenual AC	0.06	-0.03	0.18	-0.07	-0.25	-0.13	-0.21	-0.30	0.28	-0.09
R Pregenual AC	0.12	-0.11	0.12	-0.19	-0.20	-0.03	-0.05	-0.41	0.44	0.09
L Mayberg	0.00	-0.05	0.16	-0.14	-0.25	-0.09	-0.19	-0.26	0.33	-0.06
R Mayberg	0.17	-0.08	0.20	-0.26	-0.20	-0.05	-0.08	-0.44	0.46	0.11
L Supragenual AC	0.14	-0.05	0.25	-0.07	-0.34	-0.17	-0.29	-0.36	0.42	-0.02
R Supragenual AC	0.25	-0.08	0.29	-0.24	-0.28	-0.04	-0.16	-0.40	0.42	0.06
L Dorsal AC	0.05	-0.08	0.20	-0.08	-0.16	0.09	-0.19	-0.17	0.32	0.00
R Dorsal AC	0.16	-0.16	0.20	-0.22	-0.18	0.10	-0.14	-0.21	0.35	-0.02
L Post AC	-0.27	-0.23	-0.02	-0.19	-0.13	-0.12	-0.04	0.30	0.12	-0.21
R Post AC	-0.17	-0.08	0.10	-0.37	-0.24	0.00	-0.11	0.30	-0.02	-0.06

Appendix I. Cont'd.

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	-0.04	-0.17	-0.06	-0.14	0.16	-0.02	0.31	-0.30	-0.39	-0.10	
R Ant Temporal	-0.20	-0.15	-0.15	0.03	0.34	0.18	0.13	-0.22	-0.23	0.13	
L Post Temporal	-0.07	-0.09	-0.18	0.14	0.25	0.16	0.23	-0.22	-0.13	0.17	
R Post Temporal	0.09	0.06	-0.06	0.14	0.22	-0.07	0.27	-0.01	-0.13	-0.04	
L TP	-0.17	-0.33	-0.27	-0.11	0.41	0.33	0.17	0.26	-0.15	-0.02	
R TP	-0.22	-0.29	-0.22	-0.17	0.27	0.10	0.22	0.38	-0.19	-0.09	
L Inf Parietal	0.00	0.04	-0.06	0.00	-0.11	0.04	0.04	-0.29	0.22	0.22	
R Inf Parietal	0.15	0.12	0.07	-0.02	0.00	0.01	0.10	-0.09	0.12	0.11	
L Occipital	-0.17	-0.10	-0.17	0.06	-0.05	-0.07	-0.09	0.04	0.06	-0.03	
R Occipital	-0.13	0.14	-0.09	-0.14	-0.05	-0.27	0.05	-0.03	0.23	0.05	
L DLPFC	0.05	0.14	0.20	0.10	-0.18	-0.20	-0.25	-0.44	0.24	0.13	
R DLPFC	0.13	0.08	0.26	-0.15	-0.36	-0.44	-0.14	-0.29	0.23	-0.18	
L VLPFC	0.02	-0.17	0.04	0.03	0.05	0.19	-0.04	-0.55	0.30	0.17	
R VLPFC	-0.08	-0.21	-0.08	0.13	0.01	0.00	0.03	-0.11	0.16	-0.11	
L MOFC	-0.09	-0.22	-0.27	-0.21	0.08	0.23	0.25	-0.03	0.13	0.15	
R MOFC	-0.07	-0.28	-0.25	-0.20	0.09	0.25	0.37	-0.02	0.13	0.13	
L LOFC	-0.08	-0.26	-0.15	-0.07	0.29	0.30	0.20	0.06	-0.01	0.11	
R LOFC	0.08	-0.21	0.07	0.00	0.16	0.10	0.19	0.15	-0.16	-0.16	
# r > .45	0	0	0	0	1	0	3	0	1	0	5
# r < -.45	0	0	0	0	0	0	1	1	0	0	2
Total	0	0	0	0	1	0	4	1	1	0	7
# r > .5	0	0	0	0	0	0	0	0	0	0	0
# r < -.5	0	0	0	0	0	0	1	1	0	0	2
Total	0	0	0	0	0	0	1	1	0	0	2
# Positive corrs	36	16	32	13	26	41	24	17	47	36	288
# Negative corrs	27	47	31	50	37	22	39	46	16	27	342
Total	63	63	63	63	63	63	63	63	63	63	630

Euphoria		0.40	0.74	-0.32	-0.39	-0.25	-0.07	-0.12	0.05	-0.05
Calmness	0.40		0.54	-0.14	-0.39	-0.58	-0.33	0.00	-0.05	-0.11
Good/Bad	0.74	0.54		-0.46	-0.64	-0.54	-0.41	0.05	-0.15	-0.31
Depression	-0.32	-0.14	-0.46		0.28	0.12	0.03	0.00	-0.07	-0.06
Fear	-0.39	-0.39	-0.64	0.28		0.68	0.41	0.01	0.11	0.28
Anxiety	-0.25	-0.58	-0.54	0.12	0.68		0.31	-0.08	0.19	0.46
Anger	-0.07	-0.33	-0.41	0.03	0.41	0.31		0.19	0.00	0.33
Tiredness	-0.12	0.00	0.05	0.00	0.01	-0.08	0.19		-0.48	-0.34
Auditory	0.05	-0.05	-0.15	-0.07	0.11	0.19	0.00	-0.48		0.55
Visual	-0.05	-0.11	-0.31	-0.06	0.28	0.46	0.33	-0.34	0.55	

Appendix J. Correlations Between Brain ROIs and Emotional and Sensory Responses in 32 Healthy Controls - Delta CBF / Delta Rating

