THE ROLE OF DOPAMINE IN MULTIPLE MEMORY SYSTEMS IN NAVIGATION: AN ACUTE PHENYLALANINE/TYROSINE DEPLETION STUDY

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Abstract

Multiple memory systems in the brain work independently and in parallel to acquire different forms of knowledge. In human navigation, this translates behaviourally to different spontaneous wayfinding strategies in a given environment. Participants who spontaneously use the spatial strategy show greater activity and grey matter in the hippocampus and those that spontaneously use the response strategy show corresponding increases in function and grey matter in the dorsal striatum. Beyond the functional and neural correlates of these strategies, much remains unexplored including translational work in determining the neurochemistry of these memory systems. Dopaminergic systems are believed to affect both memory systems independently but their relative role in the context of strategies is unexplored. Whether dopamine (DA) affects navigation strategies ubiquitously or differentially is a question open to debate. The current study seeks to determine a) whether DA has a general effect in navigation and b) whether DA may be implicated differently between those that spontaneously employ one strategy over the other. We studied the effect of DA precursor depletion on navigational strategies in healthy young adults by means of an established method of experimentally depleting precursors of dopamine, acute phenylalanine/tyrosine depletion (APTD), validated to produce transient decreases in dopamine synthesis and release. Thirty one healthy young adults with no history of psychiatric or neurological disorders took part in a total of three pilot studies- two within subject (Study 1, N = 9 and Study 2, N = 11) and one between-group (Study 3, N = 11) design studies using double-blind, counter-balanced, placebo-controlled procedures. In the within subject studies, participants took part in two testing days one month apart where they ingested an amino acid (AA) mixture that was i) nutritionally balanced (BAL), or ii) devoid of the DA precursors, phenylalanine and tyrosine (APTD). In the between-group study, participants were randomized to either the BAL or APTD AA mixture group. Participants then completed a virtual navigation task (the 4/8 Virtual Maze) that dissociates spatial learners from response learners. There was similar learning under APTD and BAL sessions in both spatial and response learners concordant with literature that dopamine is not involved in learning per se. We report

preliminary findings that striatum-based response strategies may be more susceptible to decreased dopamine neurotransmission as shown by greater reliance of landmarks under APTD in response learners suggesting a possible shift to spatial strategies. These data do not support the hypothesis that global reduction in DA transmission causes a general effect in navigation but rather that specific effects are related to spontaneous strategies.

Résumé

Il existe multiples systèmes de mémoire dans le cerveau qui fonctionnent indépendamment et en parallèle pour acquérir différentes formes de connaissances. Dans le domaine de la navigation humaine, cela se traduit de façon qu'il existe différentes stratégies spontanées pour se déplacer dans l'environnement. Les gens qui utilisent spontanément la stratégie spatiale démontrent plus d'activité cérébrale et de matière grise dans l'hippocampe et ceux qui utilisent spontanément la stratégie d'associations stimuli-réponse démontrent plus de fonction et de matière grise dans le noyau caudé du striatum. Au-delà des corrélats neuronaux et fonctionnels de ces stratégies, il reste encore beaucoup d'inexploré y compris les travaux de translation en ce qui concerne la détermination de la neurochimie de ces systèmes de mémoire. Il est présumé que les systèmes dopaminergiques affectent les systèmes de mémoire de façon indépendante mais leur rôle relatif dans le contexte des stratégies est inconnu. Si la dopamine (DA) affecte les stratégies de navigation de façon générale ou bien différentielle est une question ouverte au débat. L'étude actuelle vise à déterminer a) si la DA a un effet général sur la navigation humaine et b) si les implications de la DA sont différentes entre ceux qui emploient spontanément une stratégie de navigation par rapport à l'autre. Nous avons étudié l'effet de l'épuisement des précurseurs de la DA sur les stratégies de navigation chez les jeunes adultes en bonne santé par moyen d'une méthode établie d'épuisement expérimentale des précurseurs de la DA, « acute phénylalanine / tyrosine depletion » (APTD), validée pour produire une diminution transitoire de synthèse de la DA. Trente et un jeunes adultes en bonne santé sans antécédents de troubles psychiatriques ou neurologiques ont pris part à un total de trois études pilotes - deux mesures répétées (étude 1, N = 9 et l'étude 2, N = 11) et une avec modèle intergroupes (étude 3, N = 11) en utilisant les procédures double aveugle contre placebo et contre-équilibrées. Dans les études à mesures répétées, les participants ont participé à deux jours de test séparés d'un mois d'intervalle où ils ont ingéré un breuvage protéiné d'acide aminé (AA) qui était soit i) nutritionnellement équilibré (BAL), ou ii) dépourvu des précurseurs de la DA, la phénylalanine et la tyrosine (APTD). Dans l'étude intergroupes, les participants ont été randomisés pour recevoir soit le breuvage d'AA BAL soit APTD. Les participants ont ensuite complété une tâche de navigation virtuelle (4/8 Virtual Maze)

qui dissocie les stratégies. L'apprentissage fut ressemblante sous l'effet des breuvages APTD et BAL pour les participants utilisant la stratégie spatiale et les stratégies d'association stimuli-réponse ce qui est en accord avec la littérature suggérant que la DA n'est pas impliqué dans l'apprentissage en tant que tel. Nos résultats préliminaires constatent que les stratégies qui repose sur le striatum sont plus sensibles à la diminution de la neurotransmission de la DA, suggéré par une dépendance plus exagérée sur les points de repères suite au breuvage d'AA APTD chez les apprenants des associations stimuli-réponse ce qui suggèrerait une transition possible aux stratégies spatiales. Ces données ne confirment pas l'hypothèse que la réduction globale de la transmission DA provoque un effet général de la navigation, mais plutôt que les effets spécifiques sont liés aux stratégies spontanées.

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Chapter One: Introduction

Navigation behaviour is vital for survival across species including migrating birds, rodents and humans who need to find their way through space to food, water, a mate, shelter and other target locations in their environments. It is a complex behaviour requiring the integration of multiple cognitive capacities and neural systems. When these systems are compromised, the effects can be severely disabling with patients limiting activities and staying in their homes for fear of getting lost. Navigational impairments (topographical/spatial disorientation) are seen in diverse neuropsychiatric disorders including Parkinson's disease, Alzheimer's disease, and Huntington's disease, following some forms of brain damage (Agid, 1991, Laczo et al., 2009, Aguirre & d'Esposito, 1999), and in the course of normal aging (Iaria, Palermo, Committeri, Barton, 2009). The impairments are thought to result from any of several cognitive deficits including memory. Since there are multiple memory systems, the specific deficit plausibly affects the particulars of the navigational impairment, including which components of the environment are used for orientation. Given this, achieving a greater understanding of navigational behaviour and the brain systems at play is essential to identifying the root of these impairments and potentially providing etiologically relevant targets for treatment or cognitive interventions to improve patients' quality of life.

