EFFECTS OF BRANCHED CHAIN AMINO ACID DOPAMINE DEPLETION ON MEMORY, ATTENTION, AND EXECUTIVE FUNCTIONS IN HEALTHY MALES

by

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Abstract

EFFECTS OF BRANCHED CHAIN AMINO ACID

DOPAMINE DEPLETION ON MEMORY, ATTENTION, AND EXECUTIVE

FUNCTIONS IN HEALTHY MALES

by

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This dissertation examined the effects of catecholamine depletion on memory, attention, and executive functions in 43 healthy male volunteers between 19 and 49 years of age (M = 33.6, SD = 8.9). A branched chain amino acid (BCAA) mixture composed of valine, isoleucine and leucine, reduces brain catecholamine precursor availability by increasing competition with tyrosine and phenylalanine for transport across the blood brain barrier and stimulating protein synthesis. The aims of this study were to examine the utility of BCAA as a method of global dopamine (DA) depletion and to investigate BCAA effects on cognition in healthy males. Subjects received BCAA and placebo on two separate days and completed cognitive tests at baseline, 3-4 hours and 5-6 hours post-drink in a double-blind placebo-controlled crossover design. It was hypothesized that BCAA would cause a significant sharp increase in plasma prolactin (PRL) levels, reflecting decreased DA neurotransmission. It was further expected that BCAA would impair performance on working memory, attention, and inhibitory control if these functions depend upon intact DA and that PRL response to BCAA would be related to cognitive performance. BCAA caused significant PRL level increases. Data revealed a trend for improved performance on attention and executive function measures reflected

as decreased reaction time (RT) variability. BCAA did not affect memory measures. These findings indicate that in healthy males, BCAA catecholamine precursor depletion does not significantly impair cognition and may improve aspects of attention.

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List of Abbreviations

AA = amino acid

ACC = anterior cingulate cortex

ACh = acetylcholine

ADHD = attention deficit/hyperactivity disorder

APTD = acute phenylalanine and tyrosine depletion

ATD = acute tryptophan depletion

ATP = adenosine triphosphate

AUC = area under the curve

BBB = blood brain barrier

BCAA = branched chain amino acid

BMI = body mass index

BPRS = Brief Psychiatric Rating Scale

cAMP = cyclic adenosine monophosphate

CANTAB = Cambridge Neuropsychological Test Automated Battery

CFF = critical flicker fusion

COMT = catechol-O-methyltransferase

CPT = Continuous Performance Test

CPT-IP = Continuous Performance Test – Identical Pairs Version

DA = dopamine

DAT = dopamine transporter

5,6-DHT = 5,6-dihydroxytryptamine

DLPFC = dorsolateral prefrontal cortex

DMI = desmethylimipramine

DOPAC = 4-dihydroxyphenylacetic acid

DV = dependent variable

EDS = extradimensional shift

ERP = event related potential

fMRI = functional magnetic resonance imaging

FTT = Finger Tapping Test

5-HT = serotonin

GABA = gamma aminobutyric acid

Glu = glutamate

GPe = globus pallidus externa

GPi = globus pallidus interna

HRSD-24 = Hamilton Rating Scale for Depression – 24 item

HVA = homovanillic acid

HVLT = Hopkins Verbal Learning Test

IDS = intradimensional shift

IV = independent variable

L-DOPA = levodopa

MAO = monoamine oxidase

MDmc = mediodorsal thalamus

Met = methionine

MFB = medial forebrain bundle

NE = norepinephrine

6-OHDA = 6-hydroxydopamine

OWM = Cogtest Object Working Memory

PD = Parkinson's disease

PET = positron emission tomography

PFC = prefrontal cortex

phe = phenylalanine

PKU = phenylketonuria

PPI = prepulse inhibition

POM = profile of mood states questionnaire

PRL = Prolactin

rCBF = regional cerebral blood flow

RAVLT = Rey Auditory Verbal Learning Test

RM ANOVA = repeated measures analysis of variance

RT = reaction time

RVIP = Rapid Visual Information Processing

SANS = Scale for the Assessment of Negative Symptoms

SCID-I/NP = Structured Clinical Interview for DSM-IV-TR Axis I Disorders Non-patient Edition

SNpc = substantia nigra pars compacta

SNr = substantia nigra pars reticulate

SPDA = spatial delayed alternation

SPECT = single photon emission computerized tomography

STN = subthalamic nucleus

TI = tuberoinfundibular

tyr = tyrosine

Val = valine

VAS = visual analogue scale

VRM = visual recognition memory

VTA = ventral tegmental area

WCST = Wisconsin Card Sorting Test

WRAT-3 = Wide Range Achievement Test -3 Reading Subtest

CHAPTER 1

Introduction

Pharmacologic, lesion, imaging, and genetic data suggest that aspects of memory, attention, and executive functions depend at least in part upon adequate dopamine (DA) neurotransmission. Although an overwhelming amount of animal and human research implicates DA involvement in cognition, the exact nature of DA's role remains unclear. Animal study findings are difficult to interpret in terms of their generalizability to human cognition, and some of the reward paradigms utilized may be confounded by DA involvement in hunger and satiety. Human research also faces several limitations (e.g., difficulty in manipulating DA in healthy samples and lack of specificity of neuropsychological measures). The exact nature of DA involvement in cognition is also difficult to study due to the complex and diffuse interactions with numerous brain structures, neurotransmitters, and neuropeptides. Recently, studies have investigated global DA depletion effects on cognition in healthy human samples.

The use of a simple branched chain amino acid mixture (BCAA) lacking DA precursors offers a relatively novel approach to investigating the role of DA in healthy human cognition. BCAA mixtures composed of three essential amino acids (valine, isoleucine, and leucine) are commercially available, well-tolerated, and orally administered. BCAA administration reduces brain catecholamine precursor availability by increasing competition with tyrosine and phenylalanine for transport across the blood brain barrier (BBB) and stimulating protein synthesis. The resultant precursor availability reduction decreases DA synthesis and availability causing global decreases in DA neurotransmission. A few preliminary studies in healthy humans have demonstrated

cognitive impairment following BCAA administration; however, small sample sizes and inconsistent findings limit the studies.

The aims of this study were 1) to examine the utility of BCAA administration as a method of globally depleting DA and 2) to investigate global DA depletion effects on cognition in healthy human males. Study aims were examined using a double-blind placebo controlled crossover design in which healthy males received BCAA and placebo on two separate days and completed cognitive tests at baseline, 3-4 hours and 5-6 hours post-drink. The first study aim was addressed through analysis of changes in plasma prolactin (PRL) levels, which reflect altered DA function. The second aim of investigating the effects of global DA depletion on cognition was achieved through measuring performance on memory, attention and executive function tests prior to and following placebo and BCAA administration. The following hypotheses were tested:

Primary Hypotheses:

- BCAA administration will cause significant sharp increases in PRL levels, reflecting decreased DA system function.
- If BCAA primarily affects DA system function, and working memory, attention, and inhibitory control depend upon intact DA system function, then BCAA administration will impair performance on these cognitive tasks as reflected by significant Treatment x Time interaction effects.

Secondary Hypotheses:

- Differences in PRL response to BCAA will be related to changes in performance on cognitive measures.

CHAPTER 2

DA System Review

DA Pathways

The DA system is highly topographically organized and consists of three major pathways including the mesocortical, mesolimbic, and nigrostriatal systems (described in Kandel, 1991; Swartz, 1998). The mesocortical pathway projects from the DA neurons in the ventral tegmental area (VTA) of the midbrain to the frontal, temporal and anterior cingulate cortices (ACC). The mesolimbic system, which also projects from the VTA, terminates in structures of the limbic system (including the nucleus accumbens, amygdala, septal nuclei, and hippocampus) as well as in the mesial frontal, ACC and entorhinal complex. The nigrostriatal system originates in the substantia nigra pars compacta (SNpc) and projects to the striatum. A secondary DA pathway consists of the tuberoinfundibular projections originating in the arcuate nucleus of the periventricular area of the hypothalamus. This pathway projects to the infundibulum and the anterior pituitary gland where DA action inhibits PRL release. Each DA system is likely relevant when examining DA involvement in various aspects of cognition. The organization of the mesolimbic, mesocortical, and nigrostriatal connections facilitates DA modulatory influence on aspects of attention, memory, and executive functions.

DA Projections and Cognition

The mesocortical and mesolimbic projections are of particular interest because they project to brain areas that are involved in the maintenance of goal-directed behaviors, planning, and attention (e.g., Goldman-Rakic, Muly & Williams, 2000; Miller, 2000; Nieoullon & Coquerel, 2003). Therefore, disruption of DA system functioning is

hypothesized to modulate input to these areas, which should in turn disrupt cognitive processes involved in goal-directed behaviors. DA may also modulate cognitive functions through interactions with frontosubcortical pathways.

Together, the mesocortical and mesolimbic DA projections likely work in concert to influence working memory, reward learning, and executive functions. For example, both pathways project to ACC areas that are involved in executive functioning. Imaging research has shown that the lateral prefrontal (PFC) and rostral ACC cortices are specifically engaged during tasks that require response selection in the presence of competing response options (e.g., Bunge, Hazeltine, Scanlon, Rosen & Gabrieli, 2002; Fan, Flombaum, McCandliss, Thomas & Posner, 2003; vanVeen, Cohen, Botvinick, Stenger & Carter, 2001). DA neuron organization and projection areas and mechanisms of action support a DA role in modulation of PFC mediated processes (Goldman-Rakic, Muly & Williams, 2000; Sawaguchi & Goldman-Rakic, 1994).

While the mesocortical projections modulate PFC functioning, Jackson & Moghaddam (2001) provide data that also support a role of DA mesolimbic involvement in PFC-mediated behavior modulation. Data suggest that the PFC may work with the amygdala to produce lasting modulation of neurotransmission by inhibiting mesoaccumbens DA neuron output. Specifically, mesocortical PFC projections may modulate responses to emotional amygdala input via the PFC-mediated inhibition of nucleus accumbens DA, which is necessary for converting cognitive and emotional information into appropriate responses.

The high level of interaction with multiple brain areas and neurotransmitters such as norepinephrine (NE), serotonin (5-HT), acetylcholine (ACh) as well as

neuropeptides (e.g. Arnsten, 1997) suggests that the role of DA in cognition is complex and likely modulatory. DA projections from the midbrain are contained within the medial forebrain bundle (MFB) and grouped with other neurotransmitter projections (NE and 5-HT). These interactions further complicate the question of how DA specifically affects cognition. There is substantial evidence of complex modulatory interactions between DA and other neurotransmitters and neuropeptides affecting cognitive functions (e.g. Ellis & Nathan, 2001; Luciana, Collins & Depue, 1998).

The nigrostriatal pathway is believed to modulate cortical activity through its connections with the frontosubcortical circuits that are described later. Nigrostriatal neurons innervate the dorsal striatum containing the head of the caudate and the putamen. The dorsolateral frontosubcortical circuit, which projects to the dorsal part of the caudate, subserves executive functions; therefore, through its inputs to the dorsal caudate, DA acts to modulate executive function (Chow & Cummings, 1999). This pathway has been implicated in influencing behavioral adaptability and regulating motor responses (see Feifel, 1999).

The functioning of the tuberoinfundibular (TI) pathway serves as the primary indicator of the effect of the BCAA method on DA functioning in the brain. The DA neurons of the TI DA pathway inhibit the release of PRL from the anterior pituitary gland. Therefore, if BCAA decreases DA synthesis and availability for release, the inhibitory influence on the anterior pituitary would also be decreased, producing an increase in PRL release.

Frontal Subcortical Circuits and DA

There are five frontal-subcortical circuits, three of which are related to neurobehavioral functions (described in Alexander, DeLong & Strick, 1986; Chow & Cummings, 1999; Masterman & Cummings, 1997) (see Figure 1). These three major pathways originate in the dorsolateral, orbitofrontal, and anterior cingulate regions. The circuits share a general projection pathway from the frontal lobe to the striatum, globus pallidus and substantia nigra to the thalamus and back to the frontal lobes. Each pathway has a direct loop and an indirect loop, passing through the subthalamic nucleus. The direct pathways topographically project through the structures described above and disinhibit the thalamus. Overlap in the projections and associated anatomical structures suggest that DA likely interacts with and influences frontal subcortical circuits.

The dorsolateral circuit, originating in the dorsolateral frontal lobe, projects to the dorsolateral region of the caudate. The orbitofrontal circuit has parallel lateral and medical subcircuits, which respectively project to the ventromedial caudate and the ventral striatum. Both subcircuits project to the medial portion of the mediodorsal globus pallidus interna and the rostromedial substantia nigra pars reticulata (GPi/SNr). These axons then project to the medial and inferomedial section of the magnocellular division of the ventral anterior thalamus and then back to the lateral or medial orbitofrontal cortex. The anterior cingulate pathway originates within the ACC, projects to the ventral striatum (ventromedial caudate, ventral putamen, nucleus accumbens, olfactory tubercle) and then to the rostromedial GPi, rostrodorsal substantia nigra, and ventral pallidum. Then from the magnocellular division of the mediodorsal thalamus (MDmc), the pathway projects back to the anterior cingulate.

Each circuit also has an indirect pathway from the striatal output neurons. The indirect loops inhibit the excitatory connections of the thalamus. From the striatum, the indirect pathway sends inhibitory projections to the external segment of the globus pallidus (GPe) (which inhibits the subthalamic nucleus (STN)) and the STN then heightens inhibition of the thalamic efferents through glutamatergic efferents to the GPi/SNr. Input and activity of the frontal lobes is moderated through the direct and indirect pathways. Several neurotransmitters modulate these pathways.

DA exerts modulates inhibitory and excitatory influences in frontosubcortical circuit regulation through complex interactions with other neurotransmitters. Striatal output is regulated by DA actions on cholinergic interneurons, which in turn modulate cortical activation. DA also directly influences glutamate (Glu) cortical and thalamic efferents. SNpc DA projections innervate the entire striatum at dendritic spines. D1 receptors stimulate whereas D2 receptors inhibit production of postsynaptic cyclic adenosine monophosphate (cAMP). ACh and Glu facilitate striatal DA release. Glu, ACh, and DA are related in a corticostriatal-thalamocortical negative feedback loop. DA actions are also modulated by 5-HT; midbrain SN DA cells are innervated by 5-HT projections from the medial and dorsal raphe nuclei. DA input regulates neuropeptide levels along the gamma aminobutyric acid (GABA) efferents to the striatum. The DA-ACh interactions are relevant to cognition because of the involvement of ACh in regulating and enhancing signal-to-noise in the cortex ACh and NE have been shown to increase the signal-to-noise ratio through their interaction with DA (Hasselmo & Linster, 1999).

DA Metabolic Pathway and Receptor Types

In order to understand prior research findings and the present study, it is necessary to understand DA metabolism. DA is a catecholamine synthesized from the precursor tyrosine. Tyrosine is an essential amino acid (AA) which cannot be synthesized by the body. Phenylalanine is also an essential AA which can be converted to tyrosine. These essential AAs must be transported into the body and across the BBB where they are utilized in the synthesis of catecholamines (including DA, NE, and epinephrine). Therefore, by limiting precursor availability it is possible to alter DA synthesis and availability for release.

The DA metabolic pathway involves as the rate-limiting step, the conversion of tyrosine to levodopa (L-DOPA) via tyrosine hydroxylase (described in Cooper, Bloom & Roth, 1996). DOPA decarboxylase then converts L-DOPA to DA. DA can be further converted to NE via dopamine beta-hydroxylase. DA action is terminated through DA transporter reuptake into the presynaptic terminal, where it is converted to 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by monoamine oxidase (MAO), and the enzyme catechol-O-methyltransferase (COMT). In rats, DOPAC is the primary metabolite whereas in humans, HVA is the major metabolite.

In most DA neurons, autoreceptors located on the soma regulate firing rate, synthesis, and release; however, some neurons, particularly those projecting to the PFC and anterior cingulate, do not have release-regulating autoreceptors. Because DA transporter (DAT) is not expressed in the PFC (Lewis, Melchitzky, Sesack, Whitehead, Auh & Sampson, 2001), DA action is terminated extracellularly by the COMT enzyme,

which is located on the postsynaptic neurons. Multiple DA projections are associated with a variety of mechanisms of action and degradation.

DA receptor subtypes are varied in abundance, distribution and pharmacological characteristics. Understanding DA receptor types is relevant to understanding the literature on DA and cognition because of the heterogeneity of receptor action and distribution. DA receptors have been classified into 5 different subtypes (D_1, $D_{2a/2b}$, D_3, D_4, and D_5) (reviewed in Kandel, 1991). The five subtypes can be classified into two general groups: 1) D_1, D_5, and 2) D_2, D_3, D_4. D2 receptors are concentrated on postsynaptic neurons in areas that receive innervation from DA neurons (i.e., the PFC, the sensory motor part of the striatum, and the limbic system). D_1 and D_5 receptors stimulate adenylate cyclase activity via a receptor-linked G-protein converting adenosine triphosphate (ATP) to cAMP and modify protein function. D_1 receptors are primarily expressed in the striatum and have been shown to decrease sodium currents, increase potassium currents, and affect calcium currents. D_5 receptors are primarily located in the hypothalamus and hippocampus (Ciliax et al., 2000; Meador-Woodruff et al., 1992).

There are two types of D_2 receptors. Post-synaptic D_{2a} receptors are linked to an inhibitory G-protein that results in adenylyl cyclase inhibition. There are also pre-synaptic D_{2a} receptors located on most DA neurons that serve as inhibitory autoreceptors and control neuron firing rate as well as action potential DA release. D_{2b} receptors are also linked to a G-protein and regulate action potential DA release, but cause phosphoinositide turnover. D_2 receptors, located in the neurons of the nucleus accumbens, amygdala, hippocampus and parts of the cortex, generally depress target neuron activity (Mengod et al., 1989). G-protein coupling of DA receptors suggests that

DA signaling is slow and long lasting as opposed to fast and short acting (Nieoullon, 2002). D_3 and D_4 receptors are found in the limbic system and cortex with weak expression in the basal ganglia (Van Tol et al., 1991). D_4 receptors may inhibit Glu activity (Rubinstein et al., 2001). Understanding the heterogeneity of DA receptor actions and distributions is particularly relevant to the present study in appreciating the complexity of global DA manipulation on cognition.