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	-0.20	0.06	-0.14	0.16	-0.10	-0.03	0.09	0.11	0.08	0.34
L Tegmentum	-0.14	0.01	-0.12	0.13	0.15	0.19	0.32	-0.07	-0.16	0.09
R Tegmentum	-0.50	-0.32	-0.37	0.01	0.24	0.33	0.26	-0.17	0.05	0.13
L SI	0.42	-0.01	0.26	-0.17	-0.30	0.08	-0.17	-0.26	0.37	0.07
R SI	0.27	-0.14	0.18	-0.21	-0.17	0.09	-0.26	-0.16	0.29	-0.06
L Septal n	0.28	-0.12	0.14	-0.13	-0.22	0.23	0.01	-0.26	0.31	0.16
R Septal n	0.20	-0.08	0.13	-0.09	-0.24	0.13	-0.16	-0.24	0.27	0.02
L BF	0.35	-0.06	0.14	-0.14	-0.20	0.20	-0.07	-0.19	0.32	0.16
R BF	0.23	-0.06	0.14	-0.16	-0.22	0.10	-0.14	-0.15	0.32	0.08
Medulla	-0.27	-0.24	-0.23	-0.03	0.24	0.29	0.49	-0.38	-0.26	0.16
Caudal Pons	-0.17	-0.21	-0.18	-0.06	0.10	0.22	0.21	-0.10	0.20	-0.06
Rostral Pons	0.18	-0.10	0.01	-0.05	-0.01	0.25	-0.02	-0.20	0.36	0.12
L Cerebellum	-0.37	-0.22	-0.26	0.08	0.43	0.36	0.43	-0.12	-0.24	0.19
R Cerebellum	-0.44	-0.25	-0.18	0.11	0.30	0.13	0.42	-0.08	-0.40	-0.03
L Cerebellum n	-0.13	-0.18	-0.08	-0.09	0.16	0.19	0.30	-0.33	-0.03	0.14
R Cerebellum n	-0.18	-0.24	-0.09	-0.12	0.11	0.19	0.09	-0.17	0.08	0.05
L M Thalamus	0.15	-0.01	0.08	-0.20	-0.17	0.05	0.00	-0.26	0.25	0.11
R M Thalamus	0.07	-0.05	0.06	-0.19	-0.21	-0.01	-0.04	-0.25	0.36	0.10
L Caudate	0.50	0.26	0.32	-0.14	-0.35	-0.09	-0.13	-0.02	0.35	0.18
R Caudate	0.19	0.02	-0.14	-0.01	0.08	0.21	0.05	-0.02	0.20	0.34
L Putamen	0.30	-0.01	0.06	-0.16	-0.23	-0.01	-0.05	0.09	0.34	0.09
R Putamen	0.28	0.23	0.25	-0.21	-0.21	-0.23	-0.24	-0.22	0.19	-0.02
L Globus Pallidus	0.34	0.23	0.15	-0.05	-0.28	-0.06	-0.27	0.11	0.34	0.01
R Globus Pallidus	0.21	0.17	0.27	-0.25	-0.15	-0.05	-0.09	-0.12	0.26	0.06
L Hippocampus	0.10	-0.03	0.06	-0.15	0.19	0.31	0.10	-0.13	-0.03	0.11
R Hippocampus	-0.08	-0.11	-0.33	0.08	0.30	0.43	0.46	0.00	-0.06	0.33
L Amygdala	0.16	-0.12	0.04	-0.22	-0.02	0.42	-0.07	0.01	0.42	0.16
R Amygdala	0.08	-0.24	-0.01	-0.15	0.05	0.24	0.10	-0.12	0.19	-0.03
L Ant Insula	-0.07	-0.02	0.05	-0.13	-0.03	0.05	-0.18	-0.07	0.24	-0.22
R Ant Insula	0.26	0.11	0.17	-0.15	-0.06	0.15	-0.32	-0.14	0.25	-0.01
L Post Insula	0.29	-0.04	0.23	0.03	-0.11	-0.16	-0.33	-0.02	0.20	-0.19
R Post Insula	0.22	0.09	-0.12	-0.01	0.08	0.13	-0.02	0.22	0.52	0.11
L Subgenual AC	0.19	-0.06	0.11	-0.37	-0.21	0.17	-0.06	-0.28	0.23	0.17
R Subgenual AC	0.11	-0.02	0.08	-0.32	-0.02	0.22	0.17	-0.23	0.01	0.22
L Drevets	0.29	0.05	0.25	-0.22	-0.33	-0.08	-0.25	-0.21	0.15	0.02
R Drevets	0.31	-0.08	0.11	-0.14	0.09	0.34	-0.06	-0.54	0.33	0.20
L Pregenual AC	0.41	0.06	0.35	-0.38	-0.24	-0.04	-0.31	-0.17	0.30	-0.01
R Pregenual AC	0.39	0.05	0.29	-0.31	-0.25	0.11	-0.33	-0.15	0.33	0.07
L Mayberg	0.35	0.00	0.30	-0.34	-0.19	-0.03	-0.29	-0.07	0.31	-0.10
R Mayberg	0.40	0.13	0.34	-0.39	-0.26	0.09	-0.38	-0.13	0.33	0.05
L Supragenual AC	0.34	-0.06	0.31	-0.23	-0.30	-0.13	-0.26	-0.23	0.24	-0.15
R Supragenual AC	0.36	-0.01	0.26	-0.28	-0.27	0.07	-0.29	-0.24	0.26	0.01
L Dorsal AC	0.28	-0.03	0.18	-0.12	-0.18	-0.05	-0.17	-0.10	0.29	-0.03
R Dorsal AC	0.26	-0.07	0.16	-0.34	-0.21	0.02	-0.17	-0.13	0.26	-0.05
L Post AC	-0.30	0.03	-0.27	0.08	0.12	-0.15	0.30	0.04	0.02	-0.01
R Post AC	0.14	0.00	0.21	-0.13	-0.16	-0.18	0.02	-0.02	0.09	-0.11

Appendix J. Cont'd

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	-0.04	-0.17	-0.06	-0.14	0.16	-0.02	0.31	-0.30	-0.39	-0.10	
R Ant Temporal	-0.20	-0.15	-0.15	0.03	0.34	0.18	0.13	-0.22	-0.23	0.13	
L Post Temporal	-0.13	-0.08	-0.02	0.15	0.20	-0.02	0.36	-0.19	-0.54	-0.08	
R Post Temporal	-0.20	-0.11	-0.23	0.15	0.26	0.04	0.31	0.00	-0.30	0.02	
L TP	0.16	-0.18	-0.02	-0.40	0.30	0.30	0.33	-0.33	-0.06	0.11	
R TP	-0.23	-0.45	-0.34	-0.09	0.37	0.44	0.28	-0.09	-0.06	0.15	
L Inf Parietal	0.44	0.26	0.38	-0.29	-0.28	-0.14	-0.17	-0.14	0.18	-0.02	
R Inf Parietal	0.28	0.18	0.11	-0.25	-0.01	0.25	-0.05	-0.14	0.28	0.35	
L Occipital	-0.20	-0.07	-0.20	0.14	0.12	0.05	0.05	0.12	0.13	-0.03	
R Occipital	0.09	0.07	-0.07	0.11	0.07	-0.11	0.00	0.09	0.22	-0.06	
L DLPFC	0.24	0.24	0.26	0.10	-0.31	-0.43	-0.26	-0.06	-0.09	-0.33	
R DLPFC	0.22	0.16	0.31	-0.17	-0.32	-0.32	-0.27	-0.16	0.24	-0.26	
L VLPFC	0.13	0.12	0.08	0.07	0.04	0.05	-0.09	-0.15	-0.24	-0.04	
R VLPFC	-0.08	-0.13	-0.05	0.07	-0.12	0.07	-0.16	-0.14	0.13	-0.01	
L MOFC	0.11	0.04	0.12	-0.38	-0.26	-0.05	-0.25	-0.11	0.08	-0.11	
R MOFC	0.02	-0.24	-0.07	-0.15	0.15	0.45	0.10	-0.50	0.00	0.19	
L LOFC	-0.17	0.02	-0.07	0.14	0.11	0.11	0.14	-0.02	-0.16	0.20	
R LOFC	-0.18	0.00	0.07	-0.05	0.14	0.10	0.13	-0.01	-0.15	0.12	
# r > .45	1	0	0	0	0	0	2	0	1	0	4
# r < -.45	1	0	0	0	0	0	0	2	1	0	4
Total	2	0	0	0	0	0	2	2	2	0	8
# r > .5	0	0	0	0	0	0	0	0	1	0	1
# r < -.5	1	0	0	0	0	0	0	2	1	0	4
Total	1	0	0	0	0	0	0	2	2	0	5
# Positive corrs	43	23	39	16	27	42	26	9	46	38	309
# Negative corrs	20	40	24	47	36	21	37	54	17	25	321
Total	63	63	63	63	63	63	63	63	63	63	630

Euphoria		0.40	0.65	-0.28	-0.24	-0.05	-0.03	-0.19	0.27	0.16
Calmness	0.40		0.59	-0.17	-0.40	-0.51	-0.35	0.10	0.01	0.04
Good/Bad	0.65	0.59		-0.42	-0.53	-0.49	-0.34	-0.16	-0.09	-0.30
Depression	-0.28	-0.17	-0.42		0.28	0.07	0.03	0.16	-0.06	-0.02
Fear	-0.24	-0.40	-0.53	0.28		0.67	0.36	-0.07	0.10	0.28
Anxiety	-0.05	-0.51	-0.49	0.07	0.67		0.33	-0.19	0.32	0.59
Anger	-0.03	-0.35	-0.34	0.03	0.36	0.33		-0.20	-0.05	0.34
Tiredness	-0.19	0.10	-0.16	0.16	-0.07	-0.19	-0.20		0.03	-0.07
Auditory	0.27	0.01	-0.09	-0.06	0.10	0.32	-0.05	0.03		0.49
Visual	0.16	0.04	-0.30	-0.02	0.28	0.59	0.34	-0.07	0.49	