1. Navigation & Multiple Memory Systems

Successful wayfinding can be achieved through distinct parallel cognitive processes and involves multiple memory systems that acquire different forms of knowledge. This translates to differential representation of space and behaviourally into navigation strategies that are qualitatively different from one another but can be employed to achieve a common goal. Rodent (Packard & McGaugh, 1992; McDonald & White, 1994), primate (Zola-Morgan, Squire & Mishkin, 1982, Mishkin & Petri. 1984) and human (Graf & Schacter, 1985) studies enabled functional dissociation between the hippocampus and the dorsal striatum/caudate nucleus with respect to their contribution to learning and memory. These structures are involved independently and subserve two distinct navigational strategies (Packard et al., 1989; Packard & McGaugh, 1992; McDonald & White, 1993, 1994, 1995; Knowlton et al., 1996). The hippocampus is centrally involved in declarative memory (Cohen & Squire, 1980; Squire & Zola 1996; Scoville & Milner, 1957), allocentric frame of reference representations, spatial learning where the locations of objects are remembered in relation to external cues or landmarks in the environment, and acquiring stimulus-stimulus information. The discovery of place cells within the rat hippocampus that fire preferentially when the animal is in a specific position in its environment (O'Keefe and Dostrovsky, 1971**)** was followed by the suggestion that these were implicated in spatial orientation and that firing patterns of place cells occurred with respect to the cues in the environment signaling the animal's location (O'Keefe & Conway, 1978). This information enables the formation of a cognitive representation of space that is termed a spatial or a cognitive map acquired by means of learning relationships between landmarks in the environment (O'Keefe & Nadel, 1978). Additional key notions pertaining to these cognitive maps include that they

are rapidly acquired during exploration and that they allow flexibility, for example, in allowing the use of detours and shortcuts (O'Keefe & Nadel, 1978). The dorsal striatum on the other hand is involved in stimulus-response learning, which involves making a series of stereotypic stimulus-response associations (e.g., right/left turns) from a given position that acts as a stimulus. Contrary to the spatial strategy, response strategies are characterized by rigidity. The striatum is also involved in habit (Hirsh, 1974; Mishkin & Petri, 1984) and procedural memory (Cohen & Squire, 1980). In young adult populations, approximately 50% of participants spontaneously use a spatial strategy and 50% use a response strategy. Human neuroimaging studies reveal structural and functional differences dependent on the default navigational strategies employed. When young adult participants were tested on a virtual navigation task, those who spontaneously engaged in a spatial strategy were found to have increased functional activity (Iaria et al., 2003) and grey matter (Bohbot et al., 2007) in the hippocampus while those who spontaneously used a response strategy showed analogous increases in function and grey matter in the caudate nucleus (Iaria et al., 2003; Bohbot et al., 2007).

2. Dopamine biochemistry and dopaminergic neurotransmission

The neurochemistry of these distinct processes remains largely unknown, especially in the human literature. Dopamine, however, is a candidate given its established role in motivated behavior and seminal work in the two decades that affected the field of cognitive neuroscience. Some classic experiments by Schultz and colleagues demonstrated that dopamine neurons respond to surprising or unexpected events (Schultz

et al., 1993, 1998, 2002; Fiorillo, Tobler & Schultz, 2003**)**. These dopamine neurons signal not reward per se but a signal widely referred to as a prediction error. This prediction error has implications in learning and memory because it allows us to evaluate how much an expected value deviated from an outcome. The prediction error then allows us to update our expectations based on these outcomes and, more broadly, from experiences in the world (Daw, O'Doherty, Dayan, Seymour & Dolan, 2006). This is not to mean that dopamine has a causal role in learning. The idea that surges in dopamine act as teaching signals in and of themselves is not well supported in the literature. Rather, the role of dopamine has come to be better understood with some experiments that parsed processes of learning about rewards, hedonic "liking" and "wanting" of rewards. These experiments demonstrated that though dopamine was not necessary for liking or learning about rewards, wanting, seeking or incentive motivation for goal-directed behaviour did necessitate dopamine (Berridge & Robinson, 1998; Robinson et al., 2005; Hnasko et al., 2005; Leyton et al. 2005). This suggests that dopamine is not necessary for learning per se but could influence learning and memory by attributing salience to otherwise intrinsically neutral events or stimuli rather than bearing any direct effects on all forms of learning (Flagel et al., 2011). Interest in these signals and their role in learning and memory has since increased and taken several directions. Dopaminergic systems are therefore good contenders to instigate investigation into the neurochemistry of structures involved in learning and memory systems in navigation. This is especially true given that dopamine is an important neuromodulator in both the striatum and the hippocampus.

It wasn't until the later half of the 1950s that dopamine received attention as a neurotransmitter in its own right. Before this, dopamine was merely thought of as a

precursor to epinephrine and norepinephrine and an intermediary in catecholamine synthesis (Cooper, Bloom & Roth, 2003). Arvid Carlsson and colleagues discovered that regional concentrations of dopamine differ significantly from those of norepinephrine in the central nervous system (Cooper, Bloom & Roth, 2003). Shortly thereafter, Parkinson's disease came to be associated with decreased striatal dopamine levels (Ehringer & Hornykiewicz, 1960). From there, implications including the treatment with L-DOPA in Parkinson's disease came about and its role in other disorders like schizophrenia became apparent (Birkmayer & Hornykiewicz, 1961; Heinz & Schlagenhauf, 2010).

2.1 Dopamine synthesis

Dopamine biosynthesis occurs both peripherally and within the central nervous system. In the pathway proposed by Blaschko (1939), this occurs in a two-step process in the cytosol of catecholaminergic neurons. As for all catecholamines, synthesis begins with the amino acid precursor tyrosine. Available phenylalanine obtained from diet can also be enzymatically converted to tyrosine in vivo (Womack & Rose, 1934; Clark & Bier, 1982). In the first step, tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (L-DOPA) by enzyme tyrosine hydroxylase. Then in the second step, L-DOPA is rapidly decarboxylated to dopamine by the enzyme DOPA decarboxylase, also called L-aromatic amino acid decarboxylase because it does not show specificity to the DOPA substrate exclusively (Lovenberg, Weissbach, Udenfriend, 1962). This proposed process was confirmed when tyrosine hydroxylase was directly observed to hydroxylate tyrosine

(Nagatsu, Levitt & Udenfriend, 1964). Evidence for the reaction of the second step has also been reported in vivo (Holtz, Credner & Koepp, 1942). The first step implicating enzyme tyrosine hydroxylase in this process is rate-limiting. In other words, the rate of dopamine synthesis is determined by this step. Synthesis rate can be regulated by inhibitory action on tyrosine hydroxylase via pharmacological manipulation, the availability of the cofactor tetrahydrobiopterin (BH4) and dopamine presynaptic receptors among others.

2.2 Dopamine metabolism

After dopamine is released into the synapse in response to a presynaptic action potential and has acted on postsynaptic dopamine receptors, it can be inactivated and recycled through several mechanisms. Primarily, extracellular dopamine can be actively taken back into the presynaptic neuron via sodium-dependent dopamine transporter (DAT), where it can then be sequestered into synaptic vesicles again (Eriksen, Jørgensen & Gether, 2010). Dopamine can also be broken down enzymatically into its major metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), via enzymes monoamine oxidase (MAO), catechol-*O*-methyltransferase (COMT), and aldehyde dehydrogenase (ALDH). MAO deaminates dopamine leading to an intermediate 3,4-dihydroxyphenylacetaldehyde (DOPAL) which can be further oxidized by ALDH to DOPAC (Eisenhofer, Kopin & Goldstein, 2004). In an alternate pathway, dopamine is first 3-*O*-methylated by COMT leading to the intermediate 3-methoxytyramine which is then deaminated by MAO to form HVA.