DA Neuron Characteristics

Resting DA neurons have a slow regular discharge rate (Ljungberg, Apicella & Schultz, 1992). When resting DA neurons are activated, they exhibit a bursting response, which efficiently releases DA from the nerve terminals. Mesocortical DA neurons that project to the PFC and ACC have a faster firing rate, more bursting activity, and increased transmitter turnover compared to those of the mesolimbic and nigrostriatal pathways (Roth & Elsworth, 1995). If impulse flow in mesolimbic or nigrostriatal pathways is disrupted, the neurons rapidly increase DA nerve terminal concentrations by decreasing release, and increasing synthesis (Cooper, Bloom & Roth, 1996). Acute agonist stimulation of these neurons decreases cell activity, DA turnover, and catabolism, while acute antagonist administration increases activity, DA turnover, catabolism, and synthesis. Chronic blockage of the DA receptors results in tolerance in the nigrostriatal and mesolimbic system but not in the mesocortical system. The differences between the mesocortical and mesolimbic and nigrostriatal systems may be due to the lack of autoreceptors in the mesocortical system. In the absence of autoreceptors, neuropeptides (e.g., substance P and substance K) may modulate activity in the mesocortical system.

CHAPTER 3

Empirical Evidence of DA Involvement in Cognition: Animal Research

Considerations

All three DA projections (mesocortical, mesolimbic and nigrostriatal) have been implicated in cognition regulation in different capacities. The association of DA with cognition has been reported and extensively studied in animals; however, the exact nature of the relationship and the neurochemical mechanisms remain unclear (for reviews see Nieoullon, 2002; Kulisevsky, 2000; Carbon & Marie, 2003). Animal research allows the use of lesion, neurochemical, and genetic techniques that would not be feasible in humans. Findings from studies using these techniques have provided evidence of more specific involvement of DA in efficient attention, memory, and executive functioning.

Animal research of DA involvement in cognition is limited by difficulties in interpreting how the paradigms translate to human behaviors and cognition. Interpreting results from animal studies is also limited by the common use of cognitive paradigms involving food rewards. Decreased DA activity after haloperidol (DA antagonist) administration and 6-hydroxydopamine (6-OHDA, a catecholamine neuron and nerve terminal toxin) injections into the MFB both reduces rate and time spent on feeding in food-deprived rats (Salamone, Zigmond & Stricker, 1990). DA-depleted rats show abnormal feeding (feeding with the use of one or no paws), suggesting that striatal DA is necessary in motor control associated with proper feeding. In rats, 6-OHDA induced ventrolateral striatum lesions reduce water intake and body weight (Dunnett & Iversen, 1982). These findings complicate extrapolation of DA effects on feeding behavior and alterations on cognition.

Another potential limitation in animal research is the use of behavioral paradigms that are commonly based on changes in motor activity. Locomotor activity is significantly reduced in DA-deficient (Kim, Szczypka & Palmiter, 2000) and D2 knockout mice (Baik et al., 1995), while it is significantly increased in D3 knockout mice (Accili et al., 1996). These findings must be taken into consideration in designs and interpretation of results from studies of DA in behavior and cognition. Despite these limitations, animal research has yielded evidence supporting a role DA in modulation of attention, memory, and executive functions (e.g., sensorimotor integration, inhibitory control processes, planning, sequencing, self-monitoring, inhibiting, initiating, controlling or altering behavior). The following section presents an overview of the vast animal literature supporting DA involvement in cognition.

Attention and DA

Subcortical and cortical DA systems appear to regulate specific aspects of selective attention. Selective attention refers to the ability to focus on specific important aspects of a task without becoming distracted by irrelevant information. Specific components of selective attention shown to be modulated by DA function include the ability to maintain focus on a given task or set of stimuli for a period of time as well as disengaging and re-engaging attentional focus. Efficient DA modulation of attention appears to be dependent upon an optimal level of cortical and subcortical DA functioning.

Unilateral and bilateral nigrostriatal DA lesion studies in rats, monkeys, and cats provide evidence of subcortical DA involvement in attentional processes involved in sensory neglect (Feeney & Wier, 1979; Ljungberg & Ungerstedt, 1976; Marshall &

Gotthelf, 1979). Sensory neglect is a syndrome involving loss of responsiveness to sensory stimuli contralateral to a brain injury despite spared classical sensory pathways. In cats, Feeney & Weir demonstrated contralateral neglect deficits in attention following unilateral SN lesions. Before lesioning, cats were trained to perform a modified conditioned suppression task. The task required licking (a liquid food dispenser) suppression at the onset of a light which was presented at varying angles to either side of the cat and signaled imminent foot shock. Cats who exhibited sensory neglect failed to suppress licking at the onset of light in the visual field contralateral to the SN lesion. The deficits in performance were not attributable to motor deficits, as the cats performed the tasks appropriately when the reward was presented in ipsilateral space. These findings implicate the nigrostriatal system in sensory neglect, but the method of electrolytic lesioning permits only indirect conclusions of DA involvement. Rat and monkey studies using more specific 6-OHDA DA lesioning techniques confirm cat findings and indicate that DA is involved in visual and somatosensory neglect (Ljungberg & Ungerstedt, 1976; Marshall & Gotthelf, 1979). Administration of apomorphine (a nonselective DA agonist) reverses these sensory attention deficits in rats (Marshall & Gotthelf, 1979), further supporting specific DA involvement.

Focused attention deficits after ventral mesencephalic tegmentum lesions (Montaron, Bouyer, Rougeul & Buser, 1982) have also been found in cats, suggesting cortical DA influence. Cats focused on a target within their environment exhibit mu-type rhythms in the fronto-parietal cortical areas. Mu-type rhythms are associated with neurophysiological mechanisms of attention (Montaron, Bouyer & Rougeul-Buser, 1979). Montaron and colleagues have shown that bilateral electrolytic lesioning of the

VTA results in mu-type rhythm suppression and hyperactivity, suggesting that the DA system is involved in aspects of attention. Bouyer, Dedet, Joseph & Rougeul (1979) found that injecting monoamine precursors (L-DOPA, 5-HTP, L-tyrosine, L-tryptophan, and tryptamine) enhanced the rhythms that are associated with high levels of alertness and vigilance and quiet wakefulness. The rhythms that were associated with alertness and vigilance were controlled by the catecholaminergic systems, while those associated with quiet wakefulness were controlled by the 5-HT system, suggesting a role of DA in attention, but not ruling out involvement of other catecholamine neurotransmitters such as NE. These findings along with those of Roberts and colleagues (1994) suggest that mesocortical DA projections play a role in aspects of attention.

Recent rodent studies have provided evidence of specific PFC D1 receptor activation involvement in visual attention modulation. Rats subjected to bilateral PFC infusions of the D1 agonist SKF81297, exhibit improvement on a visual attention task (Chudasama & Robbins, 2004). Rats were trained to perform a five-choice serial reaction time (RT) task in which they are required to respond to a briefly presented light randomly presented in one of 5 spatial locations. Rats received a food reward if they correctly poked their nose into the illuminated hole for 5 seconds. Low doses (0.01 micrograms) did not affect attention, while medium doses (0.06 micrograms) and high doses (0.3 micrograms) increased attention accuracy to the target stimulus. These data suggest that D1 receptor stimulation facilitates attentional processes.

Primate data suggest that DA nigrostriatal activity is involved in spatial attentional shifting processes (Boussaoud & Kermadi, 1997; Evenden & Robbins, 1985). For example, in marmosets, Crofts et al. (2001) have demonstrated opposing roles of DA

within the frontostriatal catecholamine system in modulating aspects of selective attention including developing, maintaining, and shifting attentional set. Catecholamine depletion via 6-OHDA lesioning in the frontal cortex impaired attentional abilities and increased susceptibility to distraction from task-irrelevant stimuli. However, similar catecholamine depletion within the caudate nucleus did not adversely affect these aspects of attention, and actually reduced susceptibility to distraction from task-irrelevant stimuli.

Similar to the findings of Crofts et al. (2001), 6-OHDA lesions of the PFC in monkeys improve Wisconsin Card Sorting-like attentional set-shifting task performance (Roberts et al., 1994). Extradimensional shifting (EDS), which requires attention shifting from one stimulus dimension to another (e.g., shifting from responding to shapes, to responding to lines), was significantly improved after 6-OHDA lesions. Contrary to previous findings, in lesioned monkeys, EDS was also relatively unaffected by the introduction of exemplars from the irrelevant stimulus dimension. 6-OHDA lesions did not affect intradimensional shifting (IDS) (the ability to maintain attention to a specific stimulus dimension). Results revealed marked PFC DA depletion with a smaller loss of PFC NA. Pretreatment with citalopram prevented PFC 5-HT from being affected by 6-OHDA injections (which have previously been shown to reduce 5-HT by 60-70%). In vivo microdialysis measures of cortical DA showed significant reductions accompanied by significant extracellular subcortical ventromedial caudate increases. Long-term adaptive striatal changes were evident such that cortical DA depletion resulted in extracellular caudate DA elevations. Together, these results suggest that a reactive increase in DA nigrostriatal transmission may have improved attentional set shifting ability.

Other research suggests subcortical DA modulates attention through influencing signal-to-noise ratios via neuromodulation in the striatum. Nicola and colleagues (2004) have suggested that DA may facilitate selection among competing neurons by enhancing the contrast between stronger and weaker excitations (signal-to-noise ratio). Toan and Schultz (1985) investigated dopaminergic influences on corticopallidal impulse transmission through systemic administration of haloperidol and fluphenazine, DA receptor-blocking neuroleptics, as well as by conditioning electrical stimulation of the SN. Results consistent with data from conceptually different studies, demonstrated that DA influences restrict the flow of information from the cortex to the pallidum. Toan and Schultz (1985) suggest that the findings may constitute a DA-modulated focusing mechanism by which only information from the strongest cortical inputs is allowed passage to the pallidum.

DA mesolimbic and mesocortical balance has also been suggested to regulate sensorimotor gating. Pre-pulse inhibition (PPI) is regarded as a sensorimotor gating measure. 6-OHDA lesioning of the PFC (Bubser & Koch, 1994) and mesolimbic system overactivity (Swerdlow et al., 1990) alter PPI. Nieoullon (2002) notes that D2, D3 and D4 agonists but not by D1 agonists also affect PPI, suggesting a subcortical mechanism of action. He adds that DA influence on the PFC may modulate behavioral inhibitory control, but alone does not induce premature response. A disruption in cortical-subcortical network DA influence would likely result in deficient sensorimotor stimuli gating and inappropriate competing motor response inhibition.

Although much of the evidence supports DA involvement sensorimotor integration, some data from rodent studies suggests that DA may not be involved in

higher cognitive functions such as attention and memory. Investigation of the sensorimotor neglect syndrome in rats with unilateral nigrostriatal lesions has provided evidence suggesting that DA depletion produces primarily motor deficits in alert orientation, not necessarily in attention functions. Sensorimotor neglect syndrome occurs when rats subjected to unilateral nigrostriatal lesions do not respond and orient to contralateral sensory events. These lesions do not impair sensory attention to contralateral space, but impair motor initiation necessary for contralateral response (Carli, Evenden & Robbins, 1985). These findings suggest that DA mediates motor components of alert orienting behavior but not necessarily sensory attention components.

Memory and DA

There is extensive evidence of DA involvement in aspects of working and delayed spatial memory through mesocortical and mesolimbic projections to the PFC (e.g., Watanabe, Kodama & Hikosaka, 1997; see Seamans & Yang, 2004 for a review of DA modulation in the PFC).

Lesioning PFC DA of rhesus monkeys produces deficits in spatial delayed alternation performance similar to those produced by surgical ablation of the area (Brozoski, Brown, Rosvold & Goldman, 1979). Monkeys were trained to perform a spatial delayed alternation task (7-60 second delay periods) as well as a visual pattern discrimination task. Prior experiments have shown that the delayed alternation task depends on intact PFC, while the pattern discrimination task is not PFC dependent (e.g., Goldman & Rosvold, 1970). Animals were trained and tested and then lesioned with either intracortical injections of 6-OHDA (with or without desmethylimipramine (DMI) a NE reuptake blocker) or 5,6-dihydroxytryptamine (5,6-DHT), a 5-HT toxin. Animals

were then retested and later sacrificed for neurochemical assays. 6-OHDA injections accompanied by DMI injections produced an 87% DA and 76% NE depletion (without DMI, there was a 56% DA and 85% NE depletion). 5,6-DHT injections produced 70% 5-HT and 48% DA depletions. Monkeys with 6-OHDA + DMI lesions exhibited deficits comparable to those seen in monkeys with surgical resection of the injected areas. Only slight and insignificant delayed alternation deficits were seen in monkeys with primarily NE depletion. No differences in performance were seen after 5,6-DHT or DMI injections. There were no deficits on the discrimination task. Optimal doses of DA agonists (L-DOPA and apomorphine) reversed the deficits. Together, these findings provide direct evidence of DA involvement in memory functions and suggest that significant DA loss is necessary to impair performance on the delayed alternation task.

Cortical DA lesions specifically affect spatial delayed alternation (SPDA) performance. In marmosets, Collins and colleagues (1998) demonstrated that PFC DA lesions produce SPDA performance deficits. Excitotoxic lesions to the PFC negatively impacted self-ordered sequencing task performance; however, these were not affected by DA and NE depletions in the same area. These same large depletions impaired performance on an active working memory and spatial delayed response task. Although these findings implicate DA in aspects of working memory, they indicate that DA is not solely responsible for these functions. Similar findings of impaired spatial delayed response performance have been shown in a more recent monkey study examining 6-OHDA PFC lesions (Roberts et al., 1994). Attention and set-shifting tasks were also impaired, complicating understanding of which working memory task components are primarily affected by 6-OHDA lesions.

Studies utilizing more specific DA manipulation techniques have yielded further evidence supporting a modulatory role of DA in more specific PFC related working memory functions. For example, a study in rhesus monkeys using selective D1 antagonist (SCH23390 and SCH39166) and haloperidol PFC injections yielded dose and delay dependent working memory impairments, while sulpiride (D2, D3, D4 receptor blocker) and raclopride (D2 antagonist) did not affect performance. Monkeys were trained to fixate on a central spot, while a visual cue was briefly presented (300 ms) in one of several peripheral locations. After varied 1.5-6 second delay, the monkeys were required to move their eyes to the target location that had been previously cued. Selective D1 antagonist and haloperidol administration caused increased errors and latency on the oculomotor memory task requiring memory (Sawaguchi & Goldman-Rakic, 1991; Sawaguchi & Goldman-Rakic, 1994). Antagonist administration did not affect performance on a similar task requiring only visually guided saccades. These findings suggest a specific involvement of D1 receptors in working memory functions related to PFC functioning.

Sawaguchi, Matsumara and Kubota (1988) investigated the effects of manipulating PFC DA on spatial delayed response task performance in monkeys. Iontophoretically applied DA enhanced task-related neuronal activity in the PFC, while D1 (fluphenazine) and non-specific (haloperidol) antagonist administration had the opposite effect. Sulpiride (a D2, D3, D4 receptor blocker), did not affect neuronal activity. Using identical neurochemical techniques, these findings were replicated in further studies by Sawaguchi, Matsumara and Kubota (1990), which also provided evidence that DA is involved in specific cue, delay, and go period components of a

delayed memory task. The task involved a precue stimulus, followed by the cueing stimulus in which monkeys are shown a light on either the right or left, and then, the monkeys must withhold their response for a specified delay before finally responding by moving a handle to the correct previously cued position. Haloperidol and fluphenazine significantly decreased the signal-to-noise ratio of the task-related activity to background activity during performance of the delayed response task.

In a separate study using similar techniques, Sawaguchi (2001) trained monkeys to make a visual saccade to a previously cued target location (right, up, left, or down with 15 degrees of eccentricity) after a brief 4-second delay period. Iontophoretically applied SCH23390 (D1-antagonist) to the dorsolateral prefrontal cortex DLPFC neurons (that show increased activity during the delay period of the task) altered neuronal activity. Most of the neurons showed a decrease in neuronal activity. Sulpiride (D2-antagonist) however did not alter performance or neuronal activity. These data suggest that DA promotes spatial short-term memory processing by increasing memory-related activity in the primate prefrontal cortex, possibly via D1-type dopamine receptor action and signal-to-noise enhancement. The delayed response paradigms used in these studies involve motor planning as well as attention and spatial memory; therefore, results may reflect a relation between DA and components of motor planning and attention.

Excessive or insufficient PFC DA activity is detrimental to spatial working memory functioning. Administration of FG7142 (an anxiogenic beta-carboline that selectively increases PFC DA turnover) selectively impairs rat and monkey performance on spatial working memory tasks while delayed response and spatial discrimination performance are spared (Murphy, Arnsten, Goldman-Rakic & Roth, 1996). The FG7142

related impairments were prevented by pretreatment with DA antagonists (haloperidol, clozapine, and SCH23390). In rats, similar dose-dependent working memory impairments due to excessive PFC DA activity occur when VTA mu-opioid receptors are stimulated with [D-Ala2,N-Me-Phe4,Gly-ol5] enkephalin (Romanides, Duffy & Kalivas, 1999). This impairment is reversed by PFC D1 antagonist administration. Administration of varied D1 receptor agonist SKF81297 doses to mice reveal an inverted "U" shaped dose-response curve on spatial working memory performance (Lidow, Koh & Arnsten, 2003). These data demonstrate that increased excessive dopamine tone in the PFC disrupts working memory.