Appendix K. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder at Baseline

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	-0.19	-0.18	-0.02	-0.34	-0.01	0.16	-0.16	-0.37	0.03	0.11
L Tegmentum	-0.16	-0.11	-0.39	0.07	0.42	0.32	0.45	0.01	0.36	-0.12
R Tegmentum	-0.02	0.25	-0.46	0.47	0.35	0.18	0.23	0.05	0.62	-0.16
L SI	0.29	0.09	0.07	0.27	0.17	-0.13	0.50	0.43	-0.14	-0.05
R SI	0.07	-0.21	-0.24	0.38	-0.07	-0.11	-0.07	-0.29	0.15	0.15
L Septal n	0.36	0.30	0.03	0.42	0.23	-0.11	0.54	0.48	-0.08	-0.02
R Septal n	0.29	0.25	-0.23	0.27	0.40	0.27	0.32	0.09	0.22	0.10
L BF	0.21	-0.12	-0.14	0.50	0.24	-0.01	0.49	0.10	0.00	0.09
R BF	0.05	-0.04	-0.37	0.50	0.07	-0.03	0.09	-0.20	0.32	0.04
Medulla	0.03	-0.01	-0.37	0.63	0.23	0.16	-0.12	-0.20	0.46	-0.09
Caudal Pons	0.19	0.26	-0.40	0.43	0.50	0.29	0.54	0.25	0.41	0.03
Rostral Pons	0.13	0.15	-0.50	0.57	0.70	0.43	0.67	0.23	0.44	-0.09
L Cerebellum	0.17	0.37	-0.32	0.37	0.10	0.02	0.19	-0.05	0.48	0.19
R Cerebellum	0.11	0.24	-0.24	0.23	0.39	0.37	0.17	-0.11	0.54	-0.09
L Cerebellum n	0.07	-0.02	-0.28	0.15	0.47	0.51	0.41	-0.06	0.51	-0.07
R Cerebellum n	0.08	0.11	-0.28	0.37	0.40	0.30	0.14	-0.06	0.59	-0.21
L M Thalamus	0.02	-0.21	-0.39	0.66	0.52	0.10	0.34	0.15	0.20	-0.20
R M Thalamus	0.02	-0.17	-0.39	0.27	0.70	0.37	0.63	0.17	0.18	-0.16
L Caudate	0.20	-0.04	0.19	0.09	-0.06	-0.13	0.39	-0.03	-0.18	0.21
R Caudate	-0.39	-0.43	-0.31	-0.11	0.26	0.19	0.24	-0.45	0.13	0.00
L Putamen	-0.17	-0.26	-0.35	0.27	0.51	0.23	0.64	-0.01	0.32	-0.17
R Putamen	-0.17	-0.32	-0.29	0.14	-0.17	-0.18	-0.25	-0.10	0.06	-0.17
L Globus Pallidus	0.22	-0.05	-0.23	0.22	-0.06	0.03	0.18	-0.20	0.15	0.34
R Globus Pallidus	-0.27	-0.13	-0.40	0.04	0.07	0.09	-0.03	-0.38	0.37	-0.14
L Hippocampus	0.05	0.08	-0.01	0.39	-0.09	0.09	-0.26	-0.45	0.34	0.02
R Hippocampus	-0.11	-0.19	0.15	-0.27	0.18	0.24	0.36	-0.02	-0.20	-0.11
L Amygdala	0.22	0.06	-0.25	0.50	0.28	0.27	-0.01	0.01	0.42	-0.08
R Amygdala	0.32	0.38	0.10	0.33	0.13	-0.08	0.27	0.53	-0.02	-0.23
L Ant Insula	0.09	0.49	0.15	0.24	-0.01	-0.16	0.09	-0.01	0.31	-0.10
R Ant Insula	-0.27	0.15	0.09	0.08	-0.08	-0.38	0.12	0.10	0.17	-0.34
L Post Insula	0.06	0.05	0.07	0.04	0.28	0.13	0.30	0.05	0.24	-0.29
R Post Insula	0.05	0.04	0.09	0.14	0.18	0.03	0.15	0.24	0.18	-0.34
L Subgenual AC	0.15	-0.23	0.37	-0.01	-0.33	-0.11	-0.34	-0.42	-0.21	0.34
R Subgenual AC	-0.17	-0.40	-0.19	0.11	-0.08	0.04	-0.29	-0.63	0.21	0.16
L Drevets	-0.05	0.12	-0.01	0.21	-0.04	-0.27	0.16	-0.12	0.10	-0.15
R Drevets	0.23	0.30	-0.01	0.11	-0.04	-0.22	0.28	0.03	0.03	0.16
L Pregenual AC	-0.15	-0.16	-0.14	0.05	0.08	0.07	0.28	-0.41	0.36	0.03
R Pregenual AC	-0.17	-0.29	-0.46	0.40	0.34	-0.06	0.46	0.09	0.03	-0.17
L Mayberg	0.03	-0.12	-0.04	-0.03	0.17	0.10	0.49	-0.23	0.16	0.14
R Mayberg	-0.27	-0.39	-0.45	0.34	0.38	-0.07	0.56	0.19	-0.08	-0.28
L Supragenual AC	-0.01	-0.36	0.09	-0.18	0.19	0.34	0.39	-0.40	-0.08	0.13
R Supragenual AC	-0.10	-0.23	-0.02	-0.28	0.38	0.22	0.64	0.06	-0.17	-0.13
L Dorsal AC	0.29	-0.02	0.28	-0.15	0.12	0.19	0.29	0.05	-0.09	0.07
R Dorsal AC	0.12	-0.01	0.27	-0.14	0.02	-0.03	0.21	-0.10	-0.15	-0.09
L Post AC	-0.26	-0.04	-0.18	0.26	-0.30	-0.32	-0.36	-0.21	0.24	-0.26
R Post AC	-0.24	-0.25	-0.07	0.10	-0.24	-0.14	-0.37	-0.36	0.09	-0.13

Appendix K. Cont'd

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	-0.17	0.11	0.13	-0.31	0.02	0.20	-0.03	-0.51	0.19	-0.03	
R Ant Temporal	-0.11	0.04	0.04	-0.04	0.06	0.16	-0.14	-0.59	0.38	-0.01	
L Post Temporal	0.14	0.55	0.37	-0.14	-0.38	-0.51	-0.26	0.22	-0.28	-0.09	
R Post Temporal	-0.26	-0.04	-0.18	0.26	-0.30	-0.32	-0.36	-0.21	0.24	-0.26	
L TP	0.22	0.19	-0.10	0.47	0.23	0.06	0.02	0.16	0.30	-0.09	
R TP	0.24	0.31	-0.02	0.38	0.27	0.01	0.14	0.24	0.07	-0.07	
L Inf Parietal	0.20	-0.04	-0.07	-0.18	0.35	0.30	0.50	0.34	-0.19	0.16	
R Inf Parietal	-0.14	-0.49	-0.08	0.00	0.11	-0.09	0.30	0.13	-0.19	-0.18	
L Occipital	-0.27	0.32	-0.18	-0.03	0.07	-0.19	0.17	0.52	0.03	-0.48	
R Occipital	-0.20	0.25	-0.27	-0.01	0.27	-0.13	0.38	0.74	-0.04	-0.37	
L DLPFC	-0.08	0.00	0.41	-0.42	-0.02	-0.19	0.12	-0.07	-0.21	-0.07	
R DLPFC	0.20	0.25	0.22	-0.10	0.09	-0.15	0.02	0.46	-0.31	0.08	
L VLPFC	-0.10	-0.33	-0.16	-0.25	0.20	0.25	0.41	-0.23	-0.13	0.27	
R VLPFC	0.43	0.08	0.42	-0.06	-0.34	-0.39	-0.08	0.14	-0.39	0.37	
L MOFC	0.16	0.39	0.17	0.08	-0.29	-0.38	-0.12	-0.18	-0.03	0.10	
R MOFC	0.37	0.46	0.15	0.02	-0.26	-0.29	-0.08	-0.19	0.03	0.38	
L LOFC	0.03	-0.18	-0.14	0.05	-0.27	-0.21	-0.30	-0.09	-0.09	0.33	
R LOFC	0.07	-0.27	0.14	-0.25	-0.39	-0.08	-0.59	-0.19	-0.27	0.33	
# r > .45	0	3	0	8	6	1	12	5	6	0	41
# r < -.45	0	1	4	0	0	1	1	4	0	1	12
Total	0	4	4	8	6	2	13	9	6	1	53
# r > .5	0	1	0	4	4	1	7	3	4	0	24
# r < -.5	0	0	1	0	0	1	1	3	0	0	6
Total	0	1	1	4	4	2	8	6	4	0	30
# Positive corrs	38	30	22	44	42	33	44	29	40	25	347
# Negative corrs	25	33	41	19	21	30	19	34	23	38	283
Total	63	63	63	63	63	63	63	63	63	63	630