2.3 Dopaminergic systems

Dopamine has been implicated in learning and memory consolidation, cognitive processes at the root of navigation, however its role seems to be distributed to encompass multiple mechanisms. The majority of dopamine cells originate from the same embryological source, at the junction of the mesencephalon and the diencephalon and then project to different targets as well as some overlapping ones (Wise, 2009). These cell groups are often studied as anatomically subdivided into a number of dopamine systems including the nigrostriatal and mesocorticolimbic systems, which are two subdivisions particularly well studied. It is important to note however that though these systems are often talked of as segregate in the literature, there is much commonality in both their function and anatomy. As their common origin and overlapping targets would suggest, their functional and anatomical properties also share similarities (Wise, 2009). Here we discuss the differences between these classical divisions but this is not to suggest that they are completely independent. For instance some evidence of their shared properties include that both population of dopaminergic neurons respond to unexpected stimuli (Schultz 1998). The nigrostriatal dopamine system is a population of neurons that originate from the substantia nigra pars compacta (SNc) and innervate primarily the dorsal part of the striatum (the human analogue of the caudate-putamen in the rat). This system is primarily associated with motor functioning and as Urban Ungerstedt (1971) described it, "bilateral, complete denervation of the [rat] nigrostriatal dopamine pathway produces severe, long lasting adipsia and aphagia, hypoactivity, difficulties to initiate activity and loss of exploratory behaviour and curiosity." Further functional dissociation

among the projections to the dorsal striatum have been shown in rodents and in primates to have different roles such that the projections from the SNc to the *dorsomedial* striatum influence goal-directed operant learning while projections from the SNc to the *dorsolateral* striatum contribute to stimulus-response learning (Featherstone & McDonald, 2004; O'Doherty et al. 2004; Nakamura & Hikosaka, 2006). The mesocorticolimbic populations of dopamine neurons originate from the ventral tegmental area (VTA) and project primarily to the ventral part of the striatum (the nucleus accumbens and the olfactory tubercle) and these play an important role in reward, conditioned reinforcement/building Pavlovian associations and in motivation (Wolterink et al., 1993; McFarland & Ettenberg, 1995). There are also projections from the VTA to other limbic structures as well including the septum, the amygdala and the hippocampus and cortical regions including medial prefrontal, cingulate and perirhinal cortex (Wise, 2004).

The contributions of these neural mechanisms in navigation have yet to be unraveled. Specifically, whether dopamine depletion would cause impairments in navigation behaviour due to changes in neural processing of local circuits and/or that navigation behaviour in general would be impacted due to distributed brain regions that act in concert with local circuits (*i.e.* "distributed network effect") remains largely unknown. In the case of the former, determining the relative, or more broadly, possible differential contributions of individual neural systems would need to be determined.

3. Support for the role of Dopamine in the striatum

The striatum juncture is vastly composed of medium spiny neurons (MSN; 90%) and given their unique architecture they receive dense cortical as well as dopaminergic input. Dopamine neurons interface the base of the dendritic spines of these MSNs (Parent and Hazrati, 1993). This is a strategic position that permits modulation of signaling and plasticity of synaptic connections and, given that the striatum receives both sensory and motor input from the cortex, these together with other reward signals can guide behaviour (i.e. through the association of stimuli to responses). Circuits responsible for motivation and cognition affecting decision-making include the striato-midbrain-striatal connection. This connects the ventral striatum/nucleus accumbens to the dorsal striatum (motor output) through reciprocal ascending spirals to midbrain dopamine neurons. These spiral interconnections allow information to be transmitted from the limbic to the cognitive to the motor parts of the brain and underlie the development of habits or a shift from action outcome to stimulus-response behaviour **(**Haber et al., 2000**)**. Effect on the striatum itself occurs via the integrated cortico-striatal projections. Three key players have been identified including the ventromedial prefrontal cortex (vmPFC), the orbitofrontal cortex (OFC) and the dorsal anterior cingulate cortex (dACC). These have different functions including encoding of value (Hare, Camerer & Rangel, 2009), coding of the relative value of the stimulus (Eliott, Agnew & Deakin, 2008), monitoring including processing errors or conflict between actions and expected outcome for behavioural adjustment like shifting attention (Egner, 2011; Hayden et al., 2011), respectively. These interact with the striatum where their terminals interface forming a network between the cognitive and motor circuits necessary for planning behavior based on motivation and forming habits **(**Haber et al., 2006**).** The regulation of striatal input occurs by tonic and phasic changes in

dopamine (Schultz et al., 2007) and changes in tonic dopamine within the striatum can alter the exploration-exploitation trade-off, in favour of behaviour whose outcome is either certain or one that is uncertain but with the possibility of being more favourable (Humphries et al., 2012). These reward-related signals have been reported in the dorsal striatum (Cooper et al., 2011; Apicella et al., 2011**)** and are not exclusive to the ventral striatum. Dopamine has been proposed to have many roles. This also includes its role in reinforcement of the association between an otherwise neutral stimulus and either a response or another stimulus following a reward is well known and influential. Another such hypothesis is one of dopamine's role in reward where midbrain dopamine neurons have been found to transmit important reward-related information regarding external stimuli through phasic bursts of activity (ex. stimulus-specific responses that predict future rewards and motivation) (Cohen, Braver & Brown, 2002; Wise, 2004). Given these varied roles, how might dopamine influence caudate-dependent learning in navigation? There have been several proposed hypotheses about what the role of dopamine is including one of a direct contributor to learning and in hedonic processes. As mentioned before, hypotheses of dopamine directly relating to learning and hedonic effects are not well supported. Of the more consistently plausible suggestion has been the role that dopamine plays in incentive salience and actively approaching stimuli that come to have value. In this context compelling work comes from a number of studies where dopamine is selectively involved in incentive motivation for goal-directed behaviour rather than learning an association, rewarding or not (Berridge 2012; Berridge & Robinson, 2007; Leyton et al., 2005; Liggins, Pihl, Benfelfat & Leyton, 2012; Flagel et al., 2011). What is more, dopamine-dependent long term potentiation (LTP) and long term depression (LTD)

also occur in the dorsal striatum/caudate nucleus, in that we see an effect on dopamine receptor activation from these learning mechanisms. Both dopamine receptor types of dopamine, D1 and D2, mediate these effects but in seemingly different ways. While D1 receptor activation enhances NMDA-mediated flow of ions to cause excitatory post-synaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs), D2 receptor activation does the contrary (Cohen, Braver & Brown, 2002). Given this distinction in addition to the differences in time course over which these receptors take effect, it has been postulated that it may help to understand both the maintenance or tonic and learning or phasic functions of dopamine that would guide behavior (Cohen, Braver & Brown, 2002).