DA may also be involved in modulating functions necessary for efficient retrieval of information after a delay. While decreasing D1 receptor activity impairs memory performance, PFC D1 agonist administration enhances radial maze task performance in rats depending upon the strength of the memory. Floresco & Phillips (2001) investigated the effects of PFC D1 agonist (SKF81297) administration on memory retrieval. Rats were tested with recall delays of either 30 minutes or 12 hours. D1 agonist infusions improved task performance after the 12-hour delay, but impaired performance after the 30-minute delay. These findings suggest that D1 receptor activity plays a role in delayed memory retrieval.

Chudasama & Robbins (2004) also investigated the effects of PFC D1 agonist infusions on attention and memory in rats. Rats were required to visually attend to a target stimulus and remember the location over a variable delay (0-16 sec). Similar to the findings of Floresco & Phillips, data demonstrated that under higher attentional demand, high agonist doses produced a delay-dependent modulation of memory for the stimulus

target location. Specifically, good memory at the shorter delay was impaired while poor memory at the longer delay was improved. It is not clear why D1 agonist administration would impair performance after the shorter delay period; however, these convergent data suggest that an optimal level of DA receptor activation is necessary for efficient short term and delayed memory processes.

D1 receptor knock-out mice exhibit spatial learning and working memory deficits which are related to the presence of the receptors in the PFC and hippocampus (El-Ghundi, Fletcher, Drago, Sibley, O'Dowd & George, 1999). Knock-out mice for the D1 receptor (located in hippocampus and PFC) showed longer Morris water maze escape latencies compared to intact mice in the absence of sensorimotor reflex, locomotor activity, spontaneous alternation and contextual learning differences. These findings demonstrate evidence for a specific spatial memory deficit induced by D1 receptor manipulation.

Although considerable evidence suggests DA involvement in aspects of memory functions, it is likely that the role is modulatory and involves D1 receptor activity. Other neurotransmitters are also involved in memory functions. For example, hippocampal cholinergic antagonism has been demonstrated to affect delayed non-matching to position performance in rats (Dunnett, Wareham & Torres, 1990). In experiments similar to those investigating the role of DA in memory functions, noradrenergic PFC antagonist administration has also been shown to impair performance on oculomotor working memory performance in monkeys (Sawaguchi, 1998). Working memory functions appear to be under complicated control of several neurotransmitters possibly via their interaction with one another and through their individual modulatory roles on signal-to-noise ratios

and information gating. Nevertheless, there is clear evidence of DA involvement in working memory functions.

Executive Functions and DA

Although somewhat difficult to study in animals, data suggest that nigrostriatal, mesocortical, and mesolimbic DA projections modulate aspects of higher cognitive functions and goal-directed behavior. Studies of rodents and non-human primates have examined the effects of DA manipulations on measures of executive functions including inhibitory control (suppression of motor response or irrelevant task information), temporal organization, cognitive flexibility, and goal-directed behavior.

Findings provide relatively consistent evidence that nigrostriatal DA is necessary for efficient sensorimotor integration. DA neuron lesion studies in rodents reveal decreased sensorimotor integration reflected as deficits in alert orienting behavior, visual spatial discrimination, and skilled forepaw use in a reaching task (e.g., Amalric, Moukhles, Nieoullon & Daszuta, 1995). Lesions were accomplished via 6-OHDA administration, a DA neuron and nerve terminal toxin. In rodents, sensorimotor integration is reflected by performance on conditioned motor tasks in which animals are trained to release a lever in response to a visual cue within a reaction-time limit to receive a food reinforcer. After performance on the task stabilizes, animals are subjected to various neurotransmitter manipulations. In rodents, motor RT increases following (SNpc) DA neuron and dorsolateral striatum DA nerve terminal lesions. Primate studies of baboons involving bilateral lesioning of the SN DA neurons have revealed similar slowed motor movements and worse RT on a conditioned motor task (Vaillet, Trouche, Nieoullon, Beaubaton & Legallet, 1984). Similar RT slowing was found specifically with

D2 receptor antagonist (eticlopride) administration (Amalric, Berhow, Polis & Koob, 1993; Smith, Smith, Zigmond, Amalric & Koob, 2000), while D1 (A69024) and D3 (nafadotride) receptor antagonists did not affect RT.

Studies in which techniques normalize previously depleted DA neurons and improve sensorimotor task performance lend further support for the role of nigrostriatal DA in sensorimotor processes. For example, Moukhles and colleagues (1994) investigated the functional effects of DA-rich ventral mesencephalic suspension grafts transplanted into partially DA-depleted striatum on conditioned motor RT task performance in rats. Animals that received DA grafts showed a large number of DA fibers in the reinnervated area and most of them (73%) showed significant RT improvement. Results demonstrate that restoring DA functioning via intrastriatal ventral mesencephalic transplants results in substantial or complete recovery of performance on a complex RT task (Moukhles, Amalric, Nieoullon & Daszuta, 1994). These results also suggest that there is an optimal level of DA transmission necessary for efficient cognitive function.

Baunez, Nieoullon, and Amalric (1995) examined the effects of enhancing and decreasing DA activity on time estimation and complex sensorimotor integration in rats. Rats were trained to perform a task in which they depressed a lever, waited for a light stimulus occurring after various intervals (either equiprobable or variable), and then responded by releasing the lever within a required RT in order to received a food reward. The task required not only time estimation for efficient performance, but also required attention and vigilance while waiting for the cue and motor preparation. Rats exhibited increased premature lever releases when DA was stimulated through systemic d-

amphetamine and striatal DA administration. Alternately, decreasing DA transmission via raclopride (D2 antagonist) decreased premature lever releases. Time estimation and motor preparation were not impaired (rats continued to display shorter RT with longer delay).

The conditioned motor task (in which animals are trained to release a lever in response to a visual cue within a RT limit to receive a food reinforcer) can be administered with different delay periods; under normal circumstances, the longer the delay period, the shorter the response RT. Extensive nigrostriatal DA lesioning results in increased RT on the conditioned motor task described previously as well as increased numbers of anticipated responses (premature lever release before the visual cue occurs) (Amalric et al., 1995). Unilateral striatal DA depletion also causes deficits on another task requiring sensorimotor integration in which rats were trained to perform a visual spatial discrimination (Brown & Robbins, 1991). The visual stimuli, which provided information regarding the required response direction, were presented either in advance of a temporally unpredictable auditory imperative stimulus (simple RT condition) or simultaneously with the imperative stimulus (choice RT condition). Unilateral striatal 6-OHDA DA depletion caused spatial response bias towards the side of the DA depletion and abolished the delay-dependent speeding of RT that reflects motor readiness, on the side contralateral to the lesion. Contrasting the findings of Baunez, Nieoullon, and Amalric (1995), D2 antagonist administration and extensive bilateral lesioning of the striatal DA terminals produce time estimation and motor planning deficits. The results demonstrate the importance of nigrostriatal DA in motor planning and readiness.

Taken together, findings generally imply that the nigrostriatal projections are involved in movement control and execution. An optimal level of nigrostriatal DA function is necessary for appropriate early stage motor processing as well as later stage response selection and execution. Animal research findings are consistent with the movement deficits seen in Parkinson's disease, in which there is significant loss of DA neurons from the SN and nigrostriatal projections.

In addition to nigrostriatal DA, mesocortical DA has been implicated in aspects of executive functioning. Cortical DA depletion causes locomotor hyperactivity and other symptoms of impaired motor inhibition, and temporal organization of behavior sequences (Jones and Robbins, 1992; Tassin et al., 1978). In non-human primates, excitotoxic PFC lesions have also been shown to affect general behavioral organization abilities and caudate function. Wilkinson and colleagues (1997) studied DA involvement in an object retrieval task in which monkeys had to gain access to a food reward. The task demands included behavior organization and prepotent response inhibition. Marmosets subjected to lateral and orbitofrontal PFC lesions exhibited difficulty and less efficiency gaining access to the food reward following D-amphetamine administration. Error analysis indicated that poorer performance was not due to prepotent response inhibition deficits, but due to general behavioral disorganization. Neurochemical analysis revealed that lesions caused attenuation of amphetamine induced DA release in the caudate. These data suggest that balanced frontostriatal DA interaction is necessary for efficient accomplishment of goal directed behavior.

Mesocortical DA has also been implicated in attentional set shifting, which is regarded as an executive function reflecting mental flexibility. In monkeys, 6-OHDA

(which mimics the effects of ablation procedures) PFC administration, causes deficits in attentional set shifting (e.g., Roberts et al., 1994). Lesioned monkeys previously trained to perform a Wisconsin Card Sorting like task requiring extradimensional shifting (EDS) of attention from one stimulus dimension (shapes) to another (lines) show marked PFC DA depletion accompanied by impaired set shifting ability. Deficits are reversible with the administration of L-DOPA (a DA precursor) or apomorphine (a DA agonist). These data provide evidence for DA involvement in attentional set shifting ability, but it is unclear whether impaired performance on the set-shifting task is due to executive or attentional dysfunction. As discussed earlier, DA appears to influence attention processes.

Further evidence for DA involvement in executive functions is from genetic studies in rats. There is a functional Val158Met polymorphism for the COMT gene which codes for variations in enzymatic activity of COMT, the brain enzyme responsible for the catabolism of DA in the PFC. Tunbridge, Bannerman, Sharp & Harrison (2004) have demonstrated that COMT inhibition with tolcapone affects rat attentional set shifting. COMT inhibition with tolcapone significantly improved EDS ability and augmented clozapine-induced increases in extracellular DA release, measured with microdialysis in awake rats. These data indicate that COMT activity influences EDS and influences PFC DA release under conditions of increased catecholaminergic transmission.

Mesolimbic DA involvement in executive functions related to goal-directed behavior, reward, learning, and novelty detection. DA has long been accepted as integral to the brain's reward circuitry. Research has shown that intact mesolimbic dopamine transmission to the nucleus accumbens and the mesial forebrain are necessary for

efficient brain stimulation reward to occur (e.g., Olds & Milner, 1954, Corbett & Wise, 1980). The relationship between the DA system and the nucleus accumbens has been investigated in terms of reward systems and learning. Schultz and colleagues have provided extensive evidence of DA's role in establishing learned associations. Neuronal recording techniques have provided evidence that DA exerts a modulatory influence on learning, motivational behavior, and neuronal activity at cortical, subcortical and subthalamic nucleus levels (Apicella, Legallet & Trouche, 1996; Schultz, Apicella & Ljungberg, 1993; Schultz, Dayan & Montague, 1997).

Schultz, Apicella & Ljungberg (1993) examined DA neuron involvement in areas previously shown to be involved in attentional and motivational behaviors. DA neuron activity (163 neurons) in areas previously shown to be involved in attention and memory processes were recorded while monkeys were trained to perform a spatial delayed response task by learning two intermediate tasks. During the learning phase of each task, 25% of the DA neurons (more pronounced response area A10) responded phasically during the delivery of the primary liquid reward during learning of the task, while only 9% of the neurons responded once task performance was established. Neurons also showed responses to the instruction cue (which indicated the target of the upcoming arm movement) and to the trigger stimulus (an incentive stimulus, which predicted reward and elicited a saccadic eye movement and an arm reaching movement). DA neurons were non-responsive during the delay between the instruction and trigger stimuli. These findings indicate DA involvement in attention and motivation processes associated with learning and goal-directed behavior. The data argue against a role of DA in working memory, and encoding processes in learning processes.

Mirenowicz and Schultz (1994) further investigated the role of DA neurons in the establishment of reward prediction involving novel stimuli. Neuronal firing was recorded in two monkeys during administration of liquid not associated with reward and also during administration of liquid associated with reward either during learning or established auditory RT task performance. Liquid given not as a reward caused 75% of the neurons to fire. These same neurons fired in response to the liquid given as a reward during learning of the task. Once task performance was established, these neurons fired in response to the reward-predicting stimulus. Data indicate that DA is involved in the establishment of unpredicted stimulus occurrence as reward predicting and support the role of DA in reward driven learning.

More recently, Yun, Nicola, and Fields (2004) demonstrated that following nucleus accumbens selective D1 receptor antagonism, rats showed increased latency and reduced responding to a stimulus that was predictive of impending food reward. General nucleus accumbens inactivation resulted in increased latency to respond and increased extraneous behaviors (e.g., responding when cue was absent). DA transmission is integral to efficient functioning of a reward facilitating association learning so that novel or unexpected events become predictable. In this reward system, suppression of DA neuronal activity results in deficient novelty detection and behavioral adaptation. D4 (localized in limbic system) knockout mice exhibited decreased exploration of novel stimuli within their environment, which reflects a negative impact on goal-directed behavior (Dulawa, Grandy, Low, Paulus & Geyer, 1999).

Alternately, Seamans, Floresco & Phillips (1998) suggest that PFC DA modulates executive functions by influencing short-term working memory functions that guide

behavior. Microinjections of SCH-23390 (a D1 antagonist) and sulpiride (a D2 antagonist) affected performance on rat foraging in a radial arm maze task. Rats either received prior training in which spatial information was used in the later test phase (delayed task), or they were deprived of prior information about location of food reward and only performed the test phase of the maze task (non-delayed). SCH-23390, but not sulpiride into the prelimbic PFC, prior to testing disrupted performance on the delayed task but did not change response latencies. However, neither drug affected the non-delayed task. The second part of the study investigated the effects of PFC SCH-23390 and hippocampal lidocaine injections on delayed responding. Results revealed impaired performance after injections. These data indicate that PFC D1 receptor activation and D1 modulation of hippocampal input to the PFC are involved in the ability to use previously acquired information to guide behavior.

Evidence from lesion, neurochemical manipulations, and neuronal recording studies in rats, monkeys, and cats suggest a modulatory role of DA in attention, memory, and executive functioning. Specific aspects of attention including shifting of attention are clearly affected by DA inputs. DA involvement in shifting of attention may play a role in the effects that DA manipulations have on memory functions. DA modulates information maintained in working, short term, and delayed memory by regulating attentional shifting, and the information that is attended to. Executive functioning may also be affected by DA's primary modulation of attentional processes. Alternately, DA may be specifically involved in sensorimotor gating and may affect all aspects of cognitive functioning through its modulation of what information is enhanced and what information is suppressed in further cortical processing. DA exerts influence on goal-

directed behavior through its role in reward, motivation and learning. Evidence also consistently points to an optimal level of DA neurotransmission and a necessary balance between subcortical and cortical DA activity for efficient cognitive functioning. Although the ability to translate animal data of DA and cognition to human cognition is limited, it can be expected to depend upon an optimal level of subcortical and cortical activity. Excessive or insufficient DA activity would be predicted to be reflected in altered cognitive functions.

CHAPTER 4

Empirical Evidence of DA Involvement in Cognition: Human Research

Considerations

Because of the vast number of disorders in which there is a presumed disturbance of DA processes (e.g., schizophrenia, Parkinson's disease (PD), phenylketonuria (PKU), attention deficit/hyperactivity disorder (ADHD), Alzheimer's disease, Huntington's disease, and autism), the role of DA in human cognition has received much attention. However, complexity of neurochemical and neuroanatomical dysfunction in clinical populations prevents studies from providing information regarding the specificity of DA involvement in the cognitive deficits. As with animal studies, human studies of DA and cognition are limited by various factors (e.g., ethical and DA manipulation methods limitations). Nevertheless, advances have been made in the understanding of the role of DA in cognition.

Clinical Disorders and DA

Although motor deficits related to significant nigrostriatal neuronal loss are predominant features of PD, DA is clearly involved in the associated cognitive deficits (for reviews see Nieoullon, 2002; Kulisevsky, 2000; Carbon & Marie, 2003; Remy & Samson, 2003). In PD, frontosubcortical DA circuits are associated with motor, simultaneous task processing, attentional set (formation, maintenance and switching), and working memory deficits (Cools, Barker, Sahakian & Robbins, 2001; Cools, van den Bercken & Horstinck, 1984; Downes, Roberts, Sahakian, Evenden, Morris & Robbins, 1989; Harrington & Haaland, 1991; Malapani, Pillon, Dubois & Agid, 1994; Zgaljardic, Borod, Foldi & Mattis, 2003). Significant PD deficits in EDS attention shifting (ability to

adapt to changes in dimensional relevance of the stimuli) (Downes, Roberts, Sahakian, Evenden, Morris & Robbins, 1989) suggest that frontostriatal DA balance is necessary for efficient attentional functions. L-DOPA administration significantly improves task switching, visuospatial, and object working memory abilities in patients with PD (Costa et al., 2003). Imaging data indicates a positive correlation between striatal (18F)-L-DOPA accumulation and performance on executive function tests (Wisconsin Card Sorting Test (WCST) and Stroop Test). L-DOPA ameliorates high-level executive planning deficits in PD, which PET studies have revealed is accompanied by blood flow changes in the right DLPFC (Cools, Stefanova, Barker, Robbins & Owen, 2002).

Recent theories of schizophrenia propose disturbances in distinct DA circuits and receptors are responsible for information processing, attention, backward masking (when a distraction causes a person to forget a preceding event), and memory deficits. The DA disturbance has been characterized as involving hypo-DA mesocortical (D1) pathway functioning and hyper-DA mesolimbic pathway (D_2, D_3, D_4 receptors) (e.g., Davis, Kahn, Ko & Davidson, 1991). D1 and D2 receptors may contribute to cortical stability of external and internal representations through pyramidal and local circuit neuron effects mediating neuronal excitability and recurrent inhibition, optimizing signal-to-noise ratios (Winterer & Weinberger, 2004). PET binding studies provide direct evidence of DA involvement in schizophrenia working memory deficits (e.g., Abi-Dargham et al., 2002; Arnsten & Goldman-Rakic, 1998; Sawaguchi & Goldman-Rakic, 1994). Reduced D1 and D2 receptor linkage may play a part in schizophrenia symptomatology. Excessive D1 receptor activity may increase cognitive stability through tonic DA increases, but may limit cognitive flexibility (Nolan, Bilder, Lachman & Volavka, 2004).