Euphoria		0.50	0.60	0.02	-0.17	0.07	-0.08	0.09	-0.22	0.82
Calmness	0.50		0.37	-0.07	-0.21	-0.21	0.13	0.28	0.08	0.26
Good/Bad	0.60	0.37		-0.58	-0.60	-0.33	-0.36	-0.15	-0.52	0.54
Depression	0.02	-0.07	-0.58		0.29	0.07	0.11	0.16	0.46	-0.13
Fear	-0.17	-0.21	-0.60	0.29		0.77	0.67	0.24	0.33	-0.25
Anxiety	0.07	-0.21	-0.33	0.07	0.77		0.35	-0.16	0.41	0.15
Anger	-0.08	0.13	-0.36	0.11	0.67	0.35		0.41	0.14	-0.17
Tiredness	0.09	0.28	-0.15	0.16	0.24	-0.16	0.41		-0.33	-0.30
Auditory	-0.22	0.08	-0.52	0.46	0.33	0.41	0.14	-0.33		-0.16
Visual	0.82	0.26	0.54	-0.13	-0.25	0.15	-0.17	-0.30	-0.16	

Appendix L. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder with Procaine Administration

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	0.13	0.04	-0.43	0.21	0.51	0.54	-0.20	-0.49	0.49	0.21
L Tegmentum	-0.06	-0.14	0.25	-0.39	-0.10	0.04	0.48	-0.18	-0.39	-0.13
R Tegmentum	0.12	0.09	0.31	-0.20	-0.01	0.10	0.01	-0.28	-0.20	0.07
L SI	0.01	0.51	0.27	0.23	-0.22	-0.33	0.13	0.46	-0.42	0.14
R SI	0.37	0.79	0.47	0.04	-0.13	-0.43	-0.13	0.00	-0.05	-0.04
L Septal n	0.25	0.72	0.44	0.02	-0.14	-0.36	0.12	0.03	-0.16	-0.03
R Septal n	0.29	0.57	0.27	0.07	0.01	-0.10	0.19	0.04	-0.30	0.18
L BF	0.20	0.73	0.46	0.04	-0.27	-0.47	0.12	0.03	-0.20	-0.09
R BF	0.34	0.75	0.43	0.05	-0.14	-0.36	-0.08	0.00	-0.21	0.12
Medulla	0.09	0.03	-0.11	0.02	0.12	0.25	-0.08	-0.31	-0.21	0.38
Caudal Pons	-0.10	0.07	0.32	-0.20	-0.23	-0.04	0.31	0.08	-0.51	-0.01
Rostral Pons	-0.04	0.28	0.22	0.12	-0.11	-0.08	0.36	0.27	-0.55	0.17
L Cerebellum	-0.06	-0.41	0.27	-0.44	0.17	0.16	0.10	-0.37	-0.16	0.06
R Cerebellum	-0.05	-0.42	-0.06	-0.23	0.21	0.32	0.30	-0.18	-0.50	0.28
L Cerebellum n	0.06	-0.02	0.13	-0.19	-0.12	0.02	0.20	-0.14	-0.53	0.31
R Cerebellum n	0.07	0.05	0.09	-0.12	0.03	0.04	0.03	0.00	-0.52	0.35
L M Thalamus	0.11	0.41	0.08	0.34	-0.13	-0.03	0.16	0.12	-0.42	0.31
R M Thalamus	0.14	0.54	0.33	0.21	-0.05	-0.11	0.11	0.13	-0.41	0.26
L Caudate	-0.06	0.35	0.37	-0.20	-0.45	-0.53	0.29	0.02	-0.23	-0.46
R Caudate	-0.27	0.19	0.16	-0.15	-0.20	-0.14	0.24	-0.16	-0.26	-0.14
L Putamen	0.11	0.26	0.03	0.15	0.05	-0.01	0.43	0.42	-0.32	0.19
R Putamen	0.29	0.29	0.05	0.18	-0.24	-0.28	0.20	0.28	-0.43	0.38
L Globus Pallidus	-0.30	0.28	0.19	-0.08	-0.57	-0.52	0.06	0.14	-0.23	0.06
R Globus Pallidus	0.31	0.50	0.42	-0.07	-0.12	-0.44	-0.21	-0.05	-0.12	0.22
L Hippocampus	0.05	0.08	0.53	-0.35	-0.21	-0.29	0.30	0.28	-0.38	-0.21
R Hippocampus	0.12	-0.15	-0.24	0.25	0.32	0.38	0.28	0.60	-0.31	0.17
L Amygdala	0.28	0.42	0.45	-0.03	-0.16	-0.21	0.12	0.20	-0.49	0.04
R Amygdala	0.18	0.20	0.00	0.37	-0.04	-0.15	0.03	0.62	-0.30	0.49
L Ant Insula	-0.29	-0.25	-0.20	-0.17	0.23	0.33	0.15	0.12	-0.20	-0.12
R Ant Insula	-0.30	-0.33	-0.24	-0.01	0.19	0.15	0.24	0.28	-0.06	-0.14
L Post Insula	0.17	-0.06	-0.02	0.01	0.02	-0.05	0.41	0.43	-0.56	0.31
R Post Insula	0.22	0.19	0.16	0.18	0.00	-0.12	0.31	0.60	-0.50	0.08
L Subgenual AC	0.15	0.28	0.17	-0.18	-0.02	-0.12	0.10	-0.40	0.08	-0.43
R Subgenual AC	0.25	0.53	0.24	-0.21	-0.30	-0.36	-0.02	-0.38	0.07	-0.18
L Drevets	0.24	0.62	0.16	0.21	-0.11	-0.22	0.04	0.41	-0.30	0.11
R Drevets	0.31	0.68	0.48	0.00	-0.25	-0.40	-0.07	0.21	-0.13	-0.03
L Pregenual AC	0.33	0.70	0.32	0.00	-0.05	-0.23	-0.05	0.11	-0.22	0.04
R Pregenual AC	0.25	0.57	0.18	0.31	-0.17	-0.22	-0.05	0.26	-0.24	0.25
L Mayberg	0.34	0.72	0.36	-0.02	0.01	-0.24	-0.05	0.00	-0.15	0.06
R Mayberg	0.28	0.53	0.08	0.35	-0.09	-0.18	0.03	0.22	-0.23	0.37
L Supragenual AC	0.43	0.53	0.35	-0.05	0.14	-0.26	0.16	0.11	-0.13	0.01
R Supragenual AC	0.27	0.31	0.22	-0.08	-0.02	-0.31	0.18	0.01	-0.04	0.10
L Dorsal AC	0.34	0.24	0.22	-0.25	0.10	-0.05	0.48	0.16	-0.17	-0.26
R Dorsal AC	0.37	0.32	0.02	0.09	0.18	-0.05	0.29	0.23	0.02	0.14
L Post AC	0.19	0.22	0.18	0.03	-0.43	-0.47	-0.13	0.27	-0.21	0.01
R Post AC	0.12	0.39	0.36	-0.15	-0.65	-0.53	-0.11	-0.18	-0.23	-0.42

Appendix L. Cont'd.