4. Support for the role of dopamine in the hippocampus

Dopamine also plays a role in the hippocampus. The hippocampus is one of the only regions in the brain with all five subtypes of dopamine receptors (Cooper, Bloom & Roth, 1996). The hippocampus receives dopaminergic projections from both the ventral tegmental area (VTA) and substantia nigra pars compacta (Scanton et al, 1980; Gasbarri et al, 1994). These afferents can account for the modulatory influence of dopamine in hippocampal memory, which has been suggested in both rodents (Gasbarri et al., 1997) and humans (Wittman et al., 2005) through mechanisms that either enhance consolidation at the cellular level at the time of learning (Dudai & Morris, 2005) or increase persistence or strength of memory traces (O'Carroll et al., 2006). A role for central dopamine systems in memory consolidation processes subserved by the hippocampus has indeed been shown. In rodent studies, hippocampal dopamine receptors are involved such that

post-training intra-hippocampal injection of dopamine D1 and D2 receptor agonists have been shown to enhance retention and to improve working memory performance in radial-arm maze tasks (Packard and White, 1991, Wilkerson & Levine, 1999). Conversely, rats with 6-hydroxydopamine injections to the subiculum and CA1 of the hippocampus, forming lesions to the mesohippocampal dopaminergic connections, exhibited poorer performance and spatial working memory deficits in the spatial version of the Morris water maze but not on the cued version of this task (Gasbarri et al., 1996) suggesting the crucial role of these connections in place navigation. D1 receptor knockout mice also show deficits in spatial memory **(**El-Ghundi et al., 1999**)** and blockade of D1/D5 receptors specifically in the hippocampus with an antagonist impaired long-term spatial memory (O'Carroll et al., 2006). Long-term memory is also enhanced following novelty exposure or environmental changes (mismatch detection between prior expectation and current sensory input), which has been associated with greater hippocampal fMRI activity (Tulving et al., 1994; Kumaran & Maguire, 2006). In turn, novelty then has a facilitating effect in inducing LTP, which can favour the storage of recently encountered environmental context (Li et al., 2003). Novelty exposure has also been shown to selectively result in greater functional activation of midbrain dopamine structures (Schott et al., 2004). This could suggest that following novelty exposure, information from the hippocampus via subiculum, nucleus accumbens, and ventral pallidum is transmitted to the VTA and in turn dopaminergic neurons in the VTA can then signal release in areas including other limbic structures and cerebral cortex (Lisman and Grace 2005). This shows that interactions or links between dopamine regions and the hippocampus are implicated in producing memory representations suited for informing future choices for

behaviour that could be adaptive (Shohamy & Adcock, 2010). In the hippocampal pyramidal cells, both LTP and LTD learning mechanisms are seen at excitatory synapses. Dopamine here has the role of reinforcing or strengthening synaptic connections in the hippocampus: a) Dopamine D1 receptor agonists facilitate hippocampal LTP (Frey, Schroeder & Matthies, 1990; Frey et al., 1991; Li et al., 2003; Swanson-Park et al., 1999) while antagonists of the same receptor hinder it (Othmakhova & Lisman, 1998) and b) D1 agonists and D2 antagonists promote LTD while both D1 antagonists and D2 agonists impede it (Chen et al., 1996).

We have reviewed that both the striatum and the hippocampus are innervated by dopamine. Given the role of dopamine in both brain systems implicated in navigation, the question arises whether dopamine might influence the hippocampal and caudate related navigation strategies. For instance, we reviewed that dopamine-dependent plasticity occurs in both the striatum and hippocampus. LTP and LTD occurs in the dorsal striatum, the neural correlate of stimulus-response learning as well as the hippocampus, the neural correlate of spatial learning along with an array of other functions within both structures that should be important in the navigation strategies discussed. Behaviourally, in a study comparing the effect of post-trial injections of *d*-amphetamine into the hippocampus and the caudate nucleus of rats in both a spatial water maze task and a cued water maze task, a clear dissociation in retention was observed. While intra-hippocampal injections enhanced memory retention in the spatial task, intra-caudate injections of *d*-amphetamine did not and, similarly, intra-caudate injections selectively enhanced memory retention in the cued task (Packard, Cahill & McGaugh, 1994). Some dopamine depletion studies in rodents have shown deficits in both allocentric and egocentric navigation (Braun et al.,

2012). What remains unexplored though is the relative contribution of dopamine in both brain systems, which could help explain the root of cognitive impairments like spatial disorientation observed in pathologies characterized by degeneration of the dopaminergic system such as Parkinson's disease. There is reason to believe that although dopamine has an effect on the functioning of both systems, the role of dopamine may not be equally significant. For instance, there is sparseness in direct dopaminergic innervation to the hippocampus in comparison to the striatum in rodents (Scatton et al., 1980) and in humans (Little, Carroll & Cassin, 1995) though in primates dopamine innervation to the hippocampus is more dense (Lewis et al., 2001). In humans, additionally, response learners have been shown to have a higher lifetime use of drugs of abuse, which suggest that they may be more responsive to reward (Bohbot et al., 2013). Another trait, novelty seeking in humans has also been linked to midbrain dopamine receptor availability (Zald et al., 2008) and this could be related to spatial strategies. The preliminary evidence for this is twofold. First, novelty exposure facilitates dopamine-dependent learning processes (Li et al., 2003) and second, an imaging study with polymorphisms to the DAT gene suggests implication of midbrain dopamine in hippocampal memory (Schott et al., 2004).

What remains to be understood however is whether dopaminergic input to both systems act similarly or differently. There could be several positions on the potential effect that dopamine manipulations might have on navigation. It is critical to investigate how the functions of dopamine translate to navigational behaviour due to implications in better understanding the root of impairment in navigational ability. Specifically, in the current dissertation we first ask whether dopamine influences human navigational processing in general via a distributed network. If so, we secondly ask whether this would

translate to behavioural changes such that one navigation strategy may be affected differently than the other. If the latter were true then a secondary question would be to determine which of the two memory systems is primarily affected. These questions would provide more direct insight to disambiguate the role of dopamine in distinct memory systems that are otherwise both implicated in plasticity. It would also help answer whether we should rather think of these memory systems in a less desegregate way under certain circumstances. We predict that if dopamine is involved in response and spatial navigational processing, then decreasing dopamine synthesis using the acute phenylalanine/tyrosine depletion method will affect these behaviors in a virtual maze task. Specific effects may depend on the default navigational strategy that participants use.

5. ***Hypotheses***

A) If dopamine affects both human navigational processing strategies, then a decrease in dopamine synthesis should affect performance on a virtual navigational task for all learners irrespective of spontaneous strategy employed. In other words, we would expect a strategy non-specific disruption navigation behaviour.

B) If dopamine affects only one of the navigation strategies, then precursor depletion should differentially affect performance on a navigational tasks depending on the default strategies employed during learning.

6. Acute Phenylalanine/Tyrosine Depletion Method

Acute phenylalanine/tyrosine depletion (APTD) is a method that uses a dietary manipulation to reliably, safely and rapidly produce a transient decrease in dopamine

synthesis and dopamine transmission in the brain. This method is based on the acute tryptophan depletion method (Young et al., 1985), wherein a selective deficiency is induced of the precursor tryptophan required to produce serotonin in the brain. The APTD method was later modified by Leyton et al. 1999 to experimentally deplete the catecholamine dopamine's amino acid precursors, phenylalanine and tyrosine. This method has been validated in human PET studies (Leyton et al. 2004; Montgomery, McTavish, Cowen & Grasby 2003) and rodent microdialysis studies (McTavish et al 1999; Le Masurier et al 2013) to reduce dopamine release.