Phenylketonuria (PKU) research provides evidence of DA involvement in executive functions. PKU involves a gene mutation causing inadequate conversion of phenylalanine (PHE) to tyrosine with consequent Phe accumulation and low tyrosine levels (Scriver, Kaufman, Eisensmith, & Woo, 1995). Increased BBB transport competition combined with low Phe to tyrosine conversion significantly decreases the amount of tyrosine available to serve as the DA, 5-HT, NE, and epinephrine precursor. PKU executive functioning (e.g., prepotent response inhibition and attentional flexibility), attention, verbal memory, expressive naming and verbal fluency deficits (Brumm et al., 2004; Diamond, Prevor, Callender & Druin, 1997; Griffiths, Ward, Harvie, & Cockburn, 1998; Huijbregts, deSonneville, Licht, Sergeant & vanSpronsen, 2002) are associated with phe-tyr ratios (Luciana, Sullivan & Nelson, 2001). Relative to age-matched healthy controls, individuals with PKU exhibit exacerbated impairments in executive functioning when given haloperidol, which is consistent with frontostriatal dysfunction (Luciana, Hanson & Whitley, 2004). In mice, Dyer has shown a direct relationship between returning phe levels to normal and normalizing DA levels and myelination (2003).

Attention deficit/hyperactivity disorder (ADHD) attention and executive function deficits are associated with DA function alterations. Sonuga-Barke (2003) has proposed a dual DA pathway model of ADHD involving two independent deficits: 1) a cognitive inhibition deficit and 2) a reward sensitivity deficit. Mesocortical DA alterations are responsible for cognitive inhibition deficits, while mesolimbic DA alterations are responsible for reward sensitivity deficits. Genetic studies have shown an association between ADHD and gene polymorphisms (i.e., DAT1, DRD4, DBH, & DRD5t) affecting

DA transmission (Comings, 2001; Manor et al., 2004). Single photon emission computed tomography (SPECT) investigation of DRD4 and DAT1 genes in ADHD boys during continuous performance testing revealed highly significant right middle temporal gyrus perfusion in the group with DRD4 and DAT1 risk alleles (Szobot, Roman, Cunha, Acton, Hutz & Rohde, 2005). There is also evidence that methylphenidate is effective in treating the symptoms of ADHD through its blockade of the DA transporter which increases DA availability in the synapse (Volkow & Swanson, 2003).

Numerous clinical disorders involve DA disturbances associated with cognitive deficits; however, complexity of neurochemical and neuroanatomical dysfunction in clinical populations prevents studies from elucidating the specificity of DA involvement in cognitive deficits. The following section discusses investigations of DA and cognition in healthy humans that have provided additional but limited information (hindered by ethical and DA manipulation methods).

COMT Genetic Evidence

Genetic data indicate that DLPFC DA functioning is essential for certain attention, executive and working memory abilities. There is an association between the specific gene that codes for the enzyme responsible for PFC DA catabolism and cognition. The COMT gene has a functional polymorphism involving a valine (Val) substitution for methionine (Met) at the 105/108 peptide sequence locus. The Met allele protein has a four-fold reduction in enzymatic activity (slower DA catabolism) compared to that of the Val allele (Mannisto & Kaakkola, 1999). As mentioned earlier, the PFC depends on COMT instead of DAT to terminate DA actions (Lewis et al., 2001).

Researchers have proposed cognitive consequences associated with the COMT polymorphism.

Diamond and colleagues have shown that children homozygous for the met polymorphism of the COMT gene perform better on the dots mixed task, which engages the DLPFC and is sensitive to the level of DA there (Diamond, Briand, Fossella, & Gehlbach, 2004). Diamond et al. also report that COMT genotype is not related to performance on the self-ordered pointing task, which engages the DLPFC but is insensitive to DA levels. It is interesting that only specific tasks are affected by DA manipulations within the DLPFC. This provides indirect evidence of DA involvement in very specific aspects of DLPFC driven cognitive functioning.

Adult studies have also provided evidence that DA is related to performance on executive functioning tasks that have been shown to recruit the DLPFC. Consistent with these findings in children, studies have shown that the Val/Val COMT polymorphism is associated with worse performance on the Wisconsin Card Sorting Test (as reflected by greater perseverative errors) than Val/Met or Met/Met genotypes (Joober et al., 2002; Malhotra, Kestler, Mazzanti, Bates, Goldberg & Goldman, 2002). The COMT genotype may also be related to increased risk of developing schizophrenia (Egan et al., 2001; Weinberger et al., 2001).

Goldberg et al. (2003) have investigated the relationship between COMT genotype and working memory. They examined specific working memory subprocesses within a control sample (n=68), patients with schizophrenia (n=74), and their siblings (n=108). Subjects completed a version of the n-back task that manipulated information load and a version of the continuous performance test (CPT) that required attention and

vigilance. The n-back task involved the presentation of a number between 1 and 4 being presented for 200 msec every 1.8 sec on a computer monitor. Three conditions included: 1) 0-back condition in which subjects pressed a button corresponding to the number that appeared on the screen; 2) 1-back condition in which subjects viewed the first number, viewed the next number, and then responded by pressing the button that corresponded with the previous number that was shown; 3) 2-back condition in which subjects continually responded by pressing the button that corresponded to the number they saw "two-back". The information load in working memory was increased from the 0- to 1-back conditions and 1- to 2-back conditions. The CPT "1-9 Distractibility Version" required the subjects to view a continuous stream of digits and respond only to the sequence of a "1" followed by a "9". The index group and siblings performed significantly worse than controls on the 1- and 2-back conditions. There was a trend for a Val allele load effect of COMT genotype and poorer n-back task performance, but no effect on the CPT. The Val/Val genotype is related to poorer working memory processing in schizophrenia. There was a trend for a Val allele load effect of COMT genotype and poorer n-back task performance, but no effect on the CPT.

Investigation of the association of COMT genotype and brain response to amphetamine indicates an inverted u-shaped functional response curve to DA signal increase in the PFC (Mattay et al., 2003). Met-Met genotype individuals with superior working memory baseline performance exhibited deteriorated performance under high load on amphetamine. Alternately, Val-Val individuals exhibited enhanced PFC efficiency (reflected by less activation on fMRI) and improved performance (reflected as an improvement in RT on the WCST) at all loads on amphetamine. The data support the

theory of an inverted u-shaped curve for DA transmission and PFC-dependent cognitive functions (Arnsten, 1997). Optimal levels of DA transmission are necessary for efficient cognitive functioning.

Bilder and colleagues (2004) have proposed that COMT regulates the tonic-phasic subcortical DA balance and overall cortical DA activity, which has been linked to attention, working memory and information gating functions. They hypothesize that the Val allele on a subcortical level increases phasic and reduces tonic DA activity and on a cortical level, decreases DA activity. DA transmission associated with the Val allele causes working memory maintenance and attention deficits, but enhances task switching and behavioral flexibility. In contrast, the Met allele, associated with less COMT activity, has opposite DA tonic and phasic effects, which facilitate working memory maintenance but impair context information updating and behavioral flexibility. There have been studies of the relationship between COMT polymorphisms and simple reversal and conflict paradigms that have yielded evidence in support of COMT regulation of tonic-phasic DA activity (reviewed in Bilder et al., 2004).

Neurochemical Manipulations

DA receptor agonist and antagonist research indicates DA involvement in cognition (Ellis and Nathan, 2001). Luciana, Collins and Depue (1998) investigated the effect of bromocriptine (a selective D2 receptor agonist) on working memory performance. Bromocriptine facilitated spatial delayed memory performance, but did not affect immediate memory. A separate study found that bromocriptine administration improved spatial memory span but impaired probabilistic reversal learning (Mehta et al., 2001), while recognition memory and planning were unaffected. The D2 antagonist,

sulpiride has been shown to impair performance on spatial recognition, planning, spatial working memory, and attentional set-shifting tasks in healthy volunteers (Mehta, Sahakian, McKenna & Robbins, 1999). Müller, von Cramon, and Pollman (1998) investigated differential contributions of D1 and D2 receptors to visuospatial working memory. In a placebo-controlled cross over design, either the D1/D2 receptor agonist pergolide or bromocriptine was given to healthy humans who performed a spatial delayed working memory task. Only pergolide facilitated performance on the visuospatial working memory task, suggesting an important role of D1 receptors in working memory modulation. D1 and D2 receptor activity may modulate memory and executive functions; however, findings are conflicting. Collectively, data suggest an inverted u-shaped dose-related response with optimal activity facilitating and decreased activity impairing working memory performance.

Pharmacological methods increase overall DA activity (such as D-amphetamine) have also been used to investigate the role of DA in cognition. Methylphenidate is an indirect catecholamine agonist that increases synaptic DA (and NE) concentrations via reuptake blockade (Seeman & Madras, 1998). In a sample of healthy volunteers, using PET imaging, Mehta and colleagues (2000) investigated the effects of acute methylphenidate administration on regional cerebral blood flow (rCBF) and spatial working memory performance. Methylphenidate improved working memory performance accompanied by task-related reductions in rCBF in regions of the dorsolateral prefrontal and posterior parietal cortices. These findings provide evidence of a DA influence on spatial working memory through alteration of DLPFC functioning, but do not preclude NE contributions.

DA neurotransmission can be increased globally through acute methamphetamine administration, which increases extracellular DA via reverse DA transport and displacement from vesicular stores. Behavioral effects of methamphetamine in naïve users include heightened sense of alertness, attentiveness and energy. Some investigators have found that acute use improves RT and specific attention and visual memory task performance (see Nordahl, Salo & Leamon, 2003). Mattay and colleagues (2000) found that in a group of healthy controls, acute administration of dextroamphetamine improved working memory task performance, but only in individuals who had relatively low baseline working memory capacity, suggesting an inverted u-shaped dose response.

Chronic amphetamine abuse has a neurotoxic effect on DA neurons and receptors. Approximately 45% of a methamphetamine dose is metabolized into amphetamine, which has an 8-13 hour duration of action. Higher doses induce euphoria, grandiosity, increased impulsivity, impaired judgment, and psychosis. Other adverse effects are also consistent with the impact on DA, 5-HT, NE, and Glu systems. Chronic methamphetamine administration reduces striatal DA and DAT concentrations in multiple brain areas and decreases DA neurotransmission. Imaging studies have revealed that permanent degeneration of the striatal DA system is unlikely since they have found that levels of DOPA decarboxylase are stable. Chronic use impairs verbal memory, motor function, memory recall, executive functions (abstract reasoning, set shifting, suppression of task irrelevant information), and attention, with preserved attentional priming (which is typically impaired in patients with schizophrenia) (e.g., Salo et al., 2002). Rogers and colleagues (1999) demonstrated specific cognitive deficits in suboptimal decision making

abilities in chronic amphetamine that likely reflect DA involvement; however, 5-HT systems may also be involved in the deficits.

ERP studies have revealed that cognitive attentional deficits associated with chronic methamphetamine use are related to DA system impairment. The P300 (P3) component of the ERP occurs in response to novel targets in a stream of frequent stimuli. P3 amplitude is related to novel target event task relevance and is inversely related to subjective stimulus probability (Courchesne, Hillyard & Galambos, 1975; Ritter & Vaughan, 1969). Alternately, P3 latency depends upon stimulus evaluation duration (Kutas, McCarthy & Donchin, 1977) and is increased by task difficulty (Friedman, Simpson, Ritter & Rapin, 1975; Squires, Donchin, Squires & Grossberg, 1977). For a review of the P3 component see Ruchkin et al. (1980). Iwanami, Kuroki, Iritani, Isono, Okajima & Kamijima (1998) found that the endogenous P3 ERP component in chronic methamphetamine users exhibited increased latency and decreased amplitude, which is consistent with impairments in selective attention indicative of decreased DA functioning. Noble and colleagues have shown that the P3 latency component is related to the D2 receptor gene (1994). There are also data demonstrating a relationship between the G1947A COMT (Val(108/158)Met) gene polymorphism and frontal P300 amplitude, such that Met carriers have a smaller amplitude reflecting genetic influence on DA involvement in noise gating (Gallinat et al., 2003).

Global DA Depletion Techniques

A less commonly used approach to understanding the relationship between DA and cognition involves depleting brain DA availability via administration of essential AA mixtures lacking catecholamine precursors. Some methods employ complex mixtures

(Sheehan et al., 1996; Young, Smith, Pihl & Ervin, 1985) that are balanced but lack phenylalanine (phe) and tyrosine (tyr) (acute phe-tyr depletion, APTD) or are tryptophan-free (acute tryptophan depletion, ATD), while others employ simple BCAA mixtures (Gijsman et al., 2002). Animal research has demonstrated that administration of phe-tyr free mixtures attenuates d-amphetamine induced striatal DA release and behavior changes (McTavish, Raumann, Cowen & Sharp, 2001). However, these balanced complex mixtures are highly unpalatable and are associated with transient yet unpleasant side effects, which have led others to use less noxious mixtures.

A simple BCAA mixture of valine, isoleucine, and leucine in a 3:3:4 ratio, produces acute and temporary reduction of catecholamine precursor availability to the brain similar to that attained with complex mixtures (Gijsman et al., 2002). Catecholamine precursor availability reduction occurs via two mechanisms: 1) stimulating protein metabolism (increasing uptake of tyr, phe, and tryptophan), and 2) increasing competition for transport across the blood brain barrier (reducing the amount of catecholamine precursor that is able to cross the BBB). This simpler BCAA mixture is relatively palatable and well tolerated (minimal side effects are comparable to those found with placebo administration) (e.g., Berry et al., 1990; Harper et al., 1984; Richardson et al., 1999).

DA depletion paradigms produce sharp increases in plasma PRL levels. The PRL peak is indicative of decreased DA production. Hypothalamic DA neuronal activity inhibits anterior pituitary gland PRL release; therefore, decreased DA function and receptor activation decreases DA inhibitory influence and increases PRL release. The increase in plasma PRL indirectly reflects the decrease in DA production and

transmission. Complex BCAA mixtures lacking tyr and phe have been shown to primarily affect DA availability and to a smaller extent NE and 5-HT (e.g., McTavish, Callado, Cowen & Sharp, 1999; McTavish, Cowen & Sharp, 1999; McTavish, Raumann, Cowen & Sharp, 2001). The phe-tyr free mixture reduces frontal cortex, hippocampus and striatum tyrosine levels. The mixture produces large decreases in DOPA accumulation in the striatum and nucleus accumbens, with smaller decreases in the cortex, hippocampus, and hypothalamus. The PRL effect is unlikely due to tryptophan (Attenburrow, Mitter, Whale, Terao & Cowen, 2000) depletion since 5-HTP accumulations are not affected in any of the brain areas and 5-HT facilitates PRL release (Jorgensen, Knigge & Warberg, 1992).

In a sample of 16 healthy volunteers (8 men, 8 women), McTavish, McPherson et al. (2001) investigated the effects of dietary depletion on amphetamine induced cognitive changes. Intravenous methamphetamine was administered with the phe-tyr free amino acid mixture (Sheehan et al., 1996) composed of isoleucine (15g), leucine (22g), lysine (17.5), methionine (5g), valine (17.5), threonine (10g), and tryptophan (2.5g) with and without tyrosine (12.5g) and phenylalanine (12.5g) in a double blind, randomized, crossover design. Subjects completed a rapid visual information-processing (RVIP) test of sustained attention from the CANTAB and were given the AA drink. They received methamphetamine injection 4 hours post-drink and completed the RVIP test 15 minutes later.

Plasma PRL levels peaked 4 hours post-drink. There was a significant methamphetamine effect on RVIP, indicating decreased response latency ($F = 29.28$, $p < .001$) and bias ($F = 6.60$, $p = .025$), and increased target sensitivity ($F = 9.19$, $p = .01$).

The phe-tyr free mixture significantly attenuated amphetamine-induced DA release. Cognitive changes were observed at the time of peak PRL response. Phe-tyr depletion globally affected DA transmission. Decreased response latency may be related to nigrostriatal effects, while decreased bias and increased target sensitivity may be related to mesocortical and mesolimbic circuitry effects. Phe-tyr free drink attenuation of the methamphetamine effect supports the u-shaped theory of DA transmission and cognition and suggests that the drink returned elevated DA levels to normal. Data suggest a perturbation of DA function is necessary for acute DA depletion to have significant effects on sustained focused/selective attention. The findings of the study are consistent with the animal literature suggesting that basal DA function must be altered for DA depletion to affect cognition.

Harmer and colleagues (2001) investigated the effects of administering a phe-tyr free mixture on DA and cognitive functioning. In a double blind cross over design, 12 participants (7 male, 5 female) between 23 and 34 years of age (mean age 26.6 yrs) consumed a balanced AA mixture or a phe-tyr free mixture (Sheehan et al., 1996). Subjects completed two study days separated by a week. Prior to each study day, subjects consumed a low-protein diet (less than 20 g of protein) and fasted from midnight the night before. Visual analogue scales (VAS) and plasma PRL and AA levels data were collected every 30 minutes during the 6-hour time period. Subjects completed pattern and spatial recognition, paired associates learning, and spatial working memory CANTAB cognitive tests between 300 and 360 min post-drink. Prior to drink administration and 255 min post-drink, subjects completed the RVIP test of sustained attention.