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	0.13	-0.42	-0.04	-0.51	0.29	0.09	0.12	-0.56	0.42	-0.38	
R Ant Temporal	0.02	-0.44	0.01	-0.43	0.35	0.20	0.04	-0.33	0.05	-0.24	
L Post Temporal	0.14	-0.51	-0.37	0.11	0.41	0.21	-0.13	0.05	0.47	0.05	
R Post Temporal	0.19	0.22	0.18	0.03	-0.43	-0.47	-0.13	0.27	-0.21	0.01	
L TP	0.22	0.10	0.30	-0.04	0.11	-0.11	0.02	0.00	-0.33	-0.03	
R TP	0.22	0.02	0.17	0.11	0.24	-0.07	0.04	-0.02	-0.13	0.23	
L Inf Parietal	0.25	0.07	0.09	0.09	0.34	0.20	0.25	0.28	-0.25	0.53	
R Inf Parietal	0.08	0.34	0.21	0.18	-0.20	-0.28	0.07	0.45	-0.48	0.15	
L Occipital	-0.31	-0.43	-0.31	0.20	-0.03	0.09	-0.12	0.19	0.02	0.37	
R Occipital	-0.41	-0.26	-0.12	0.36	-0.18	0.05	-0.10	0.38	-0.32	0.40	
L DLPFC	0.42	0.45	0.42	-0.09	0.03	-0.18	0.10	-0.14	-0.01	-0.19	
R DLPFC	0.29	0.49	0.26	0.12	0.14	-0.06	0.04	0.36	-0.13	-0.10	
L VLPFC	0.18	0.46	0.02	0.07	0.16	-0.06	0.19	-0.19	0.24	-0.07	
R VLPFC	0.35	0.24	-0.01	0.14	0.14	0.16	0.05	0.40	-0.17	0.12	
L MOFC	0.38	0.62	0.35	-0.09	0.01	-0.18	-0.22	0.04	-0.05	0.16	
R MOFC	0.61	0.81	0.58	-0.07	-0.11	-0.38	-0.31	-0.04	0.10	-0.05	
L LOFC	0.47	0.27	0.18	-0.23	0.49	0.23	-0.17	-0.70	0.74	-0.34	
R LOFC	0.53	-0.04	0.10	-0.46	0.49	0.21	0.02	-0.56	0.78	-0.33	
36	3	21	6	0	2	0	2	4	3	2	43
20	0	1	0	2	2	6	0	3	9	1	24
56	3	22	6	2	4	6	2	7	12	3	67
25	2	18	2	0	0	0	0	3	2	1	28
12	0	1	0	1	2	3	0	3	6	0	16
37	2	19	2	1	2	3	0	6	8	1	44
377	51	49	51	32	28	19	45	41	11	39	366
253	12	14	12	31	35	44	18	22	52	24	264
630	63	63	63	63	63	63	63	63	63	63	630

Euphoria	1.00	0.45	0.34	-0.18	0.40	0.05	0.00	-0.25	0.53	-0.11
Calmness	0.45	1.00	0.54	0.08	-0.08	-0.29	-0.34	-0.19	0.18	-0.19
Good/Bad	0.34	0.54	1.00	-0.50	-0.19	-0.42	-0.35	-0.36	0.05	-0.43
Depression	-0.18	0.08	-0.50	1.00	-0.14	-0.08	-0.07	0.54	-0.08	0.43
Fear	0.40	-0.08	-0.19	-0.14	1.00	0.80	0.13	-0.27	0.40	0.13
Anxiety	0.05	-0.29	-0.42	-0.08	0.80	1.00	0.26	-0.19	0.04	0.20
Anger	0.00	-0.34	-0.35	-0.07	0.13	0.26	1.00	0.29	-0.30	-0.16
Tiredness	-0.25	-0.19	-0.36	0.54	-0.27	-0.19	0.29	1.00	-0.37	0.21
Auditory	0.53	0.18	0.05	-0.08	0.40	0.04	-0.30	-0.37	1.00	-0.21
Visual	-0.11	-0.19	-0.43	0.43	0.13	0.20	-0.16	0.21	-0.21	1.00

Appendix M. Correlations Between Brain ROIs and Emotional and Sensory Responses in 15 Patients with Bipolar Disorder – Delta CBF / Delta Rating

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual
Global	0.19	0.09	-0.49	0.38	0.51	0.65	0.07	-0.37	0.46	0.03
L Tegmentum	0.43	-0.15	-0.20	0.44	0.44	0.21	0.46	-0.44	0.58	-0.08
R Tegmentum	0.65	0.28	-0.04	0.36	0.36	0.05	0.52	-0.47	0.80	-0.26
L SI	0.28	0.42	0.43	-0.19	-0.03	-0.37	-0.23	0.11	-0.13	0.15
R SI	0.34	0.31	0.19	0.19	0.02	-0.40	-0.09	0.19	0.08	0.18
L Septal n	0.38	0.66	0.54	-0.17	0.02	-0.43	-0.07	-0.11	0.20	-0.15
R Septal n	0.26	0.42	0.09	0.37	0.01	-0.32	-0.07	0.10	0.19	0.05
L BF	0.48	0.58	0.60	-0.11	-0.10	-0.55	-0.12	-0.18	0.19	-0.01
R BF	0.43	0.45	0.30	0.13	0.01	-0.40	-0.09	-0.08	0.15	0.18
Medulla	0.44	-0.27	-0.22	0.25	0.45	0.31	0.55	-0.49	0.55	-0.14
Caudal Pons	0.39	0.02	0.03	0.31	0.15	-0.04	0.43	-0.36	0.57	-0.30
Rostral Pons	0.27	0.23	0.15	0.35	0.09	-0.11	0.06	-0.20	0.47	-0.29
L Cerebellum	0.30	0.07	-0.11	0.40	0.11	0.01	-0.17	-0.18	0.14	0.59
R Cerebellum	0.19	0.01	-0.23	0.52	0.12	-0.06	-0.10	0.01	0.32	0.51
L Cerebellum n	0.36	-0.24	-0.06	0.33	0.27	0.13	0.39	-0.60	0.39	0.03
R Cerebellum n	0.19	-0.40	-0.18	0.33	0.34	0.23	0.25	-0.50	0.27	0.22
L M Thalamus	0.26	0.09	0.17	0.19	-0.20	-0.21	-0.10	-0.16	0.01	0.22
R M Thalamus	0.24	0.45	0.25	0.17	-0.04	-0.24	-0.30	0.09	0.09	0.18
L Caudate	0.34	0.26	0.65	-0.38	-0.32	-0.66	0.01	-0.14	-0.17	-0.10
R Caudate	-0.13	0.01	-0.05	0.42	-0.16	-0.33	-0.05	0.37	0.00	0.17
L Putamen	0.22	-0.14	-0.16	0.46	0.23	0.05	0.01	-0.06	0.35	0.33
R Putamen	0.11	-0.17	-0.20	0.18	0.25	0.19	-0.16	0.18	-0.16	0.52
L Globus Pallidus	0.05	-0.03	-0.08	0.42	-0.36	-0.45	0.14	-0.05	0.20	0.14
R Globus Pallidus	0.45	0.36	0.09	0.35	0.20	-0.17	0.06	0.10	0.24	-0.03
L Hippocampus	0.26	-0.05	0.37	-0.23	0.08	-0.16	0.20	-0.35	-0.01	0.00
R Hippocampus	-0.05	0.06	0.36	-0.12	-0.06	-0.17	0.02	-0.05	-0.07	-0.09
L Amygdala	0.04	0.17	0.41	0.09	-0.03	-0.28	-0.30	-0.11	0.08	0.05
R Amygdala	0.04	0.08	0.36	-0.17	0.05	0.04	-0.61	-0.16	-0.02	0.37
L Ant Insula	-0.09	0.30	0.13	-0.11	-0.07	-0.17	-0.41	0.29	-0.24	0.21
R Ant Insula	0.12	0.34	0.25	0.08	0.05	-0.18	-0.60	0.01	0.09	0.26
L Post Insula	0.04	-0.29	0.00	0.04	-0.10	-0.24	-0.14	0.11	-0.19	0.62
R Post Insula	-0.23	-0.05	0.12	0.11	-0.03	-0.05	-0.57	0.23	-0.26	0.33
L Subgenual AC	0.25	0.01	0.20	0.08	0.09	0.00	-0.32	-0.39	0.01	0.23
R Subgenual AC	0.12	0.07	-0.04	0.29	-0.24	-0.49	0.25	0.15	0.08	0.35
L Drevets	0.31	0.23	0.02	0.24	0.15	0.03	-0.08	0.22	0.07	0.16
R Drevets	0.39	0.41	0.30	0.19	-0.23	-0.62	0.02	0.13	0.11	0.23
L Pregenual AC	0.41	0.38	0.11	0.07	0.19	-0.10	-0.21	0.15	0.09	0.35
R Pregenual AC	0.36	-0.06	-0.05	0.09	0.13	0.01	0.12	0.30	-0.04	0.27
L Mayberg	0.48	0.52	0.26	-0.06	0.13	-0.22	-0.27	0.06	0.07	0.31
R Mayberg	0.43	-0.10	-0.11	0.04	0.25	0.15	0.11	0.21	-0.03	0.30
L Supragenual AC	0.50	0.32	0.35	-0.17	0.10	-0.24	-0.14	0.02	-0.04	0.10
R Supragenual AC	0.54	0.31	0.32	0.06	-0.06	-0.38	-0.32	-0.13	0.14	0.32
L Dorsal AC	0.28	0.00	0.26	-0.13	0.12	0.00	-0.21	-0.33	0.07	0.06
R Dorsal AC	0.41	0.15	0.27	0.15	-0.02	-0.17	-0.26	-0.43	0.23	0.28
L Post AC	0.11	-0.02	-0.05	0.20	0.13	0.05	0.08	0.21	-0.03	0.28
R Post AC	0.14	0.28	0.20	0.04	-0.15	-0.13	-0.15	0.21	-0.22	0.13