The McGill version of the APTD method involves ingesting a mixture of 14 essential amino acids that is deficient in phenylalanine and tyrosine. This induces peripheral protein synthesis in the liver, thereby reducing availability of tyrosine and phenylalanine in plasma stores that become incorporated into the formation of new proteins. A peripheral reduction will in turn impact the availability of tyrosine and phenylalanine centrally due to increased competition with other large neutral amino acids for transport across the blood brain barrier (Pardridge, 1977). Reduced availability of tyrosine in the brain results in decreased dopamine synthesis since the rate-limiting enzyme in this process, tyrosine hydroxylase, is otherwise incompletely saturated (75%) with its substrate (Carlsson & Lindqvist, 1978).

6.1 Dopamine Specificity of the APTD method

Tyrosine and phenylalanine depletion has consistently been shown to reduce dopamine levels without global reduction of all catecholamines including noradrenaline (McTavish et al., 1999; Sheehan et al., 1996). In a laboratory animal study using

microdialysis, administration of the tyrosine-free drink diminished amphetamine-induced dopamine release in a dose-dependent fashion. In contrast, in this study, amphetamine- or idaxozan-induced noradrenaline release was not affected following the tyrosine-free drink (McTavish et al., 1999). Neuroendocrine measures of hormones in plasma from human studies provide another form of evidence to support APTD's specificity for dopamine and are consistent with animal studies. Since dopamine has an inhibitory effect on the release of prolactin from the hypothalamus, elevated prolactin levels are indicative of diminished dopamine neurotransmission (Checkley, 1980). In multiple studies, plasma prolactin levels were elevated following administration of the tyrosine-free drink compared to the balanced mixture (Harmer et al., 2001; McTavish et al., 2001). In contrast, failure to observe changes in melatonin released from the pineal gland, an endocrine index of noradrenergic transmission, indicated that noradrenergic activity was not diminished following the tyrosine-free drink relative to the balanced drink in another study (Sheehan et al., 1996). Thus for reasons not entirely understood, tyrosine and phenylalanine depletion has preferential effects on dopamine *vs*. noradrenergic neurons. These findings suggest that the APTD method can be reliably used to attenuate global dopamine function without affecting other catecholamines.

In the work described in the next chapter, we employed the APTD method to transiently reduce the release of dopamine in a series of pilot studies to investigate how dopamine influences hippocampal dependent spatial and striatum-dependent response navigation strategies.

Chapter Two: Methodology

1. Participants

Thirty-one healthy young adults (aged 18-35) were tested in two within subject (Study 1, N=9 and Study 2, N=11) and one between-group (Study 3, N=11) design studies using double-blind, placebo-controlled procedures. Participants were screened to ensure an absence of past or present neurological or psychiatric disorders as assessed with a four-part screening procedure. Women on hormonal contraceptive medication, or who were pregnant or breastfeeding, were not eligible. A complete list of inclusion and exclusion criteria can be found in **Appendix A.** Ethics approval was obtained through the McGill faculty of medicine Institutional Review Board. Participants were recruited via advertisements placed online and in the community. Upon initial visit, before the start of the study, all volunteers were given information about the experiment and were encouraged to ask questions before they signed the study's consent form.

2. Design and description of methodology/Experimental Procedure

2.1 Research Volunteer Screening

Participants were pre-screened using a phone questionnaire. Volunteers who tentatively met the entry criteria were invited to a more detailed face-to-face interview with the Structured Clinical Interview for DSM-IV Disorders, Non-Patient edition (SCID-NP; First, Spitzer, Gibbon & Williams, 2002) to ensure absence of any past or present Axis-I disorder. Information on family and personal histories were also collected

at this time. Following this interview, eligible participants were scheduled for a brief medical exam conducted by a physician. The medical exam enabled a licensed medical doctor to ensure that only subjects with no past or current contraindicating medical illness participated in the study through an assessment of electrocardiogram, blood and urine analyses, and a standard physical exam. A summary report of the SCID-NP and personal and family history was prepared. This was compiled with all medical reports and reviewed by a project senior investigator (ML) for approval of candidates. Upon approval, participants were scheduled for the initial testing session. For participants in Studies 1 and 2, the two test days were approximately one month apart.

2.2 Testing Sessions

The study included a three-hour initial testing session where participants completed a battery of neurocognitive tests and questionnaires, and then one or two full days of dopamine manipulation testing sessions conducted approximately one month apart.

2.2.1 Session 1

All testing was performed at the Ludmer Research & Training Building at the McGill University Downtown campus. The first session lasted approximately three hours, and all participants completed baseline neurocognitive and personality questionnaires.

2.2.1.1 Tests of General Cognition

The neurocognitive tests and personality questionnaires were randomized for order (See **Appendix B**) and included:

1. Wechsler Memory Scale-Revised (Wechsler, 1984). This scale measures general memory and attention functions using both auditory and visual stimuli. The Story Recall/Logical Memory I & II test and Digit Span subscales from the Wechsler Memory Scale-Revised were administered. In the story recall task, participants were read aloud two stories (A and B) and asked to verbally recall as many details from the passages immediately after the story was read aloud and again after a time delay of 30 minutes. Recall score consisted of the number of items recalled from the passages and a thematic score was also calculated for thematic items remembered. Story A was read aloud once while Story B was read aloud two times. A 15-question yes/no recognition memory component for each story was also administered after the time delay. In the digit span test, participants were read sequences of digits and were asked to repeat these sequences in the same order that they were presented. Following correct recall, progressively longer sequences were presented and a forward digit span was determined. The same procedure was then repeated for the backward digit span test but this time participants were instructed to recall digit strings in the reverse order.
2. Test of Non-Verbal Intelligence-3 (TONI-III; Brown, Sherbenou & Johnsen, 1997). This is a language-free assessment of cognitive function. It enables both native and non-native English speakers to be tested, and avoids linguistic confounds that could be encountered with other verbal tests of intelligence.
3. Rey-Osterrieth Complex Figure (Osterrieth, 1944). This is an assessment of visuospatial processing, memory, and executive function. Participants reproduced

a complex figure and then drew it from memory immediately after copying it and once again after a 30-minute delay.

4. Rey Auditory Verbal Learning Test (Schmidt, 1996). This is an assessment of immediate memory, efficiency of learning, effects of interference, and recall following a short and long delay period. Participants were read out a list of 15 words (List A) at a rate of one word per second in five learning trials. Each time, score (number of words recalled) and total time for recall were recorded. A second novel list of words (interference List B) was then read out and scored. Participants were asked to recall as many words from List A immediately following presentation of List B and then again following a 30-minute delay. Lastly, participants completed a recognition task where they needed to correctly identify the source of words presented as either belonging to List A, B or neither.
5. Trail Making Test (Reitan & Wolfson, 1985). This is an assessment of processing speed, sequencing, mental flexibility and visual–motor skills. The task consisted of two parts. In part A, participants were asked to connect 25 number targets in sequence as fast as they could without making any mistakes. In Part B, they were asked to connect another set of 25 targets but this time alternating numbers with letters of the alphabet (*ex*. 1-A-2-B-3-C etc.). Both accuracy and speed were assessed.