Plasma PRL levels peaked at 5 hours post-drink ingestion. The phe-tyr free drink increased spatial recognition memory errors (F (1,11) = 11.2, p = .007) and worsened spatial working memory strategy scores (t (1,10) = 3.1, p = .01), but did not affect pattern recognition memory. Inconsistent with McTavish et al. (2001), there was no effect of the drink on RVIP latency; however, this is likely due to the fact that the two studies differed in administration of methamphetamine. Data suggest that this method depletes DA, and support a modulatory role of DA in spatial recognition memory functions. Acute DA depletion in healthy humans may not adequately decrease DA to induce large cognitive deficits. The effect of the phe-tyr free drink on spatial recognition memory coincided with the PRL peak at 5 hours post-drink administration.

Grevet et al. (2002) examined the effects of a phe-tyr free mixture on memory and attention in 12 healthy male volunteers (only 10 completed both sessions) in a randomized, double blind placebo controlled design. Of the 12 volunteers, 6 reported a family history of mental disorders. On two separate days, subjects received a balanced mixture (Young et al., 1985) or a mixture lacking phe and tyr. Phe and tyr levels, neuropsychological testing, and ratings were collected at baseline and 5 hours post-drink ingestion. The Rey Auditory Verbal Learning Test (a word-list learning task with immediate and delayed recall) and the Aggie figures learning test (a non-verbal analogue of the Rey that requires subjects to learn a set of abstract designs instead of words) assessed memory functions. Mesulam's cancellation task (which is a timed visual search task), Hebb's digits (assessing attention span), and the Corsi blocks test (a non-verbal attention span test) assessed attention functions.

The Phe-tyr free mixture significantly decreased plasma Tyr levels. Consistent with what would be expected from lowered 5-HT function, anxiety related mood symptoms increased after ingestion of the Phe-tyr free mixture with a trend for increased hostility. Memory and attention measures showed no depletion effect except for a significant difference in delayed recognition of the Rey word-list ($p < .05$). Although, the difference was statistically significant, the actual difference appears to have been only approximately one word less after ingesting the Phe-tyr-free mixture. Data are in conflict with the findings of Harmer and colleagues (2001). The inconsistency may be due to the family history of psychopathology in the sample, which may be associated with hereditary basal DA and 5-HT functioning alterations. Alternately, the data may suggest that the method of acute DA depletion also affects 5-HT functioning or does not produce striking cognitive effects.

Gijsman et al. (2002) used a BCAA mixture composed of valine, isoleucine, and leucine (3:3:4 ratio) in a double-blind crossover design to examine the feasibility of using the simpler AA mixture to manipulate DA and cognitive sequelae. On three separate days separated by a week, 7 men and 5 women consumed 10 g, 30 g, and 60 g of the drink. Plasma AA and PRL concentrations were collected at baseline and every 30 minutes for a 6-hour period. Subjects completed language-free visual and spatial recognition memory, paired associates learning, and rapid visual information processing computerized tests from the CANTAB 300 minutes after drink ingestion. Testing was done once to avoid possible learning effects that decrease measure sensitivity to processing changes. VAS and profile of mood states questionnaire (POMS) assessed side effects.

A significant and sharp PRL increase occurred between 4 and 5 hours after ingesting the 60 g dose as compared to the 10 g and 30 g doses. Spatial recognition memory showed a trend (F (2,22), p = .06) for response latency to increase with increasing BCAA dose. There was no correlation between spatial recognition efficiency and PRL AUC; however, the correlation between efficiency and Tyr+Phe/BCAA ratio approached statistical significance. The drink was well tolerated and there were no subjective state changes. The authors conclude that previous research has shown that tryptophan depletion, affecting primarily 5-HT, negatively impacts performance on PAL and VRM; however, the BCAA mixture did not affect performance on either of these tests, suggesting that this paradigm primarily depletes DA. The cognitive measures were administered at a time corresponding with the peak PRL response.

Harrison and colleagues (2004) investigated the effects ATD (primarily 5-HT) and APTD (primarily DA) on a memory-oriented cognitive battery. ATD and APTD were expected to adversely affect declarative and working memory, respectively. Out of a sample of 20 females, 13 female (in their early 20s) completed the double blind, placebo-controlled, three-way crossover design. Participants were given 86 g AA mixtures (Young, Smith, Pihl & Ervin, 1985) that were balanced, lacking tryptophan, or lacking Tyr and Phe, on three separate days.

Direct comparison of ATD and APTD effects revealed specific effects on memory tasks. APTD caused decreased accuracy in identifying original and novel positions (F (1,12) = 7.09, $p < .05$) compared to the balanced condition. ATD decreased accuracy in word recall compared to the balanced condition (F (1,12) = 14.21, p = .003) and the APTD condition (F (1,12) = 5.67, p = .035). ATD also improved critical flicker

fusion test (assessing perceptual processing and visual discrimination) performance compared to the balanced and APTD conditions (p <.05). The results support the hypothesis that these two methods differentially affect memory and suggest the DA depletion method affects spatial memory.

Simultaneous catecholamine and 5-HT depletion yields sustained attention deficits and no learning or memory deficits (Matrenza, Hughes, Kemp, Wesnes, Harrison & Nathan, 2004). In a double blind-placebo controlled design, 20 healthy females received a balanced drink and an equivalent mixture lacking phe, tyr, and tryptophan (combined depletion) on two separate days. Subjects completed baseline and 5 hour testing on word list (immediate, delayed, and recognition), picture, numeric and spatial working memory, digit vigilance, simple and choice RT from the Cognitive Drug Research Assessment battery. Combined depletion significantly reduced tyr, phe, and tryptophan to large neutral amino acid ratios. Digit vigilance speed and accuracy were significantly worse after the combined depletion. Other cognitive measures and POMS ratings were unaffected. Data indicate that combined depletion interactions between DA, 5-HT, and NE, influence cognitive performance in a manner that is distinct from independent depletions. Further, the findings may be unique to females, as estrogen influences monoaminergic systems (McEwen, 1999).

Complex BCAA mixtures lacking phe and tyr (Harrison et al., 2004; Sheehan et al., 1996; Young et al., 1985) appear to impair spatial memory (Harmer et al., 2001) and attention (McTavish, Raumann, Cowen et al., 2001), although some depletion studies have failed to find cognitive effects (Grevet et al., 2002). Combined catecholamine and 5-HT depletion yields sustained attention deficits and no learning or memory deficits

(Matrenza et al., 2004) in females. Studies with a simpler BCAA mixture composed of valine, isoleucine, and leucine has provided less consistent evidence for spatial recognition memory (Gijsman et al., 2002). Studies investigating the relationship between BCAA DA depletion and cognition are limited by small sample sizes and by the relatively narrow extent of cognitive domains investigated (Gijsman et al., 2002; Harmer et al., 2001; Grevet et al., 2002; Harrison et al., 2004; Matrenza et al., 2004; McTavish, McPherson et al., 2001). Generalizability of data may be limited due to the small sample sizes (no study included more than 20 participants). Most studies involve samples of men and women. Some data suggest that DA precursor depletion impairs spatial working memory. DA depletion effects on more aspects of executive functioning warrant investigation. Because of DA's influence and presence in the PFC, DA depletion should affect DA mesocortical-dependent cognitive functions. The consequences of catecholamine precursor depletion on cortical-subcortical DA system balance and cognitive functions are unclear.

CHAPTER 5

Present Study Overview

BCAA administration primarily affects the DA system and may affect attention and working memory. However, investigation of DA depletion effects on attention, working memory, and executive functions has been limited. Generalizability of previous study findings is limited by small sample sizes. Most studies have administered complex AA mixtures, which are highly unpalatable and cause gastrointestinal discomfort. The present study investigated DA precursor depletion effects on working memory, attention, list learning, motor and inhibitory control processes.

The use of a simple BCAA mixture lacking DA precursors offers a relatively novel approach to investigating the role of DA in healthy human cognition. The BCAA mixture is a commercially available, well tolerated, simple mixture composed of three essential amino acids (valine, isoleucine, and leucine) that is orally administered. The drink mixture is the same composition that Gijsman et al. (2002) employed. Gijsman, Scarna and colleagues are the only groups to date that have investigated the effects of this mixture on basal DA function and cognition in healthy humans. BCAA reduces brain DA precursor availability by increasing competition with tyrosine and phenylalanine for transport across the blood brain barrier and stimulating protein synthesis. The resultant precursor availability reduction causes decreased DA synthesis and availability leading to a global decrease in DA neurotransmission.

BCAA administration selectively decreases DA production. However, 5-HT and NE systems, which have also been associated with cognitive processes, do not appear to be significantly affected by BCAA administration. Administration of this drink to

animals and humans increases protein synthesis and obstructs tyrosine transport across the BBB (through competition with the large amino acids in the drink), both of which cause decreased DA production. BCAA decreases the available amount of DA precursor in the brain. BCAA administration results in a global decrease in DA production and activity as indicated by increases in plasma PRL levels. Hypothalamic DA neuron activity inhibits PRL release; therefore, the decrease in DA production is reflected in an increase in PRL release due to decreased DA neuron inhibitory activity. Hypothalamic DA neurons inhibit the release of PRL, thus a decrease in dopaminergic activity would be expected to result in an increase in PRL release. Animal studies and indirect cognitive evidence suggest that 5-HT and NE systems are not affected.

Studies examining the effects of specific phe-tyr depletion and more global catecholamine and monoamine depletion have demonstrated some effects on cognitive functions. Gijsman and colleagues (2002) have demonstrated that BCAA administration may adversely affect spatial recognition memory functions. However, very small sample sizes and inconsistent findings limit the studies. The aims of this study were 1) to examine the utility of BCAA as a method of global DA depletion and 2) to investigate global DA depletion effects on cognition in healthy human males. The aims of the study were met through a double-blind placebo controlled crossover design in which a sample of healthy males received BCAA and placebo on two separate days and completed cognitive tests at baseline, 3-4 hours and 5-6 hours post-drink. Specifically, the first aim of the study was addressed through analysis of changes in plasma PRL levels, which reflect altered DA function. The second aim of investigating the effects of global DA depletion on cognition was achieved through measuring performance on memory,

attention and executive function tests prior to and following placebo and BCAA administration. The following hypotheses were tested:

Primary Hypotheses:

- BCAA administration will cause a significant and sharp increase in PRL levels, reflecting a decrease in DA system function.
- If BCAA primarily affects DA system function, and working memory, attention, and inhibitory control are dependent upon intact DA system function, then BCAA administration will impair performance on these cognitive tasks as reflected by significant Treatment x Time interaction effects.

Secondary Hypotheses:

- Differences in PRL response to BCAA will be related to changes in performance on cognitive measures.

CHAPTER 6

Method

Participants

Subjects were referred to the study from the Zucker Hillside Healthy Control Study, which recruits participants from the community through the use of postings and mailings. As part of participation in the Healthy Control Study, subjects were screened for existing medical conditions as well as any Axis I pathology using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders Non-patient Edition (SCID-I/NP) (First, Spitzer, Gibbon & Williams, 2001). Subjects were excluded if they had cardiac, hepatic, pancreatic, or acute or chronic kidney disease, diabetes type 1 or 2, seizure disorder, PKU, intestinal malabsorption disorder, or history of gastrointestinal surgery. Exclusion criteria also included current substance abuse, current or a history of psychiatric illness, current treatment with an antipsychotic, psychostimulant, or anticonvulsant medication, or known reported BCAA allergy.

The participants were 43 healthy male volunteers between 19 and 49 years of age (M = 33.6, SD = 8.9). Only males were recruited in order to minimize variance in response to BCAA administration that may be attributable to sex differences (such as sex differences in gastric emptying related to hormones, systemic drug metabolism) (e.g., Datz, Christian & Moore, 1987; Hamilton & Yonkers, 1996; Harris, Benet & Schwartz, 1995). A total of 41 subjects completed both study days. Two subjects withdrew from the study after completing day 1 (both subjects received placebo). One subject was consented but later terminated from the study after his baseline assessment, during which he endorsed depressive symptom items on the behavioral ratings (data from this subject

were not included in the analyses). Data were analyzed for the 43 participants (39 right-handed, 4 left-handed), ranged from 12-20 years of education (M = 15.3 yrs, SD = 2.01). All participants had normal or corrected-to-normal vision.

The protocol was reviewed and approved by both the North Shore-Long Island Jewish Health System and the Queens College Institutional Review Board. All subjects provided written informed consent before participating in the study.

Design/Procedure

The effects of DA depletion on cognitive task performance was examined using a 2 (Drink) x 3 (Time) repeated measures design. The Drink independent variable (IV) consisted of two levels, placebo and BCAA. The Time IV consisted of three levels, cognition was assessed at baseline, 3-4 hours after drink administration, and 5-6 hours after drink administration. The effects of BCAA administration on DA (as reflected in plasma PRL levels) was examined using a 2 (Drink) x 6 (Time) repeated measures design. The Drink IV consisted of two levels, either placebo or BCAA. The Time IV consisted of six levels, which represent the plasma PRL levels measured hourly (0hrs, 1hr, 2hrs, 3hrs, 4hrs, and 5hrs after drink administration).

DA depletion was accomplished via administration of a BCAA drink mixture. The double-blind placebo controlled cross-over design was completed over two days separated by at least 48 hours (but no later than one week after the first day) in order to ensure an adequate wash-out period and to minimize carry-over effects. Subjects were instructed to fast from 12:00am before study participation. A designated individual not involved in data collection for the study randomly assigned administration order for each subject and prepared the placebo and BCAA drinks.

At 8:30am on each study day, subjects completed baseline testing, which consisted of the neuropsychological battery, behavioral and side effect ratings, body mass index (BMI), and vital signs. After baseline measures were collected, an intravenous indwelling canula was placed to facilitate hourly plasma PRL level collection. The baseline plasma PRL level was collected once the canula was in place. At 10:00am, subjects consumed either the placebo or BCAA drink. The Neuropsychological Battery and behavioral ratings were completed again between 3-4 and 5-6 hours after drink consumption in an attempt to better understand the time-course of the behavioral effect of the amino acid drink in relation to the PRL response. A second rationale for using multiple testing times was to obtain data that would facilitate controlling for possible learning and fatigue effects that may occur during the course of the almost 8-hour study duration. The procedure schedule illustrated in Figure 2 was followed to control for PRL fluctuation over the course of the day.

Branched Chain Amino Acid Mixture

The BCAA drink is a commercially available powdered nutritional drink mixture composed of three essential branched chain amino acids (BCAAs), valine, isoleucine, and leucine in a 3:3:4 (18, 18, 24g) ratio. BCAA mixtures are used as a medical food for treating extrapyramidal symptoms of tardive dyskinesia (Richardson et al., 1999; Richardson et al., 2003). BCAA has a 1.5-hour half-life; therefore, 48 hours is an adequate washout period. BCAA has a low-incidence of associated side effects (i.e., constipation, nausea, and diarrhea) that are similar to those in nature and incidence of placebo (Gijsman et al., 2002; Tarvil information insert, http://www.tarvil.com). The BCAA mixture used (Tarvil™) is only approved for use in males.

BCAA used in this study was purchased in a single batch to ensure a consistent product quality. Subjects received a standard single dose of 60 g of BCAA. Other studies have administered BCAA to healthy controls in similar dose (Gijsman et al., 2002) without the occurrence of any serious adverse events. BCAA is actually better tolerated than more complex and balanced amino acid drink mixtures used in other studies (i.e., Young, Smith, Pihl & Ervin, 1985; Harmer et al., 2001; Grevet et al., 2002; Harrison et al., 2004; McTavish, McPherson et al., 2001; Sheehan et al., 1996).

An individual not involved in the study prepared the drinks according to a randomization schedule in order to maintain the double blind. Because BMI was not taken into consideration in the dosage for each subject, this information was collected at baseline and analyzed later as a covariate. BMI was calculated using a Tanita scale, which generates the BMI based on weight and height. In general, BMI is calculated using the following formula: BMI = [(Weight in Pounds) / (Height in inches)^2] x 703.

Data suggest that BCAA administration primarily decreases DA, while only secondarily affecting other neurotransmitters such as 5-HT and NE (e.g., McTavish, Callado, Cowen & Sharp, 1999; McTavish, Cowen & Sharp, 1999; McTavish, Raumann, Cowen & Sharp, 2001).

The placebo mixture was an inactive mixture of similar consistency and flavor to the BCAA mixture.

Plasma Prolactin Levels

Previous research has demonstrated in a sample of healthy human subjects, that tyrosine and phenylalanine depletion stimulates PRL release above and beyond that seen following liquid meal ingestion and balanced amino acid drink (e.g., Carlson, 1989;

Gijsman et al., 2002; Harmer et al., 2001; McTavish, McPherson et al., 2001). The D2 receptors in the hypothalamus have an inhibitory effect on the release of PRL. If these receptors are blocked or there is a decrease in DA availability due to precursor depletion, the inhibitory effect is removed and PRL release increases. The peak PRL response to branch chain amino administration based upon prior studies appears to be at approximately 4-5 hours after BCAA administration.

To facilitate hourly blood sample acquisition, an intravenous indwelling insyte autoguard canula (22ga, 1"long) was placed in a peripheral forearm vein using asceptic procedures. The canula was flushed with saline and approximately 3cc of blood were drawn every hour. Plasma PRL assays were performed at the North Shore Long Island Jewish Core Laboratory (Lake Success, New York) using the Roche electro chemiluminescence method.

Neuropsychological Battery

The neuropsychological battery included tasks that assess functions that are hypothesized to be sensitive to DA manipulation. The battery also contained tasks that were not expected to change because they are not related to DA function.

Object Working Memory (Cogtest) (OWM) (Cogtest plc, London).

The OWM test is a computerized test of working memory consisting of 3 parts. The stimuli consist of 9 x 9 square arrays, some of which are black and some of which are white. The first part of the OWM test establishes visuoperceptual competency. The subject views two test stimuli (one of which matches the target stimulus) presented below a target stimulus. The subject must use the computer mouse to select the test stimulus that is identical to the target stimulus. This part of the test establishes intact visual perception

ability. The subject must achieve at least 80% accuracy to continue on with the next part of the test.