Appendix M. Cont'd

ROI	Euphoria	Calmness	Good/Bad	Depression	Fear	Anxiety	Anger	Tiredness	Auditory	Visual	
L Ant Temporal	0.29	0.01	0.15	-0.04	0.01	-0.05	0.36	-0.56	0.41	-0.48	
R Ant Temporal	-0.02	-0.39	-0.04	0.00	0.09	0.15	0.39	-0.37	0.19	-0.36	
L Post Temporal	0.03	-0.23	0.08	-0.10	-0.10	-0.13	0.34	-0.45	0.10	-0.08	
R Post Temporal	-0.04	-0.28	0.08	0.02	-0.04	-0.11	0.22	-0.33	0.05	-0.11	
L TP	0.25	-0.12	0.19	-0.28	0.29	0.11	0.37	-0.39	0.11	-0.41	
R TP	0.26	0.04	0.35	-0.25	-0.05	-0.18	0.40	-0.57	0.27	-0.38	
L Inf Parietal	0.34	-0.02	0.03	-0.25	0.06	-0.20	0.09	0.37	-0.40	0.37	
R Inf Parietal	-0.18	-0.30	-0.04	-0.18	-0.07	0.08	-0.33	0.26	-0.49	0.55	
L Occipital	0.08	0.18	0.05	-0.09	-0.16	-0.01	-0.22	0.06	-0.11	0.25	
R Occipital	0.01	0.25	0.05	-0.22	-0.03	0.00	-0.17	0.35	-0.20	0.11	
L DLPFC	0.31	0.47	0.51	-0.27	-0.21	-0.53	-0.19	0.13	-0.18	0.20	
R DLPFC	-0.10	0.38	0.23	-0.29	-0.21	-0.36	-0.21	0.63	-0.39	0.01	
L VLPFC	0.37	0.32	-0.28	0.37	0.21	-0.10	0.18	0.26	0.25	0.16	
R VLPFC	-0.05	-0.47	-0.11	-0.05	-0.12	-0.06	-0.17	0.23	-0.62	0.71	
L MOFC	0.46	0.36	0.23	0.00	-0.01	-0.19	0.20	0.16	-0.01	-0.19	
R MOFC	0.45	0.45	0.25	0.10	-0.25	-0.67	0.31	0.16	0.06	0.08	
L LOFC	0.42	0.02	-0.24	0.13	0.49	0.30	0.55	-0.41	0.42	-0.20	
R LOFC	-0.03	-0.32	-0.29	0.03	0.36	0.36	0.36	-0.26	0.17	-0.08	
36	8	5	4	2	1	0	4	1	5	6	36
20	0	1	0	0	0	6	3	7	2	1	20
56	8	6	4	2	1	6	7	8	7	7	56
25	3	3	4	1	0	0	3	1	4	6	25
12	0	0	0	0	0	5	3	3	1	0	12
37	3	3	4	1	0	5	6	4	5	6	37
377	53	41	42	40	35	20	30	32	40	44	377
253	10	22	21	23	28	43	33	31	23	19	253
630	63	63	63	63	63	63	63	63	63	63	630

Euphoria		0.37	0.13	0.04	0.35	-0.08	0.43	-0.31	0.46	-0.09
Calmness	0.37		0.50	-0.24	-0.16	-0.42	-0.08	-0.14	0.21	-0.30
Good/Bad	0.13	0.50		-0.64	-0.37	-0.60	-0.40	-0.33	-0.18	-0.28
Depression	0.04	-0.24	-0.64		0.25	0.26	0.13	-0.08	0.57	0.25
Fear	0.35	-0.16	-0.37	0.25		0.79	0.22	-0.09	0.50	-0.14
Anxiety	-0.08	-0.42	-0.60	0.26	0.79		0.06	-0.04	0.23	-0.01
Anger	0.43	-0.08	-0.40	0.13	0.22	0.06		0.18	0.21	-0.30
Tiredness	-0.31	-0.14	-0.33	-0.08	-0.09	-0.04	0.18		-0.45	-0.02
Auditory	0.46	0.21	-0.18	0.57	0.50	0.23	0.21	-0.45		-0.25
Visual	-0.09	-0.30	-0.28	0.25	-0.14	-0.01	-0.30	-0.02	-0.25	

Appendix N. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 32 Healthy Controls – Negative Emotions

			Multivariate Analysis			Univariate Analysis			Regression Analysis				
Model	L/R	Regions	RGR	R^2		Emotion	R^2		Model	R^2		Region	B (SE)
dCBF AChNet	L	All	0.70	0.41	*	Anxiety	0.27		AM, SI, SGAC	0.25	*	AM	.19 (.07)
Predicts dEmotion												SI	-.07 (.04)
Anxiety												SGAC	.03 (.04)
Fear									AM, SI	0.24	*	AM	.20 (.07)
												SI	-.05 (.04)
									AM	0.17	*	AM	.13 (.05)
									SI	0.01		SI	.01 (.03)
						Fear	0.18						
Alternative Models	L	BF	0.74	0.43	*	Anxiety	0.24		AM, BF, SGAC	0.20			
		Drevet	0.61	0.38	*		0.25		AM, SI, DRE	0.24	*		
		Mayberg	0.67	0.40	*		0.27		AM, SI, SGAC, MAY	0.26			
		DLPFC	1.03	0.51	*		0.36		AM, SI, SGAC, DLPFC	0.34	*		
		D, M	0.60	0.37	*		0.25		AM, SI, DRE, MAY	0.25			
		D,M, D	0.90	0.47	*		0.37		AM, SI, DRE, DLPFC	0.37	*		
		B, D, M	0.66	0.40	*		0.26		AM, BF, DRE, MAY	0.26			
		B, D,M, D	**1.10**	**0.52**	*		**0.43**	*	**BF, DRE, DLPFC**	**0.41**	*	**BF**	**.20 (.06)**
												DRE	**-.07 (.03)**
												DLPFC	**-.10 (.03)**
		BF				Fear	0.13						
		Drevet					0.21						
		Mayberg					0.17						
		DLPFC					0.17		SI, PGAC, AINS, DLPFC	0.14			
		D, M					0.20						
		D,M, D					0.21		SI, MAY, AINS, DLPFC	0.14			
		B, D, M					0.18						
		B, D,M, D					0.22						

Appendix N. Cont'd.