2.2.2 Personality questionnaires

1. BIS-11-- Barratt Impulsiveness Scale (Patton & Stanford, 1995): A self-report questionnaire that assesses the personality or behavioural construct of impulsiveness. It consists of 30 questions that are factor-structured (six first-order

factors including attention, motor, self-control, cognitive complexity, perseverance, and cognitive instability impulsiveness and three second-order factors including attentional, motor, and non-planning impulsiveness).

2. SPSRQ (Torrubia, Avila, Molto & Caseras, 2001)-- The Sensitivity to Punishment and Sensitivity to Reward Questionnaire: A yes-no questionnaire that assesses two scales: sensitivity to punishment and sensitivity to reward.
3. NEO-PI (Costa & MacCrae, 1992)-- NEO Personality Inventory: A self-administered assessment of five major domains of personality that include emotional, interpersonal, experiential, attitudinal and motivational styles. It assesses a person's extraversion, agreeableness, conscientiousness, neuroticism, and openness to experience.
4. SURPS (Woicik, Stewart, Pihl, & Conrod, 2009)-- The Substance Use Risk Profile Scale: A Self-administered questionnaire that assesses four factors- hopelessness, anxiety sensitivity, impulsivity, and sensation seeking.
5. TPQ (Cloninger, Pryzbeck, Svrakic, 1991)-- Tridimensional Personality Questionnaire: This is a true-false questionnaire that assesses three domains of personality- Novelty Seeking, harm avoidance, and reward dependence. Each personality domain has four subscales.

2.2.3 Other Questionnaires/Tests

1. Rosenberg Self Esteem Scale (Rosenberg, 1965): Ten statements, self-reported on a four-point scale to assess self-esteem.
2. Beck Depression Inventory –II (BDI-II) (Beck, Steer, Brown, 1996): Self-report inventory, Used for assessing the severity of depression.

3. History of Drug Use Chart: Consists of a table that participants fill out about their history of drug use, including age of first use, number of lifetime uses, and number of uses in the past 30 days, using a time-line follow-back procedure.
4. Test for Creative Thinking- Drawing production (Jellen & Urban, 1985): The Test for Creative Thinking - Drawing Production (TCT-DP)- Test designed to assess a holistic concept of creativity.

2.2.4 Sessions 2 & 3

2.2.4.1 Prior to Full Day Test Sessions

The day before each testing session, all participants were provided with a low protein diet that included low protein snacks, fruits, vegetables, and a pre-prepared frozen meal, and participants were instructed to eat only these foods. Participants were also given a suggested meal-by-meal plan for the foods provided. As of midnight preceding the full testing day, participants fasted and arrived at the laboratory at 8:30am having abstained from smoking or eating breakfast. This together with the low protein diet is thought to facilitate the action of the amino acid drinks since a more modest tyrosine depletion was achieved in an APTD study where participants had not fasted (Sheehan, Tharyan, McTavish, Camping & Cohen. 1996).

The morning of full testing days, participants underwent a urine toxicology screen to determine the presence of cocaine, opiates (e.g., heroin, morphine, codeine), phencyclidine (PCP, angel dust), barbiturates (downers), cannabis (hash, pot), benzodiazepines (e.g., valium, ativan), and amphetamines (e.g., uppers, speed) using the "Triage Drugs of Abuse Panel" (Biosite Diagnostics Inc.). If presence of these drugs of

abuse was detected, the full test day was rescheduled to a later day depending on the time of clearing of the particular drug in question from their system. A urine pregnancy test was also conducted for female participants on each morning of testing to ensure that pregnant women did not take part in the study. A blood sample was also collected (10 ml or 2 teaspoons) for basal amino acid levels.

2.2.4.2 Preparation of Amino Acid Mixture

The mixture was prepared immediately prior to oral administration (See **Appendix C** for the amino acid content of each drink). The following ingredients were mixed together: 1. Amino acid powder and capsules, 2. 135 mL water and 3. 45 mL chocolate syrup. If participants disliked the taste of chocolate, the following alternative ingredients were used instead: 1. Amino acid powder and capsules and 2. 180 mL orange juice from concentrate. With the exception that on the experimental day, where the mixture lacked L-phenylalanine and L-tyrosine, the amino acid powder composition in both AA Mixtures were otherwise identical. Both the participant and the experimenter were blind with regards to which drink was administered. The mixture was adapted for the average lower body weight of women such that they ingested a variation consisting of 83.3% of the mixture that men consumed (Leyton et al., 2000).

2.2.4.3 Administration of Amino Acid Mixture

Participants in Studies 1 and 2 completed two testing days and served as their own control. On one day they ingested a nutritionally balanced amino acid mixture (BAL – control) while on the other they ingested an amino acid mixture devoid of the catecholamine precursors of dopamine, phenylalanine and tyrosine (APTD –

experimental). The drink that participants received and the order in which they were given either the control or experimental amino acid mixture (for Studies 1 and 2) were randomly assigned. Some amino acids (l-arginine, l-cysteine, and l-methionine) were encapsulated in gelatin capsules and administered separately from the amino acid mixture due to their unpleasant taste and smell. Participants in Study 3 had one amino acid mixture test session only, and were randomly assigned to the APTD or BAL AA mixture group.

2.2.4.4 Post-Ingestion

Following ingestion of the amino acid mixture and capsules, subjects remained awake for 4.5 hours in a relaxed environment where they could read or watch videos that were approved for relatively neutral affective content. At four and a half hours post-ingestion, participants completed subjective state scales and then were tested on the 4-on-8 Virtual Maze (4/8VM) task to assess spontaneous navigation strategies. For participants in the within subjects design experiments (studies 1 & 2), different versions of the 4/8VM were given on the two test days, balanced for order.

Following completion of the 4/8VM, participants completed a word frequency mirror effect task and then a second blood sample (10 ml or 2 teaspoons) was collected. The latter served the purpose of comparing tyrosine levels to basal tyrosine levels from the morning blood draw. Participants then completed the POMS one last time at the end of the testing session.