The second part of the OWM test is designed to determine the necessary difficulty level in order for the subjects to achieve 80% accuracy. This part of the test equates subjects in terms of accuracy. Test stimuli differ from target stimuli in varying degrees. A titration score is calculated from this part where a higher number represents a greater difficulty level. The titration score is the average Hamming distance (the number of binary elements that are different between the targets and distractors necessary to achieve 79% percent accuracy) at which reversals took place following the up-down transform rule (i.e., there is a reversal to make the test more difficult following 3 consecutive correct responses, and reversal to make it easier following any 1 failure). This is the estimate of the difficulty level at which the subject performs at 79% accuracy.

The third part of the OWM test is designed to measure working memory. The subjects are presented with the target stimulus and then there is a period of time in which a distraction is presented. During this delay period of four seconds, the subject views a random pattern of squares being displayed. After the delay, the subject selects the original target from two choices that are presented. The delay score reflects the number of correct responses out of 20 trials.

The OWM test requires working memory and attention components that are likely associated with DA PFC functioning. Prior animal and human research has implicated DA activity in maintenance of spatial information (El-Ghundi, Fletcher, Drago, Sibley, O'Dowd & George, 1999; Murphy, Arnsten, Goldman-Rakic &Roth, 1996; Sawaguchi, 2001; Sawaguchi & Goldman-Rakic, 1991, 1994; Seamans & Yang, 2004). The OWM

test requires maintenance of spatial information for efficient performance; therefore, we hypothesized that the OWM delay score would be negatively affected by BCAA administration.

Continuous Performance Test—Identical Pairs Version (CPT-IP) (Cornblatt, Risch, Faris, Friedman & Erlenmeyer-Kimling, 1988).

This commonly used computer test assesses sustained attention and concentration. There is also a working memory component, as the subjects are required to briefly maintain each number or shape presented to determine whether the subsequent shape is an exact match. Subjects view stimuli flashing on a computer monitor and are instructed to respond as quickly as possible when they see any identical consecutive stimuli. There are two stimuli types; either 4-digit numbers or abstract shapes presented either at a fast (50 msec duration) or slow rate (150 msec duration) in a total of four test conditions. Accuracy (hits, false alarms, and log randoms), RT for hits, and d-prime and beta signal detection measures were generated from the CPT-IP.

The CPT-IP measure was included as a measure of sustained attention because prior research has suggested that DA transmission modulates sustained attention (e.g., Manor et al., 2004; McTavish, McPherson et al., 2001; Szobot, Roman, Cunha, Acton, Hutz & Rohde, 2005). Prior research of the effects of BCAA administration on sustained attention has also suggested that sustained attention can be affected if DA function is not at an optimal level. McTavish, McPherson et al. (2001) found that methamphetamine affected RVIP performance such that response latency ($F = 29.28$, $p < .001$) and response bias ($F = 6.60$, $p = .025$) decreased, while target sensitivity increased ($F = 9.19$, $p = .01$). Administration of the phe-tyr free mixture significantly attenuated the methamphetamine

effect, suggesting that administration of the phe-tyr free mixture significantly attenuated the methamphetamine effect, suggesting that the mixture specifically attenuates amphetamine-induced DA release. Alternately, Harmer and colleagues (2001) did not find an effect of phe-tyr depletion on RVIP, suggesting that basal DA depletion may not impair sustained attention.

Computerized Conflict Task (Nassauer & Halperin, 2003).

The Computerized Conflict Task assesses early perceptual inhibition of irrelevant stimulus characteristics and later motor inhibition of inappropriate responses, separately and together. The task involves six subtests in which either an arrow or a box appears in various locations on a computer monitor. Subjects respond by pressing a key on the left or right side of the keyboard. In different subtests, either arrow direction or stimulus location determines the correct response. Subjects are instructed to respond as quickly as possible while trying not to make mistakes.

Perceptual inhibition assessment requires the subject to respond to a conflicting arrow direction while ignoring the more salient characteristic of stimulus location. For the first subtest trials, the subject is instructed to respond by pressing the key that is on the same side as where a central arrow is pointing (e.g., if the arrow points to the left, the correct response the left key). For the next subtest, subjects are instructed to press the key that is on the same side as where a rectangle appears (e.g., if the rectangle appears on the left side, the correct response is the left key). For the last subtest, subjects are instructed to ignore the location of the arrow and respond to the direction of the arrow (e.g., if a left-pointing arrow appears on the right side, the correct response is the left key). Motor inhibition assessment requires subjects to respond in the direction opposite of that

indicated by a centrally located arrow (e.g., if the arrow points to the left, the correct response is the right key). The combined assessment of perceptual and motor inhibition requires subjects to respond to a conflicting arrow direction by responding in the direction opposite of that indicated by the arrow direction. For example, if a left arrow is presented on the right side, the correct response is the right key. RT, response variability and interference scores for each conflict condition were generated for this set of tasks (described in detail in Nassauer & Halperin, 2003).

Research has implicated DA in conflict paradigms that require overriding prepotent responses and switching of response set (Diamond, Briand, Fossella & Gehlbach, 2004; Nolan, Bilder, Lachman & Volavka, 2004, Bilder, Volavka, Lachman & Grace, 2004). There is also evidence that tasks requiring inhibition of prepotent responses recruit PFC and anterior cingulate resources (Schulz et al., 2005). Therefore, if BCAA administration primarily decreases DA activity, perceptual inhibitory control measures, requiring inhibition of the more salient stimulus characteristic of location, should be adversely affected by BCAA administration.

Hopkins Verbal Learning Test (HVLT) (Brandt, 1991).

The HVLT assesses list learning ability. The examiner reads the subject a list of 12 words (from 3 categories) over 3 trials. After each trial, the subject recalls as many of the words as possible in any order. Six counterbalanced alternate forms were used for this study. The cognitive requirements of the list-learning task are primarily thought to be mediated by hippocampal learning; however, because items are categorically related, which may be used as a learning strategy, task performance may also recruit PFC areas.

The total number of words recalled, total number of perseverations, and total number of intrusions were analyzed.

Grevet et al. (2002) investigated the effects of BCAA administration on the Rey Auditory Verbal Learning Test (RAVLT), which is a list-learning task similar to the HVLT, however, words on the RAVLT are not semantically related. They found a significant difference in delayed recognition of the Rey word-list ($p < .05$). Although, the difference was statistically significant, the actual difference appears to have been only about one word less after ingesting the phe-tyr free mixture. It was hypothesized that BCAA administration would not impair HVLT performance.

Finger-Tapping Test (Reitan, 1993).

The finger-tapping test assesses motor speed. The subject taps on a finger tapper as quickly as possible for 10 seconds. The subject is instructed to use only the index finger. Both hands are tested alternately and the mean of the 3 fastest trials within 5 taps of each other is scored. Because of DAs involvement in motor functioning, performance on this task may change as a result of BCAA administration. This task was administered to examine BCAA effects on motor speed that could confound changes in performance on cognitive measures. The average of the left and right finger scores was analyzed. Because of DA nigrostriatal influence on motor function (reviewed in Feifel, 1999), it was hypothesized that finger tapping would be slower after BCAA administration.

Wide Range Achievement Test-3 (WRAT-3) (Wilkinson, 1993).

The WRAT-3 is a standard achievement index of word reading ability requiring the subject to read 42 words of increasing difficulty. There are two parallel forms of the test. Single-word reading skill is considered a stable estimate of general cognitive

abilities in healthy individuals. This measure is stable and not associated with PFC functioning so performance was not expected to change after BCAA administration. The total number of correctly read words was analyzed.

Behavioral and Side Effect Measures

A clinician administered behavioral rating scales to subjects at baseline and again at 4-hour and 6-hour evaluation sessions to assess any behavioral changes that may have affected performance on the neuropsychological battery. The trained clinician was blind to the treatment condition. Ratings were not expected to change following BCAA administration. Previous studies employing the AA composition have found that it is well tolerated and not associated with side effects beyond those seen with placebo administration.

Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1981).

The SANS assesses the following negative symptoms: affective flattening, alogia, avolition-apathy, role functioning, and asociality-anhedonia. A higher score on the SANS indicates endorsement of negative symptoms.

Brief Psychiatric Rating Scale-Anchored (BPRS-A) (Overall & Gorham, 1988; Woerner, Mennuzza & Kane, 1988).

The 18-item BPRS-A scale assesses somatic concern, anxiety, emotional withdrawal, conceptual disorganization, guilt feelings, tension, mannerisms and posturing, grandiosity, depressive mood, hostility, suspiciousness, hallucinatory behavior, motor retardation, uncooperativeness, unusual though content, blunted affect, excitement, and disorientation. A trained clinician rates subjects on each of the items using a 7-point scale ranging from 1=not reported to 7=very severe.

Hamilton Rating Scale for Depression-24 Item (HRSD-24) (Hamilton, 1960).

The 24-item HRSD-24 assesses symptoms of depression (e.g., depressed mood, guilt feelings and delusions, motor retardation, agitation, psychic anxiety, somatic anxiety, appetite, energy, depersonalization and derealization, paranoid symptoms, obsessional and compulsive symptoms, helplessness, hopelessness, and worthlessness). Higher scores on the HRSD-24 are indicative of greater depression.

Simpson-Angus Scale (Simpson & Angus, 1970).

The 10-item Simpson-Angus Scale assesses side effects affecting gait, arm dropping, shoulder shaking, elbow rigidity, wrist rigidity, head rotation, glabellar tap, tremor, salivation, and akathisia. Each item is rated on a 4-point scale with a higher rating indicating more severe symptomatology.

Statistical Procedures

Statistical Power.

Estimates of means and standard deviations from the literature for spatial recognition memory latency (in ms) yielded a mean of 1685 ms +/- 506 for the 10g/placebo and 1994 ms +/- 653 for BCAA 60 g (Gijsman et al., 2002). Utilizing these estimates and assuming a standard deviation of differences of 600, a sample size of 48 subjects will yield 80% power to detect a difference in means of 309 (BCAA mean of 1994 and placebo mean of 1685), i.e., a medium effect size, using a paired t-test with a 0.01 two-sided significance level. Since several repeated factors (3 time points and 2 treatments) were compared, analyses were performed at the 0.01 level.

Prolactin Data.

PRL data were analyzed with a mixed model approach to a two-way repeated measures analysis of variance (RM ANOVA) in order to determine whether an interaction effect of drink (placebo and BCAA) with time (baseline, 1, 2, 3, 4, 5 hours post-drink) exists. If BCAA depletes DA, there should be a significant interaction such that PRL is significantly elevated at 3 and 4 hours after BCAA administration compared to after placebo administration. Area under the curve (AUC) estimations were calculated for PRL according to the trapezoidal rule. Subsequently, a ratio of BCAA AUC-placebo AUC to placebo AUC was calculated. This ratio was used to determine to what degree PRL response differed due to BCAA administration and whether the magnitude of difference was associated with any cognitive differences.

Neuropsychological Data.

A mixed-model approach to repeated measures analysis of variance (RM ANOVA) was used to analyze the crossover design containing repeated factors of time (baseline, 3-4 hours post drink, and 5-6 hours post drink) and drink (placebo and BCAA). These repeated factors of time and drink are the within subjects effects. The between subjects effect of treatment order (BCAA day 1 / placebo day 2 vs. placebo day 1/ BCAA day 2) was also analyzed. The mixed model RM ANOVA was performed on the neuropsychological endpoints of attention, memory, inhibitory control, and other cognitive measures.

CHAPTER 7

Results

Behavioral Data

The BPRS, HRSD-24, SANS, and Simpson Angus scales were completed three times, after each NP assessment. Subjects did not experience any significant side effects from BCAA administration. RM ANOVA revealed that there were no significant changes in any of the items on any of the scales across drink and time, nor was there an interaction of drink with time (all $p > 0.1$). The HRSD-24 somatic energy item showed that subjects were a bit more fatigued in the middle of the day and slightly more so after BCAA than at baseline, but the differences were not clinically (all means were less than 1.0) or statistically significant, $F(2,78) = 2.93$, $p = .06$.

Prolactin Data

The mixed model RM ANOVA on PRL levels (nanograms/ml) as the endpoint variable revealed a significant Drink x Time effect, $F(5, 196) = 36.29$, $p < .0001$, indicating a significant sharp increase in PRL levels following BCAA administration as compared to placebo (see Figure 3). PRL levels peaked 3 hours after BCAA administration compared to steady levels seen with placebo administration (see Table 1 for specific means and standard deviations).

We examined the relationship between PRL and BMI to determine whether to control BMI variability. AUC for PRL was calculated for each day and the ratio of (BCAA AUC-Placebo AUC)/Placebo AUC was correlated with BMI. There was a significant negative correlation between the PRL AUC ratio and BMI ($\underline{r}_s = -.38$, $p =$

.0171), such that a smaller response to BCAA was associated with greater BMI, which is consistent with the method of using a standard fixed dose of BCAA.

Neuropsychological Data

The RM ANOVA was done using SAS version 8.2 (Copyright 1999-2001 by SAS Institute Inc., Cary, NC, USA). Given the relatively large number of statistical tests (run on the 32 dependent variables), we chose to apply an alpha level of .01 to determine statistically significant change within the cognitive measures. None of the data were transformed, given that they were reasonably normally distributed.

Order Effects.

Although BCAA and placebo order administration was counterbalanced and randomly assigned, data were examined for order effects. Of the 43 participants, 23 received placebo first, while 20 participants received BCAA first. The only significant effect was for object working memory titration (hamming distance), $F(1,41) = 11.34$, $p = .0017$; with participants receiving placebo first requiring greater difficulty to achieve 80% accuracy than those receiving BCAA first. Although statistically significant, the OWM titration score (Hamming Distance) difference was small (a difference of 1.8 binary elements. There was a significant Order effect on WRAT-3 reading total words recalled, $F(1,41) = 9.49$, $p = .0037$; however, the difference between means was less than 1-word. These two Order effects although statistically significant, were not clinically meaningful. There were no order effects on any of the other cognitive measures (all $p > .1$).

Object Working Memory (OWM).

There were no significant interaction, Drink, or Time effects on OWM titration or delay scores (all $p > .1$, see Figure 4).

Continuous Performance Test-Identical Pairs Version (CPT-IP).

Accuracy, RT and Signal detection measures were generated from each of the four CPT-IP conditions. Accuracy measures included percentages of hits, false alarms, and log randoms. RT measures included means and standard deviations for correct responses. Signal detection measures included D' and Beta.

4-Digit Condition. In the Slow condition, there was a significant Drink by Time interaction effect on RT variability, $F(2,73) = 4.81$, $p = .01$ (all other $p > .1$), variability decreased while on BCAA at the 5-6 hour assessment, but increased while on placebo (see Figure 5). There was a similar trend for the Fast condition showed with a decrease in RT variability at the 3-4 hour assessment more on BCAA than on placebo ($F(2,73) = 2.6$, $p = .08$, see Figure 6). Signal detection measures did not show any significant effects (all $p > .2$). Response accuracy measures did not show any significant interaction effects (all $p > .1$). Both 4-digit conditions indicated significant Time effects on response accuracy percentage of hits. For the 4-digits fast ($F(2,82) = 6.58$, $p = .0022$) and slow conditions ($F(2,82) = 5.67$, $p = .0049$) showed significant Time effects such that participants performed less accurately at the 3-4 and 5-6 hour assessments compared to baseline. Neither condition showed a significant effect of Drink or Time on mean correct trial (all $p > .2$).

Shape Conditions. The accuracy measures for the Fast Shapes condition did not show any significant interaction, Drink, or Time effects. There was also no significant

interaction effect for the Shapes Slow condition. The Slow Shapes condition showed a trend for poorer accuracy on BCAA at the 3-4 hour assessment compared to placebo ($F(2,82) = 3.86$, $p = .03$), but the difference was not statistically significant. For both conditions, there was no significant interaction effect, but mean RT was significantly faster over time (fast, $F(2,82) = 5.95$, $p = .004$, slow, $F(2,82) = 4.97$, $p = .009$). RT variability and signal detection measures did not show any significant interaction, Drink, or Time effect (all $p > .2$).

Computerized Conflict Task.

Separate 2 (perceptual conflict) x 2 (motor conflict) x 2 (drink) repeated measures ANOVAs were done in SPSS version 12.0 for Windows (Copyright 1989-2003 by SPSS Inc., Chicago, IL, USA) to investigate the effect of the presence or absence of motor and/or perceptual conflict and placebo or BCAA on RT and RT variability measures. There were no significant effects on RT (all $p > .1$); however, for RT variability, there was a consistent Perceptual Conflict x Motor Conflict x Drink interaction trend for decreased RT variability on BCAA at 3-4 hours and 5-6 hours for specific conflict conditions. The interaction effect comparing RT variability at the 3-4 hour assessments for placebo and BCAA ($F(1,37) = 4.436$, $p = .042$), indicated RT variability was decreased for the combined perceptual and motor conflict condition on BCAA compared to placebo ($t(1,37) = 2.438$, $p = .020$ and see Figure 7). The interaction effect comparing RT variability for the 5-6 hour assessments for placebo and BCAA ($F(1,37) = 3.502$, $p = .069$), indicated decreased RT variability for the Perceptual Conflict condition while on BCAA compared to placebo ($t(1,37) = 2.240$, $p = .031$ and see Figure 8).

Hopkins Verbal Learning Test (HVLT).

BCAA did not significantly affect HVLT total number of correct words recalled or total number of perseverations (all $p > .1$, see Figure 9).

Finger Tapping Test (FT).

Independent and averaged right and left finger tapping scores did not show any change following BCAA administration (all $p > .1$, see Figure 10).

Wide Range Achievement Test-3 (WRAT-3).

As predicted, there was no significant interaction or Drink effect on WRAT-3 total raw score of correctly read words. There was a significant Time effect (F (2,84) = 4.33, $p = .0162$); however, the actual difference in total words across times was not meaningful (less than a 1-word difference) (see Figure 11).