			Multivariate Analysis			Univariate Analysis			Regression Analysis				
Model	L/R	Regions	RGR	R^2		Emotion	R^2		Model	R^2		Region	B (SE)
dCBF AChNet Predicts dEmotion	R	All	0.73	0.42	*	Anxiety	0.38	*	**AM, MOFC**	**0.25**	*	**AM**	**.07 (.06)**
												MOFC	**.14 (.05)**
Anxiety									PGAC, MOFC	0.22	*	PGAC	-.04 (.05)
Fear												MOFC	.16 (.06)
									MOFC	0.20	*	MOFC	.14 (.05)
									AM, PGAC, MOFC	0.28	*		
						Fear	0.21		PGAC, MOFC	0.17			
Alternative Models	R	BF	0.56	0.36		Anxiety	0.35		AM, BF, MOFC	0.33	*	AM	-.09 (.05)
												BF	.17 (.06)
												MOFC	.19 (.04)
		Drevet	**0.71**	**0.41**	*		0.38	*	AM, DRE, PGAC, MOFC	0.28			
									AM, DRE, MOFC	0.25	*		
									ADRE, MOFC	0.20	*		
									DRE, PGAC	0.15		DRE	.15 (.07)
												PGAC	-.06 (.06)
		Mayberg	0.69	0.41	*		0.38	*	AM, MAY, MOFC	0.27	*		
									MAY, MOFC	0.22	*		
		DLPFC	0.51	0.34			0.28		AM, PGAC, DLPFC	0.23			
		D, M	0.66	0.40	*		0.38	*	AM, DRE, MAY, MOFC	0.27			
									DRE, MAY, MOFC	0.22			
									DRE, MAY	0.14			
		D, M, D	0.80	0.44	*		0.44	*	AM, DRE, MAY, DLPFC	0.33	*		
		B, D, M	0.54	0.35			0.35		AM, BF, DRE, MAY, MOFC	0.34	*		
		B, D, M, D	0.73	0.42			0.41	*	AM, BF, DRE, MAY, DLPFC	0.41	*		
									BF, DRE, DLPFC	0.29	*		
		BF				Fear	0.26		BF, PGAC, MOFC	0.18			
		Drevet					0.24		DRE, PGAC, MOFC	0.21			
		Mayberg					0.21		MAY, MOFC	0.16			
		DLPFC					0.17		PGAC, DLPFC	0.14			
		D, M					0.24		DRE, MAY, MOFC	0.19			
		D, M, D					0.32		DRE, MAY, DLPFC	0.26			
		B, D, M					0.31		BF, DRE, MAY, MOFC	0.25			
		B, D, M, D					**0.38**		BF, DRE, MAY, DLPFC	0.32			

Appendix O. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 32 Healthy Controls – Positive Emotions

			Multivariate Analysis		Univariate Analysis		Regression Analysis			
Model	L/R	Regions	RGR	R^2	Emotion	R^2	Model	R^2	Region	B (SE)
dCBF AChNet Predicts dEmotion euphoria calmness	L	All	0.34	0.25	Euphoria	0.24	SGAC, PGAC, AINS, MOFC	0.19	SGAC	-.01 (.06)
									PGAC	.12 (.05)
									AINS	-.02 (.03)
									MOFC	-.04 (.11)
					Calmness	0.04	SI, SGAC, PGAC	0.02	SI	-.01 (.05)
									SGAC	-.02 (.05)
									PGAC	.03 (.06)
							SI, PGAC	0.01		
							SI	0.00	SI	.00 (.03)
Alternative Models	L	BF	0.28	0.22	Euphoria	0.20	BF, SGAC, PGAC, AINS, MOFC	0.20		
							AM, BF, PGAC, AINS	0.19	AM	-.03 (.11)
									BF	.05 (.11)
									PGAC	.09 (.06)
									AINS	-.01 (.04)
		Drevet	0.41	0.29		0.26	DRE, PGAC, AINS, MOFC	0.19		
		Mayberg	0.35	0.26		0.23	SGAC, MAY, AINS, MOFC	0.16		
		DLPFC	0.34	0.25		0.23	SGAC, PGAC, AINS, DLPFC	0.20		
		D, M	0.44	0.30		0.26	DRE, MAY, AINS, MOFC	0.16		
		D,M, D	0.45	0.31		0.26	DRE, MAY, AINS, DLPFC	0.18		
		B, D, M	0.34	0.25		0.19	AM, BF, MAY, AINS	0.18		
		B, D,M, D	0.35	0.26		0.20	BF, DRE, MAY, AINS, DLPFC	0.20		
		BF			Calmness	0.04	BF, SGAC, PGAC	0.02		
		Drevet				0.03	SI, DRE, PGAC	0.01		
		Mayberg				0.03	SI, SGAC, MAY	0.01		
		DLPFC				0.08	SI, SGAC, PGAC, DLPFC	0.07		
		D, M				0.03	SI, DRE, MAY	0.01		
		D,M, D				0.09	SI, DRE, MAY, DLPFC	0.09		
		B, D, M				0.03	BF, DRE, MAY	0.03		
		B, D,M, D				0.11	BF, DRE, MAY, DLPFC	0.10		

Appendix O. Cont'd.

			Multivariate Analysis		Univariate Analysis		Regression Analysis			
Model	L/R	Regions	RGR	R^2	Emotion	R^2	Model	R^2	Region	B (SE)
dCBF AChNet Predicts dEmotion	R	All	0.27	0.21	Euphoria	0.21	SGAC, PGAC, MOFC	0.21	SGAC	.02 (.04)
									PGAC	.16 (.06)
Euphoria									MOFC	-.11 (.08)
Calmness					Calmness	0.19	SI, AINS, MOFC	0.09	SI	-.02 (.04)
									AINS	.02 (.02)
									MOFC	-.07 (.07)
Alternative Models	R	BF	0.27	0.21	Euphoria	0.21	BF, SGAC, PGAC, MOFC	0.21		
							BF, SGAC, MAY, MOFC	0.20		
		Drevet	0.40	0.29		0.28	DRE, PGAC, MOFC	0.26		
		Mayberg	0.28	0.22		0.20	SGAC, MAY, MOFC	0.20		
		DLPFC	0.23	0.19		0.18	SGAC, PGAC, DLPFC	0.17		
		D, M	0.41	0.29		0.28	DRE, MAY, MOFC	0.27		
		D,M, D	0.23	0.18		0.18	DRE, MAY, DLPFC	0.17		
		B, D, M	0.40	0.28		0.28	BF, DRE, MAY, MOFC	0.27		
							BF, MAY, MOFC	0.20		
		B, D,M, D	0.24	0.19		0.18	BF, MAY, DLPFC	0.17	BF	.00 (.07)
									MAY	.09 (.05)
									DLPFC	.03 (.05)
		BF			Calmness	0.20	BF, AINS, MOFC	0.08		
		Drevet				0.21	SI, DRE, AINS, MOFC	0.12	SI	-.15 (.05)
									DRE	.11 (.11)
									AINS	.01 (.02)
									MOFC	-.13 (.09)
		Mayberg				0.20	SI, MAY, AINS, MOFC	0.19		
		DLPFC				0.15	SI, AINS, DLPFC	0.09		
		D, M				0.23	SI, DRE, MAY, AINS, MOFC	0.21		
		D,M, D				0.17	SI, DRE, MAY, AINS, DLPFC	0.15		
		B, D, M				0.22	BF, DRE, MAY, AINS, MOFC	0.14		
		B, D,M, D				0.16	BF, DRE, MAY, AINS, DLPFC	0.10		

Appendix P. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 15 Patients with Bipolar Disorder – Negative Emotions

			Multivariate Analysis			Univariate Analysis		Regression Analysis				
Model	L/R	Regions	RGR	R^2		Emotion	R^2	Model	R^2		Region	B (SE)
dCBF AChNet	L		1.45	0.59		Anxiety	0.29	AM, SI, SGAC	0.23		AM	-.11 (.12)
Predicts dEmotion											SI	-.10 (.07)
Anxiety											SGAC	.09 (.10)
Fear								AM, SI	0.16		AM	-.07 (.11)
											SI	-.07 (.06)
								AM	0.08		AM	-.11 (.11)
								SI	0.14		SI	-.09 (.06)
						Fear	0.13					
Alternative Models	L	BF	3.03	0.75	*	Anxiety	0.45	AM, BF, SGAC	0.39			
		Drevet	3.60	0.78	*		0.39	AM, SI, DRE	0.26			
		Mayberg	1.67	0.63			0.27	AM, SI, SGAC, MAY	0.24			
		DLPFC	1.27	0.56			0.44	AM, SI, SGAC, DLPFC	0.31			
		D, M	2.93	0.75	*		0.39	AM, SI, DRE, MAY	0.26			
		D,M, D	2.33	0.70			0.53	AM, SI, DRE, DLPFC	0.37	*		
		B, D, M	3.92	0.80	*		0.44	AM, BF, DRE, MAY	0.41			
		B, D,M, D	**5.03**	**0.83**	*		**0.58**	**BF, DRE, DLPFC**	**0.53**	*	**BF**	**-.22 (.13)**
											DRE	**.10 (.05)**
											DLPFC	**-.13 (.08)**
		BF				Fear	0.15					
		Drevet					0.11					
		Mayberg					0.08					
		DLPFC					**0.43**	**SI, PGAC, AINS, DLPFC**	**0.34**			
		D, M					0.11					
		D,M, D					0.36	SI, MAY, AINS, DLPFC	0.28			
		B, D, M					0.11					
		B, D,M, D					0.23					