3. Four on Eight Virtual Maze Task (4/8VM)

All participants were tested on the Four on Eight Virtual Maze Task (4/8VM) a computerized task to assess spontaneous navigation strategies (Bohbot et al., 2004; Bohbot et al., 2007). The 4/8 VM is a virtual navigation task created using commercially available Unreal Tournament 2003 game editor (Epic Games, Raleigh, NC). It dissociates hippocampus-dependent spatial strategies and caudate nucleus-dependent response strategies. In this task, participants navigate through a virtual eight arm radial maze starting from a central platform while the surrounding environment contains various landmarks. The task consists of two parts: the learning phase and the probe phase. The learning phase is further divided into two parts. In Part 1, participants are asked to retrieve hidden objects (not visible from the central platform) located at the end of the four accessible pathways. In this part, participants are also instructed to remember which pathways they visited to retrieve the objects. Then, in Part 2 all arms become accessible and participants are asked to avoid the previously visited pathways to retrieve the objects, now located in the arms that were previously blocked. Three learning trials were administered to all participants. Participants had to reach criterion (*i.e.,* performance on part 2 of at least one trial of the learning phase without errors) before they moved on to the probe trial. If they did not reach criterion within the first 3 trials, then extra trials were administered until they did. In the probe trial, a wall is erected around the radial maze thereby obstructing participants' view of environmental landmarks. This trial enables dissociation of spatial learners, who used landmarks to solve the task, from response learners. To remember where the objects were located, participants could: i) use two or more landmarks in the environment and learn the spatial relationships between these landmarks (spatial strategy) or ii) use a single starting position to remember a series of

right and left turns, remember a counting or numbering pattern or have associated other labels to the pathways in their sequence (nonspatial strategy). In the latter case, this single starting position could either be a landmark (response landmark strategy) or the participant's own position at the start of the task (response start position strategy). About 40% of users have also been shown to shift from a spatial strategy to a response strategy with practice over trials (Iaria et al., 2003). Spatial learners are expected to make more probe errors, which is an indication of a higher reliance on landmarks as compared to response learners who do not rely on the landmarks in the same way. Strategy employed by each participant is determined by errors in the probe trial and also by means of verbal reports recorded and transcribed verbatim. Following the probe trial, an additional trial (trial 5) identical to a trial from the learning phase is administered. This additional trial is used to determine whether participants switch strategies after the probe trial where landmarks were removed. Studies 1 and 3 used the original version of the 4/8VM (**Appendix D**) whereas Study 2 used a more challenging version of the 4/8VM (**Appendix E**). In the original version, participants completed the learning phase in three identical environments, whereas in the more difficult version used in Study 2, participants completed the learning phase in 3 novel environments on each test day.

4. Neuropsychological Test of Memory sensitive to the HPC. Following the completion of the 4/8VM, participants then completed a dual-process verbal declarative memory test, the *word frequency mirror effect task*, a variant of the remember/know task (Tulving, 1985). This task was programmed with the E Prime software using the same word stimuli used in Davidson et al. (2006) who selected them from the MRC Psycholinguistic database. They selected words for frequency classification (either high or low, using

mean occurrence per million) and matched words between categories for length and concreteness. In the learning phase of this task, participants were asked to read aloud and memorize 48 words, half of which were high frequency words (mean occurrence: 204.38/million), while the other half were low frequency words (mean occurrence: 1.23/million). These were presented sequentially in the center of a computer screen over a white background for 2500ms each with an inter-stimulus interval of 2000ms. After a filled delay of 10 minutes, participants completed the testing phase. Here, they were presented with 96 words, half from the old list they had studied and the other half being new words (distractors). Participants were asked to classify each of the words presented as "Remember" (indicating that they consciously recalled the word), "Know" (indicating they did not recall specific contextual details for the word but believed it was seen), or "New" (believed it was not a word they had studied before). This task can be traced to Reder et al. (2000) who describe a model for memory for low and high frequency items based on the fact that when an item is encountered at study, two kinds of information are coded, and recognition memory judgments can rely on: a) an assessment of the *familiarity* of stimulus or b) *recollection* of situation-specific details from the study episode (Mandler 1981; Gardiner 1988; Jacoby 1991). In a memory test, then, correct recognition judgments are greater for low-frequency items relative to false alarm rates since less common words have low level of baseline familiarity, and as a result, the item-specific information from the most recent presentation would stand out, leading to recall. In contrast, false alarm rates are higher for high-frequency items, which have a higher level of baseline familiarity since these words are more repeatedly seen over the course of a lifespan (Gregg, 1976, Glanzer & Adams, 1985).

Familiarity and recollection involve distinct memory processes (Kelley & Wixted, 2001; Rotello, Macmillan & Reder, 2001) and they show distinct spatial and temporal event related potentials (Duzel, Yonelinas, Mangun, Heinze & Tulving, 1997) suggesting they would rely on different neural correlates. A compelling body of neuroimaging and neuropsychological studies suggests that recollection relies on the hippocampus and prefrontal cortex, whereas familiarity relies on regions surrounding the hippocampus like parahippocampal cortex (Yonelinas 2002). Recognition memory deficits have been found in Parkinson's patients using this task that dissociates familiarity and recollection processes (Davidson, Anaki, Saint-Cyr, Chow & Moscovitch, 2006; Weiermann et al., 2010). These impairments were largely due to deficits in familiarity whereas recollection was either affected to a lesser extent or relatively intact. Some have suggested that dopamine is involved though this has never been directly investigated (Davidson et al., 2006; Hay, Moscovitch & Levine, 2002). The current study investigated whether this verbal memory task, in addition to a task that dissociates multiple memory systems in the spatial memory domain (4/8 VM), is also sensitive to a tyrosine/phenylalanine depletion paradigm. Scores on hit rates and false alarm rates were determined for high and low frequency words for all participants. Comparisons were made for the rate of hits and false alarms for remember and know judgments across AA mixture and across spatial learners and response learners. Following this task, a final POMS was completed (T3) to ensure a complete account of changes of mood throughout the day both prior to and following cognitive testing.

5. Profile of Mood States (POMS) & Study-Related Adverse Effects Questionnaire

To control for the effects of mood throughout the testing session, the Profile of Mood States (POMS) was administered throughout the session (Lorr et al 1982). It was administered at baseline prior to the start of the session (time 1), immediately before starting the cognitive testing, 4.5hrs post-ingestion of the AA mixture (time 2), and once again at the end of cognitive testing (time 3). The POMS is a self-report scale that requires participants to rate the degree to which a series of adjectives describes how they felt at a given moment. Similarly, the study-related adverse effects questionnaire, which assessed the negative feelings that people may have experienced throughout the day, was also administered at the end of the day.

6. Blood Samples

During each testing session, two blood samples (10 ml or 2 teaspoons each) were taken by a nurse, one in the morning upon arrival at the laboratory and another following the completion of the 4/8 VM and the Word Frequency Mirror Effect tsk, six hours following ingestion of the amino acid mixture and capsules. Blood sample centrifugation was completed by the experimenter, who also pipetted out the serum and stored it in labelled epindorphs in a -80C degrees freezer in Dr. Leyton's laboratory at the Ludmer Research & Training building. Immediately following collection of blood samples, tubes were left undisturbed sitting upright at room temperature to allow for the blood to clot for a minimum of 30 to a maximum of 60 minutes. Then, samples were centrifuged at 3000rpm for 5 minutes at 4C before storing in labeled epindorphs.

7. **Post-Testing : Meal and Follow-up**

After completion of testing, participants were given a sandwich or meal of the participant's choice that contained a balance of carbohydrates and protein to help restore amino acid levels to baseline. It was ensured that participants were feeling well before they could leave the laboratory. In order to monitor everyone's mood that evening and over the following days, participants were either telephoned at home or emailed two or three times over the following week, and encouraged to call the researchers if they experienced any adverse symptoms such as mood changes.