Exploratory Analyses

Relation Between PRL Response and Cognitive Measure Response to BCAA.

Because of the significant negative correlation between BMI and PRL, we investigated the relationship between BMI and cognition. BMI was added to the RM ANOVA as a covariate. Adding BMI as a covariate did not change the results.

Separate exploratory analyses also investigated whether PRL response to BCAA was related to changes in performance on cognitive measures after BCAA. Two approaches to investigating PRL and NP measure response to BCAA included averages and ratios. Average PRL response was calculated for placebo and BCAA days. Similarly, for each cognitive test, average cognitive measures were calculated for each day. There was no significant correlation between average PRL measures and average NP measures.

Ratio scores were also calculated for PRL levels and NP measures to compare levels and performance (respectively) at 3-4 hours with baseline and 5-6 hours with baseline. These ratios were then analyzed to determine whether a relationship between PRL ratios and NP ratios exists. Analyses showed no significant relationship between PRL and NP performance.

Performance as a Function of Baseline Choice RT, Drink, and Time.

Separate exploratory analyses were done to investigate whether initial differences in simple choice RT speed affected performance on cognitive measures after BCAA administration. Using a median split, participants were categorized as either fast (RT < 379 ms) or slow (RT ≥ 379 ms). Results showed no significant interaction, baseline choice RT, Drink, or Time effects.

CHAPTER 8

Discussion

Results demonstrate that acute BCAA administration (a 60 g standard dose) was well tolerated and did not induce changes in side effect and behavioral ratings. Two participants voluntarily withdrew participation after completion of the first study day for undisclosed reasons; however, both of these participants had received placebo. One person also became nauseous after placebo administration. Failures to complete the study and side effects were not associated with BCAA, supporting its tolerability. These findings are consistent with previous studies using the same 3-essential amino acid mixture lacking phenylalanine and tyrosine (Gijsman et al., 2002).

As predicted, PRL levels increased significantly after BCAA administration, indicating decreased hypothalamic DA function. These findings are consistent with findings from an earlier study that used a mixture identical in composition to BCAA (Gijsman et al., 2002). Although 5-HT precursor levels also decrease following BCAA, the effect on DA is greater than that on 5-HT. Since 5-HT has an excitatory effect on PRL release through its inhibitory influence on DA release (Attenburrow, Mitter, Whale, Terao & Cowen, 2001; Jorgensen, Knigge & Warberg, 1992); therefore, if 5-HT were primarily decreased, PRL release would also have decreased. Further, tryptophan supplemented phe-tyr free drinks result in a large PRL response and cognitive effects, supporting the notion that BCAA administration primarily affects DA (Scarna, McTavish, Cowen, Goodwin & Rogers, 2005). The sharp increased PRL response clearly indicates that BCAA primarily altered DA; however, it is unclear to what degree NE was

affected. The conclusion that BCAA primarily affects DA depletion is based on prior study findings and indirect PRL evidence.

PRL levels peaked at about 3 hours after drink ingestion, which was somewhat earlier than the 4-5 hour peak found by Gijsman and colleagues. By about 5 hours post-drink administration, PRL levels had returned to normal. The difference in peak time may have been due to differences in the time at which the studies administered the drink. The present study consistently administered placebo and BCAA drinks at 10a.m. on each study day (specific time of drink administration was not reported for the Gijsman study). The observed earlier PRL peak may also have been due to differences in drink composition, we used a less complex mixture than the majority of other catecholamine depletion studies. The participants were also not instructed to consume a low-protein diet prior to arriving on each study day, which may have altered the time course of the BCAA effect.

Data trends in RT variability should be cautiously interpreted in light of the number of statistical tests that were conducted. However, trends suggest that acute BCAA induced decreases in DA availability may improve attention and response consistency on cognitively demanding tasks. Differences in RT variability in the absence of differences in mean and median measures of RT suggest a subtle effect on response consistency or activation state (e.g., Chapman, Chapman, Curran & Miller, 1994). RT variability decreased for the CPT-IP 4-digits fast and slow conditions and more demanding subtests of the Conflict Inhibitory Tasks after BCAA, reflecting improved consistency in responding. There is a demonstrated relationship between RT variability and focused attention abilities (Zahn, Kruesi & Rapoport, 1991; Epstein, Erkanli, Klaric,

Costello & Angold, 2003). Decreased DA transmission improvement of focused attention and readiness to respond in healthy males is consistent with opposing roles of DA within the frontostriatal catecholamine system in modulating aspects of selective attention including developing, maintaining, and shifting attentional set (Crofts et al., 2001).

Further evidence of decreased RT variability (reflecting improved focused attention and readiness to respond) following catecholamine precursor depletion would lend support to the tonic-phasic theory of DA regulation. The tonic-phasic dopamine theory proposed by Grace (1991) differentiates tonic and phasic DA activity roles. Tonic and phasic activity are in turn related to specific aspects of cognitive function. Limbic-striatal DA regulation is controlled by phasic DA release (high-amplitude and transient regulated by burst firing), and tonic DA transmission (steady and low-level activity regulated by baseline DA neuron firing and corticostriatal glutamatergic input). Tonic DA release regulates phasic responsivity by influencing the amplitude of the phasic bursting DA response to behaviorally relevant stimuli.

D1 receptors are proposed to regulate tonic DA function while D2 receptors are critical for phasic DA regulation in the PFC and subcortical areas. Excess tonic or phasic activity is predicted to have specific cognitive consequences (Bilder, Volavka, Lachman & Grace, 2004). Excessive tonic DA activity may yield excessive stability of neuronal activation states, resulting in cognitive rigidity and negative symptoms associated with schizophrenia. Alternately, excessive phasic DA activity may cause neuronal activation state instability, which would result in poorer sustained attention and increased distractibility.

Our present findings suggest that striatal DA may be more sensitive to BCAA manipulation than mesocortical DA. RT variability decreases after BCAA may reflect an effect of decreased phasic DA activity (release) which would increase sustained attention ability and decrease distractibility according to Bilder and colleagues (2004). These findings are consistent with findings that have shown catecholamine depletion via 6-OHDA lesioning in the frontal cortex impaired attentional abilities and increased susceptibility to distraction from task-irrelevant stimuli. However, similar catecholamine depletion within the caudate nucleus did not adversely affect these aspects of attention, and actually reduced susceptibility to distraction from task-irrelevant stimuli.

It is unlikely that the trend for improved RT variability is due to a "rebound" effect, or previously depleted DA levels returning to normal and thus improving performance on the tasks. The trends were seen across the two tasks both at both the 3-4 hour assessment and 5-6 hour assessment, while the peak PRL response occurred between 3-4 hours after BCAA administration. This "rebound" effect explanation would be more feasible if the improvement in RT variability was seen only at the 5-6 hour assessment when the PRL levels were almost returned to normal.

Contrary to what was predicted, there were no effects of BCAA on memory, attention, and executive function accuracy and global RT measures. The lack of effects of BCAA on memory and attention accuracy and RT measures is not likely due to a lack of statistical power and may be explained by the inverted u-shaped DA theory, which suggests that an optimal level of DA transmission is necessary for efficient cognitive functioning. Animal and human literature provide evidence supporting an inverted u-shaped dose-response relation between DA neurotransmission and efficient cognitive

functioning (e.g., Arnsten & Goldman-Rakic, 1986; Cai & Arnsten, 1997; Goldman-Rakic, 1996; Ellis & Nathan, 2001; Luciana, Collins & Depue, 1998; Mattay et al., 2003; Murphy, Arnsten, Goldman-Rakic & Roth, 1996).

Disruption to the DA system balance would be expected to result in cognitive deficits. Global DA depletion effects are most striking when basal DA functioning is at an extreme. Arnsten reviews evidence of an inverted u-shaped curve for optimal D1 receptor stimulation and PFC functioning, D2 receptor stimulation may be detrimental to PFC functioning while NE positively influences PFC functions through post-synaptic alpha 2A adrenergic receptors, but negatively influences the same functions through alpha 1 adrenergic receptors. D1 receptor overactivity leads to cognitive rigidity, while under-activity leads to cognitive instability and unpredictable behavior. Although the acute DA manipulation in the present study decreased DA function, it may not have adequately perturbed DA levels to result in significant disruption of cognitive functioning.

Findings from prior catecholamine depletion studies have been inconsistent. Two separate studies by the same group have found both impaired and unimpaired spatial recognition memory following administration of mixtures identical to BCAA in composition (Gijsman et al., 2002); however, one study reported impaired accuracy while the other reported longer response latency on spatial recognition memory. It should be noted also that the findings from studies using more complex mixtures for phe-tyr depletion have also been inconsistent. Some data indicate significantly more errors on a spatial recognition memory task and poorer spatial working memory strategy scores (Harmer et al., 2001), while other data indicates no effect on memory and attention

measures, but impaired list-learning performance (Grevet et al., 2002). There were no effects on visual recognition memory, rapid visual information processing, or paired associates learning. These studies were limited by small sample sizes and may not have had the statistical power to detect a relatively small effect of phe-tyr depletion on cognition. However, the present study had a large enough sample for adequate power to detect an effect.

A potential weakness of the study may have been the use of multiple cognitive assessments. We tested subjects at baseline, 4 hours and 6 hours post drink ingestion, while most studies test only 5 hours post drink ingestion. The extra testing session may have added a factor of variance to the design and may have increased subject fatigue or affected participant motivation; however, there were no changes in the behavior or side effect measures. The multiple assessment design introduces the potential for carryover effects as well; however, baseline performance on placebo and BCAA days was not affected. There were significant effects of time on some of the measures, indicating a practice or learning effect. Although practice effects were noted for several of the measures, these effects were controlled by the placebo data and the repeated measures crossover design. Another limiting factor in the design was the indirect nature of measuring DA function and the lack of specificity of the catecholamine depletion technique. BCAA administration likely decreased NE and 5-HT precursor availability. Therefore, it is not possible to adequately separate DA from possible NE and 5-HT effects on cognition.

Although not statistically significant, results from the Conflict Task data show a trend for decreased RT variability on BCAA while performing tasks requiring increased

cognitive load. These results are also consistent with CPT-IP data trends for the 4-digits slow and fast conditions in which RT variability also decreased while on BCAA compared to placebo. Role of DA involvement with cognitive load may be of interest for future studies. Further investigation of trends for increasing preparatory effect of DA may be warranted since it lends support to the tonic-phasic DA theory. The current data suggest that acute BCAA administration does not adversely affect cognition; however, chronic BCAA administration, such as for tardive dyskinesia treatment, may result in receptor changes and cognitive effects. It would be interesting to examine the effects of more chronic BCAA administration on cognition.

BCAA administration may have applications in schizophrenia. Two primary DA system disturbances involving cortical and subcortical activity alterations are proposed to account for schizophrenia symptoms: 1) increased activity in the mesolimbic system (involving D_2, D_3, D_4 receptors) accounting for the positive symptoms and 2) decreased prefrontal activity accounting for the negative symptoms of the disorder (Weinberger, 1987). Other theories characterize the DA disturbance as hypo-DA mesocortical pathway functioning and hyper-DA mesolimbic pathway (e.g., Davis, Kahn, Ko & Davidson, 1991). Positive symptoms in schizophrenia are associated with the increased DA mesolimbic activity in the nucleus accumbens and striatum, while negative symptoms may be explained by hypo-DA mesocortical PFC activity.

The complexity of the DA projections and interactions with other neurotransmitters and neuropeptides potentially limits the utility of global manipulations in revealing the involvement of DA in specific cognitive domains. The BCAA administration globally affects catecholamine synthesis; therefore, in addition to DA, NE

and 5-HT synthesis are decreased. By globally decreasing neurotransmitter production, the BCAA method also affects the other neurotransmitter systems that interact with DA, NE, and 5-HT. Additionally, the BCAA approach involves manipulating global DA synthesis which in turn affects PRL levels. The increase in PRL stimulates hypothalamic DA neurons through negative feedback loops and may also possibly exert influence on cognitive processes through interaction with corticosteroid and hormone pathways, though this remains a topic of debate (e.g., Fonda, Bertrand, O'Donnell, Longcope & McKinlay, 2005; Halari, Kumari, Mehrotra, Wheeler, Hines & Sharma, 2004).

Another approach to diminishing overall DA activity would be to use specific autoreceptor-selective agonists (e.g., 3-PPP, EMD 23-448) that diminish DA synthesis, release, and impulse flow (Cooper, Bloom & Roth, 1996). Future research should target separation of NE and DA involvement in cognitive processes, perhaps supplementation of BCAA with a chemical that would prevent or attenuate subsequent NE depletion. The RT variability trends and the effect of cognitive load should be further examined in investigating DA's role in memory, attention, and executive functions possibly through the use of an N-back-like task in which cognitive load can be manipulated. It may also be interesting to investigate the effects of catecholamine in patients with schizophrenia exhibiting positive symptoms as the present results suggest that BCAA depletion may primarily affect striatal DA.

Table 1. Plasma PRL values as a function of time and treatment.

Time	**PRL Placebo (*SE*)**	**PRL BCAA (*SE*)**
10 a.m.	10.88 (0.52)	10.72 (1.11)
11 a.m.	10.68 (0.52)	10.19 (1.10)
12 p.m.	9.64 (0.52)	14.37 (1.10)
1 p.m.	11.18 (0.52)	25.68 (1.10)*†
2 p.m.	11.67 (0.52)	21.62 (1.10)*†
3 p.m.	11.66 (0.53)	15.82 (1.10)†

***** Difference between placebo and BCAA values $p < .0001$.

† Difference between BCAA value and all other BCAA values $p < .0001$.

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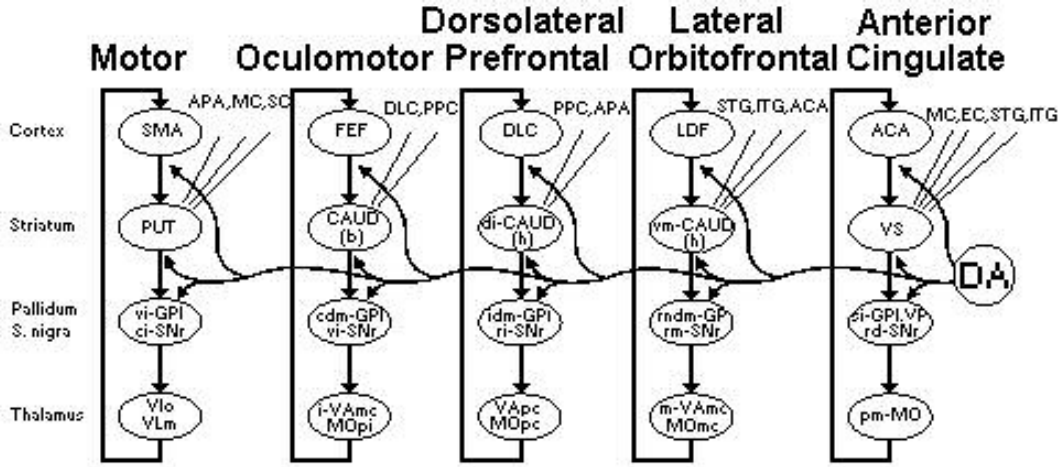
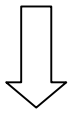


Figure 1. Frontal subcortical dopamine circuits (adapted from

http://www.unifr.ch/biochem/DREYER/%20dopamine.html)

Placebo/BCAA



Time	8:30am	10:00am	11:00am	12:00pm	1:00pm	2:00pm	3:00pm
Data	NP Battery	PRL, behavior ratings, vitals, BMI	PRL, vitals	PRL, vitals	PRL, vitals, NP Battery, behavior ratings	PRL, vitals	PRL, Vitals, NP Battery, behavior ratings

Figure 2. Schedule of procedures for subject participation on both placebo and BCAA days.

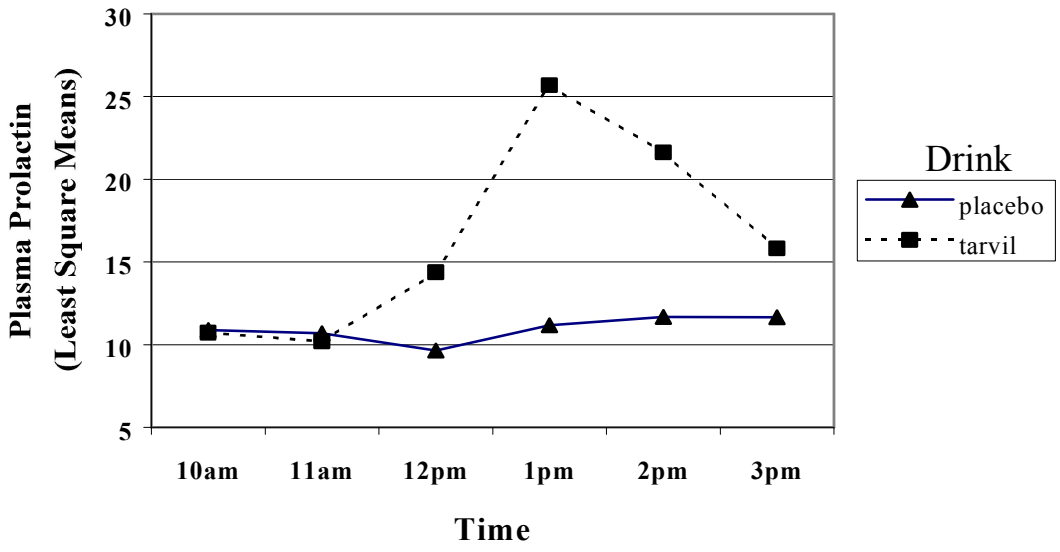


Figure 3. Plasma PRL levels as a function of drink and time. Plasma PRL levels showed a significant Drink x Time interaction, $F(5,196) = 36.29$, $p < .0001$, indicating a sharp increase in PRL, peaking at 3 hours post-BCAA ingestion ($n = 43$).