Appendix P. Cont'd

			Multivariate Analysis			Univariate Analysis		Regression Analysis				
Model	L/R	Regions	RGR	R^2		Emotion	R^2	Model	R^2		Region	B (SE)
dCBF AChNet Predicts dEmotion Anxiety Fear	R		3.82	0.64	*	Anxiety	0.61	AM, MOFC	0.45	*	AM	.00 (.11)
											MOFC	-.22 (.07)
								PGAC, MOFC	0.53	*	PGAC	.12 (.08)
											MOFC	-.27 (.07)
								MOFC	0.45	*	MOFC	-.22 (.07)
								AM, PGAC, MOFC	0.57	*		
						Fear	0.26	PGAC, MOFC	0.12			
Alternative Models	R		3.91	0.80	*	Anxiety	0.60	AM, BF, MOFC	0.45		AM	.00 (.13)
											BF	-.07 (.15)
											MOFC	-.13 (.11)
			3.83	**0.79**	*		**0.61**	AM, DRE, MOFC	0.45			
								DRE, MOFC	0.45	*		
								DRE, PGAC	**0.59**	*	**DRE**	**-.27 (.07)**
											PGAC	**.21 (.09)**
			3.50	0.78	*		0.61	AM, MAY, MOFC	0.58	*		
								MAY, MOFC	0.54	*		
			1.33	0.57			0.37	AM, PGAC, DLPFC	0.14			
			3.70	0.79	*		0.61	AM, DRE, MAY, MOFC	0.59	*		
								DRE, MAY, MOFC	0.59	*		
								DRE, MAY	0.58	*		
			2.48	0.71			0.59	AM, DRE, MAY, DLPFC	0.60	*		
			3.82	0.79	*		0.58	AM, BF, DRE, MAY, MOFC	0.59			
			2.57	0.72	*		0.62	AM, BF, DRE, MAY, DLPFC	0.61			
								BF, DRE, DLPFC	0.41			
						Fear	0.24	BF, PGAC, MOFC	0.15			
							0.29	DRE, PGAC, MOFC	0.16			
							0.28	MAY, MOFC	0.16			
							0.26	PGAC, DLPFC	0.08			
							0.30	DRE, MAY, MOFC	0.20			
							0.42	SI, DRE, MAY, DLPFC	0.41			
							0.27	BF, DRE, MAY, MOFC	0.26			
							0.33	BF, DRE, MAY, DLPFC	0.32			

Appendix Q. Multivariate Multiple Regression Analysis of the Relationship Between Change in Emotion Ratings and Change in rCBF – 15 Patients with Bipolar Disorder – Positive Emotions

			Multivariate Analysis			Univariate Analysis			Regression Analysis				
Model	L/R	Regions	RGR	R^2		Emotion	R^2		Model	R^2		Region	B (SE)
dCBF AChNet Predicts dEmotion Euphoria Calmness	L	All	1.75	0.64		Euphoria	0.63		SGAC, PGAC, AINS, MOFC	0.49		SGAC	.10 (.09)
												PGAC	.11 (.06)
												AINS	-.10 (.05)
												MOFC	.16 (.09)
						Calmness	0.29		SI, SGAC, PGAC	0.28		SI	.14 (.10)
												SGAC	-.11 (.12)
												PGAC	.08 (.09)
									SI, PGAC	0.23			
									SI	0.18		SI	.14 (.08)
Alternative Models	L	BF	5.44	0.84	*	Euphoria	**0.82**	*	BF, SGAC, PGAC, AINS, MOFC	0.55			
									AM, BF, PGAC, AINS	**0.71**	*	**AM**	**-.33 (.11)**
												BF	**.51 (.14)**
												PGAC	**.15 (.05)**
												AINS	**-.11 (.04)**
		Drevet	3.12	0.76	*		0.70		DRE, PGAC, AINS, MOFC	0.68	*		
		Mayberg	2.38	0.70			0.70		SGAC, MAY, AINS, MOFC	0.60	*		
		DLPFC	1.58	0.61			0.60		SGAC, PGAC, AINS, DLPFC	0.40			
		D, M	4.39	0.81	*		0.72		DRE, MAY, AINS, MOFC	0.70	*		
		D,M, D	1.74	0.63			0.63		DRE, MAY, AINS, DLPFC	0.42			
		B, D, M	6.03	0.86	*		0.75	*	AM, BF, MAY, AINS	0.70	*		
		B, D,M, D	5.94	0.86	*		0.78	*	BF, DRE, MAY, AINS, DLPFC	0.52			
		BF				Calmness	**0.52**		**BF, SGAC, PGAC**	**0.44**			
		Drevet					0.40		SI, DRE, PGAC	0.24			
		Mayberg					0.40		SI, SGAC, MAY	0.37			
		DLPFC					0.28		SI, SGAC, PGAC, DLPFC	0.28			
		D, M					0.48		SI, DRE, MAY	0.30			
		D,M, D					0.33		SI, DRE, MAY, DLPFC	0.33			
		B, D, M					0.50		BF, DRE, MAY	0.39			
		B, D,M, D					0.49		BF, DRE, MAY, DLPFC	0.40			

			Multivariate Analysis		Univariate Analysis		Regression Analysis			
Model	L/R	Regions	RGR	R^2	Emotion	R^2	Model	R^2	Region	B (SE)
dCBF AChNet	R	All	1.02	0.50	Euphoria	0.34	SGAC, PGAC, MOFC	0.30	SGAC	-.12 (.13)
Predicts dEmotion									PGAC	.10 (.12)
Euphoria									MOFC	.21 (.13)
Calmness					Calmness	0.50	SI, AINS, MOFC	0.42	SI	-.26 (.16)
									AINS	.24 (.12)
							AINS	0.11	MOFC	.42 (.18)
Alternative Models	R	BF	1.50	0.60	Euphoria	0.46	BF, SGAC, PGAC, MOFC	0.39		
							BF, SGAC, MAY, MOFC	0.43		
		Drevet	1.03	0.51		0.39	DRE, PGAC, MOFC	0.28		
		Mayberg	1.02	0.50		0.40	SGAC, MAY, MOFC	0.36		
		DLPFC	1.37	0.58		0.42	SGAC, PGAC, DLPFC	0.16		
		D, M	1.03	0.51		0.45	DRE, MAY, MOFC	0.38		
		D,M, D	**2.24**	**0.69**		0.44	DRE, MAY, DLPFC	0.30		
		B, D, M	0.97	0.49		0.48	BF, DRE, MAY, MOFC	0.46		
							BF, MAY, MOFC	0.34		
		B, D,M, D	1.53	0.60		**0.48**	**BF, MAY, DLPFC**	**0.45**	**BF**	**.30 (.14)**
									MAY	**.11 (.08)**
									DLPFC	**-.11 (.06)**
		BF			Calmness	**0.53**	BF, AINS, MOFC	0.29		
		Drevet				0.49	**SI, DRE, AINS, MOFC**	**0.48**	**SI**	**-.28 (.16)**
									DRE	**-.29 (.27)**
									AINS	**.31 (.13)**
									MOFC	**.74 (.34)**
		Mayberg				0.50	SI, MAY, AINS, MOFC	0.46		
		DLPFC				0.21	SI, AINS, DLPFC	0.16		
		D, M				0.50	SI, DRE, MAY, AINS, MOFC	0.49		
		D,M, D				0.40	SI, DRE, MAY, AINS, DLPFC	0.39		
		B, D, M				0.43	BF, DRE, MAY, AINS, MOFC	0.35		
		B, D,M, D				0.35	BF, DRE, MAY, AINS, DLPFC	0.33		

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