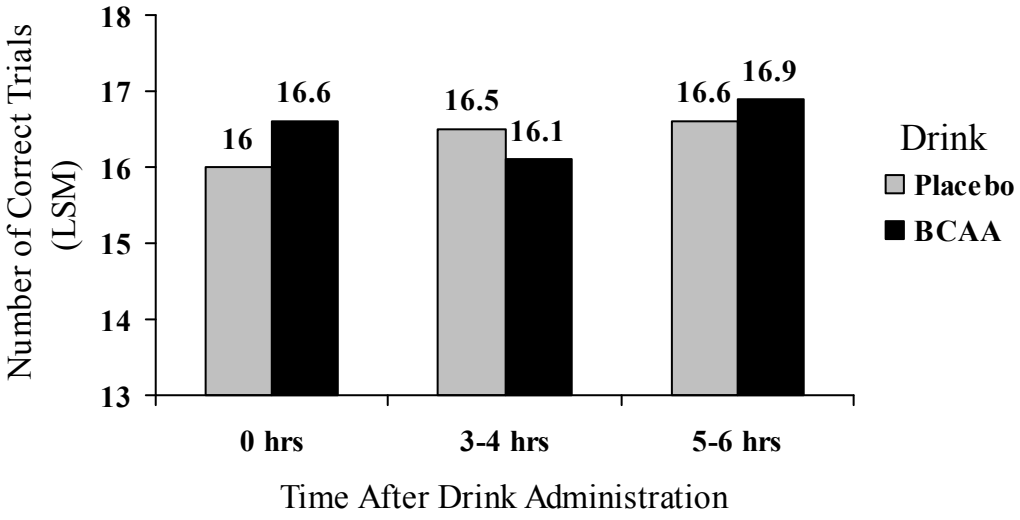


Figure 4. Object Working Memory delay score as a function of Drink and Time. There was no significant Drink x Time interaction, $F(2,75) = 1.24$, $p = .3$, on the number of correct trials ($n = 43$).

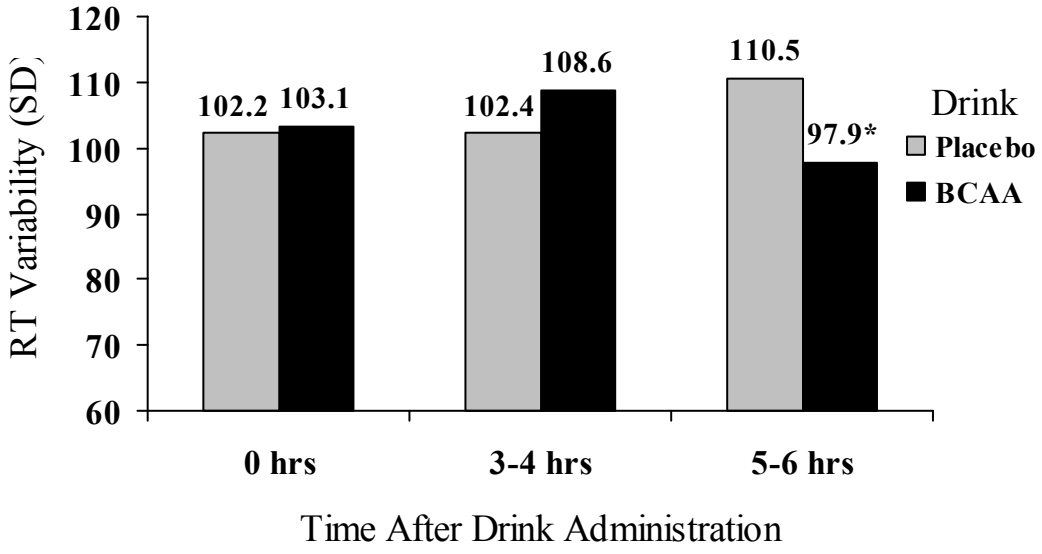


Figure 5. CPT-IP 4-Digits Slow condition RT variability as a function of Drink and Time. There was a significant Drink x Time interaction, Interaction $F(2,73) = 4.81$, $p = .01$, indicating that RT consistency significantly improved (decreased variability) after BCAA compared to placebo during the 5-6 hour assessment, $*t(1,73) = 2.56$, $p = .01$ ($n = 43$).

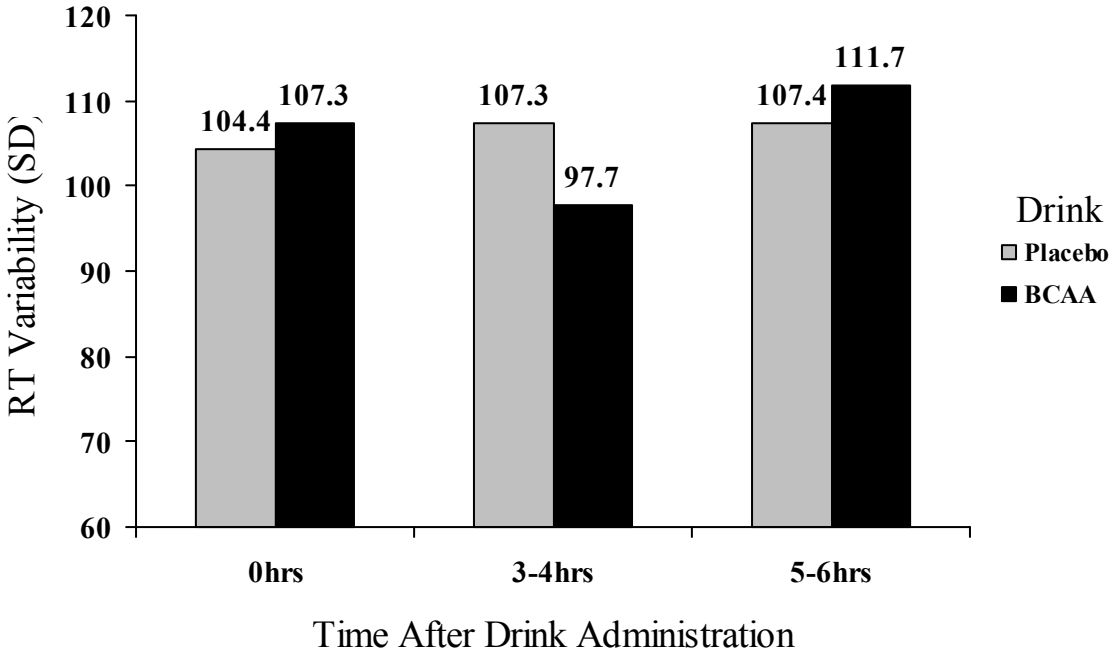


Figure 6. CPT-IP 4-Digits Fast condition RT variability as a function of Drink and Time. There was a trend for improved RT consistency (decreased variability) after BCAA compared to placebo during the 3-4 hour assessment, interaction $F(2,73) = 2.6$, $p = .08$ ($n = 43$).

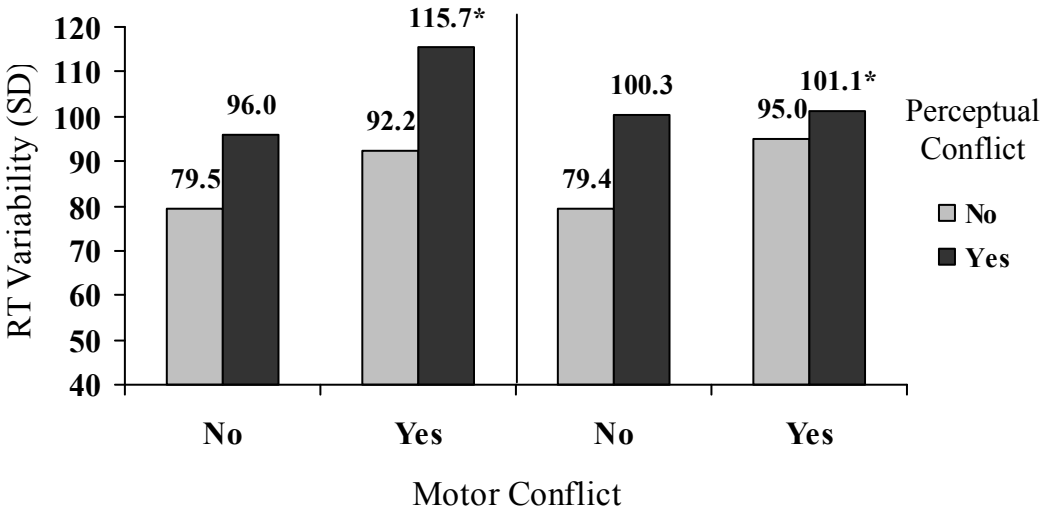


Figure 7. RT variability as a function of Motor and Perceptual Conflict and Drink during the 3-4 hour assessment of inhibitory control. There is a trend, interaction $F(1,37) = 4.436$, $p = .042$, for improved RT consistency (decreased variability) after BCAA compared to placebo for the most difficult condition requiring inhibitory control in the presence of both a perceptual and motor conflict, $*t(1,37) = 2.438$, $p = .020$ ($n = 39$).

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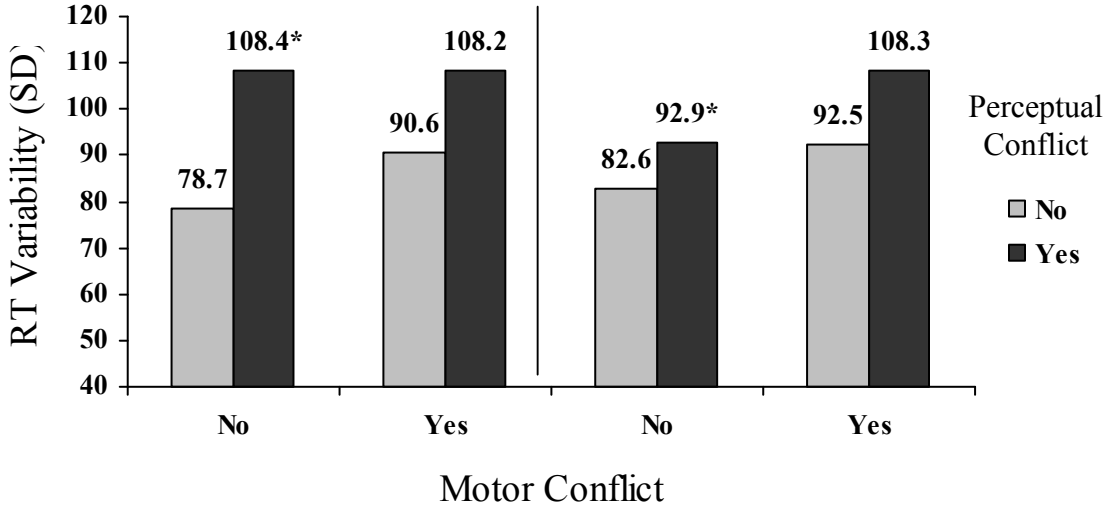

Figure 8. RT variability as a function of Motor and Perceptual Conflict and Drink during the 5-6 hour assessment of inhibitory control. There is a trend, interaction $F(1,37) = 3.502$, $p = .069$, for improved RT consistency (decreased variability) after BCAA compared to placebo for the condition requiring inhibitory control in the presence of a perceptual conflict, $*t(1,37) = 2.240$, $p = .031$ ($n = 39$). This condition required inhibition of the more salient stimulus attribute to correctly respond.

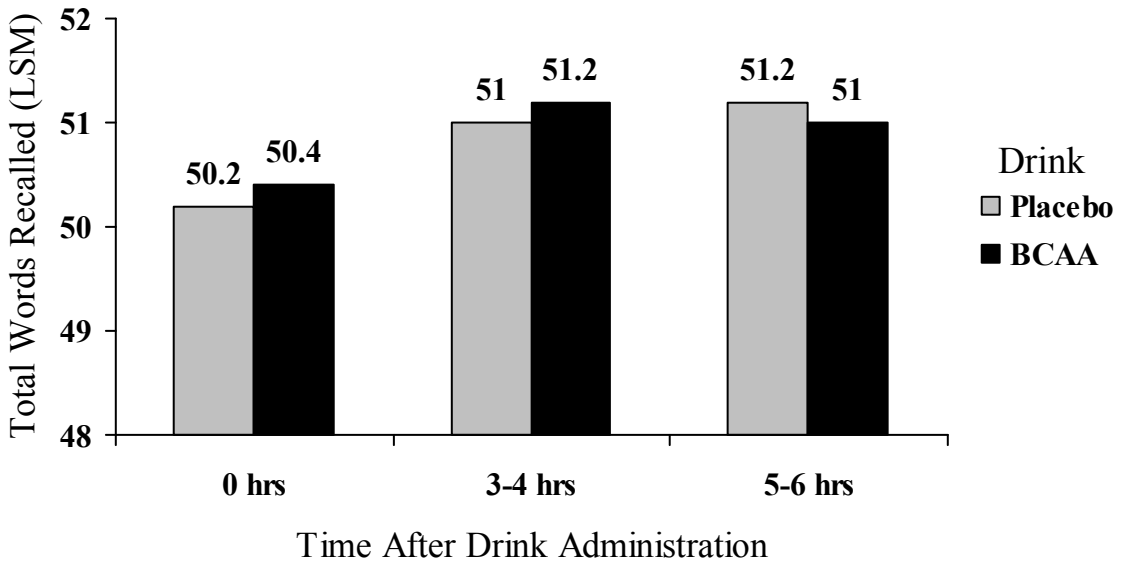

Figure 9. HVLT list learning as a function of Drink and Time. As predicted, there was no significant Drink x Time interaction, $F(2,80) = 1.75$, $p = .2$, on HVLT performance ($n = 43$).

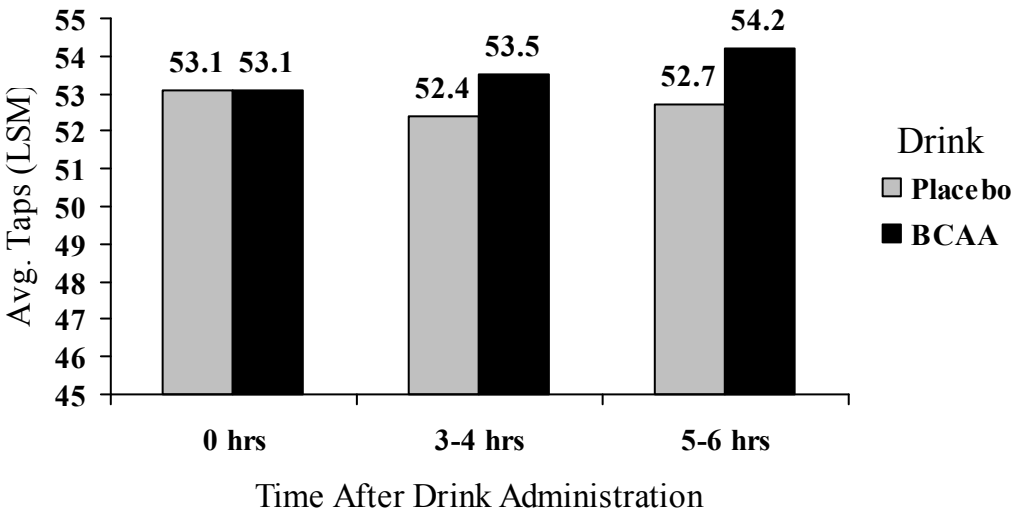

Figure 10. Finger tapping as a function of Drink and Time. Contrary to what was predicted, there was no significant Drink x Time interaction, $F(2,80) = 1.90$, $p = .2$, on Finger tapping performance ($n = 43$).

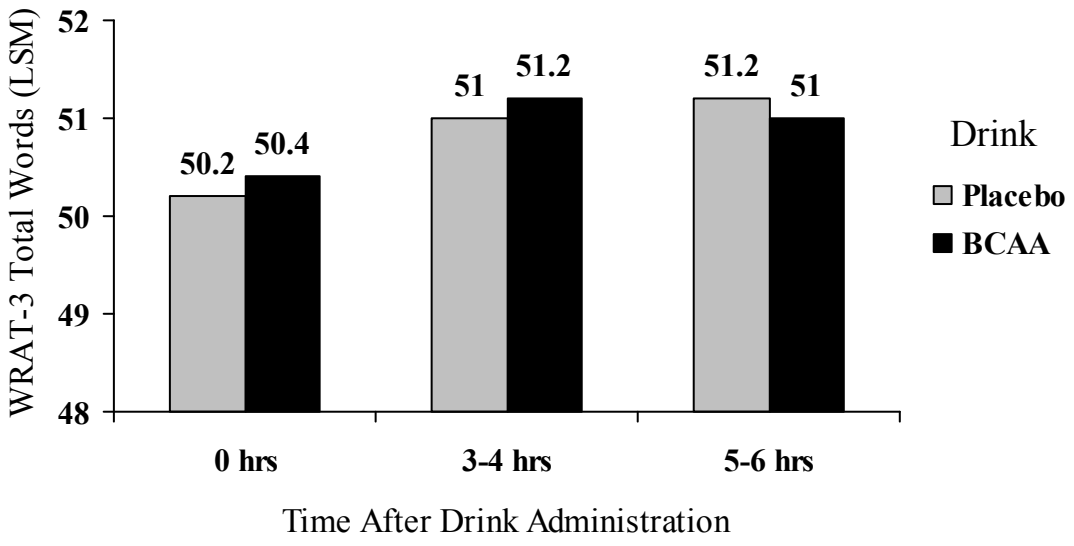

Figure 11. WRAT-3 reading as a function of Drink and Time. As predicted, there was no significant Drink x Time interaction, $F(2,80) = .2$, $p = .8$, on WRAT-3 reading performance ($n = 43$).

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