8. Statistical Analyses

All statistical analyses were performed using the SPSS software version 20 for Macintosh. To determine whether there were any effects of APTD on navigation, behavioural data analyses included general linear model for the effect of AA Mixture (APTD vs. BAL) on dependent variables including the time taken to complete trials and errors participants made in the learning phase and probe of the 4/8VM. Repeated measures analyses of variance with Trials as a repeated measure, AA Mixture as either a repeated measure (Studies 1 and 2) or as a fixed factor (Study 3) were performed for these dependent variables. Post-hoc paired comparisons were performed when significant main effects or interactions were seen.

To determine whether there were differences in performance across spatial and response learners for the AA Mixtures, data analyses included the general linear model for the effect of strategy and mixture on dependent measures including time taken to complete trials and errors on 4/8VM learning phase and probe. Chi square analyses were

also performed to determine whether there were proportional differences in the number of response and spatial learners across AA Mixtures.

Descriptive statistics were also calculated for demographic and neurocognitive measures including the Wechsler Memory Scale, Test of Non-verbal Intelligence-3, Rey Auditory Verbal Learning Task and the Rey-Osterrieth Complex Figure in order to characterize and compare study populations.

To determine whether side effects could have influenced any reported main effect or interactions, normative *t*-scores were determined for each subscale of the POMS and along with individual adverse effects (*ex*. nausea, lowered energy, gastrointestinal discomfort etc.), these were compared across AA Mixtures. If any subscale or adverse effect was significantly different across AA Mixtures and correlated with the reported significant dependent measures from analyses of variance, then these were used as covariates in the general linear model.

Chapter Three: Results

4/8 VM Navigation Measures

Pilot Study 1: Within subjects design with original 4/8VM task

Study 1 was the first of three pilot studies. The objective was to determine whether the standard (original) 4/8VM could be administered repeatedly during a within subjects APTD study. A total of nine subjects took part in Study 1 with a mean age of 25.00 ± 4.90 years. Participants had an average number of years of education at 14.56 ± 4.28 and a non-verbal intelligence quotient of 113.35 ± 15.76. Additional demographic and neurocognitive measures for this population are reported in **Table 1.**

Table 1. Demographic & baseline neurocognitive measures in Study 1.

Characteristic	Study 1 (*n* = 9) Mean ± SD
Age	25.00 ± 4.90
Years of Education	14.56 ± 4.28
Weight (kg)	61.56 ± 8.57
Height (cm)	173.06 ± 12.23
Sex	M: 3, F: 6
Non-Verbal IQ-TONI III	
Raw Score	36.11 ± 7.18
Quotient	113.00 ± 15.76
Percentile	73.78 ± 28.12
Rey Auditory Verbal Learning Test (RAVLT)	
Total recall score for trials 1-5 (List A) (Maximum score: 75)	60.00 ± 5.55
Interference score (List B). (Maximum score: 15)	6.89 ± 1.90
Recall score after interference (List A). (Maximum score: 15)	12.56 ± 2.46
Delayed recall score (List A). (Maximum score: 15)	13.11 ± 1.69
Recognition (List A)	14.44 ± 1.24
Recognition (List B)	8.78 ± 2.22
Rey Osterrieth Complex Figure (ROCF)	
Copy drawing score (Maximum score: 36)	34.72 ± 2.11
Immediate drawing score	26.833 ± 7.30
Delay drawing score	25.72 ± 6.30
Wechsler Memory Scale- Story Recall	
Story A Recall Score	11.78 ± 3.84
Story A Thematic Score	5.78 ± 1.39
Story B 2nd Recall Score	17.00 ± 2.40
Story B 2nd Recall Thematic Score	7.00± 1.00
Story A Delayed Recall	11.56 ± 2.60
Story B Delayed Recall	15.67 ± 3.67
Learning Slope Story Recall	5.22 ± 2.64
Yes/No Story Recall	25.56 ± 1.51
Percent Retention	91.80 ± 13.00
Trail Making Errors: Trail A	0.11 ± 0.333
Trail Making Errors: Trail B	0.22 ± 0.44
Digit Span (Backward & Forward)	20.62 ± 4.49

Abbreviations: TONI, Test of Nonverbal Intelligence.

1.1 Effect of AA Mixture on 4on8 VM Navigation Measures in Study 1

No effect of AA mixture was found using repeated measures analyses of variance with all primary dependent measures of the original 4/8VM. These are summarized in **Table 2** where all *p*-values for primary dependent measures were above 0.08. A description of 4/8VM navigation variables can be found in **Table 3** of the appendix**.**

Table 2. Analyses of 4/8VM variables across AA mixtures in Study 1

Analysis	Measure	Statistic	
AA Mixture x Learning Trials Repeated Measures ANOVA	Errors	Main effect of AA Mixture	$p = 0.89$
		AA Mixture x Learning Trials Interaction	$p = 0.45$
	Time to Complete Trials	Main effect of AA mixture	$p = 0.96$
		AA Mixture x Learning Trials Interaction	$p = 0.16$
Probe Errors Across AA Mixtures	Absolute Errors	Mean- APTD: 0.63 ± 1.89; BAL: 0.75 ± 1.04	$p = 0.83$
	Rotational Errors	Mean- APTD: 0.00 ± 0.00; BAL: 0.38 ± 0.52	$p = 0.08$
Trials to Criteria Across AA Mixtures	Number of Trials to Reach Criterion	Means: APTD: 1.50 ± 0.76; BAL: 1.30 ± 0.74	$p = 0.73$

1.2 Practice Effects in Study 1: 4on8 VM Test Session 1 vs. Test Session 2 Irrespective of AA Mixture

To test for practice effects, 4on8 VM variables were compared across test sessions using a repeated measures ANOVA for time taken to complete trials and the total of working memory and reference memory errors made across trials (mean error measure). A main effect of test session for time taken to complete trials was found ($F(1, 7) = 13.86$, $p = .007$) where participants took significantly less time to complete the 4/8VM on the second test session as compared to the first. A trend for a main effect of test session for errors ($F(1, 7) = 4.09$, $p < .083$) was observed also. Following these

findings, paired samples t-tests were conducted to compare performance variables on the 4on8 VM across both sessions (**Table 4**).

Table 4. Post-hoc paired samples *t*-tests comparing 4/8VM performance across test sessions irrespective of AA Mixture administered.

Variable	Test Day	Statistic		P-Value
		Mean	SD	
Trials to Criteria	1	1.75	0.89	$t(7) = 2.38$, $p = 0.049$
	2	1.13	0.13	
Probe Trials: Absolute Errors	1	1.13	0.44	$t(7) = 1.99$, $p = 0.087$
	2	0.25	0.25	
Total Errors: Part 2 of all trials	1	1.88	0.64	$t(7) = 2.39$, $p = 0.048$
	2	0.38	1.83	
Total Time, All trials	1	1299.41	190.10	$t(7) = 6.34$, $p < 0.0001$)
	2	1157.96	196.35	

Significantly fewer 4/8VM trials were needed to reach the performance criterion of zero errors on test session 2 (mean = 1.13 ± 0.125) as compared to test session 1 (mean = 1.75 ± 0.886) ($t(7) = 2.38$, $p = 0.049$) (**Figure 1**). A trend was observed between the number of absolute errors made on the probe trial across the two sessions irrespective of AA Mixture (**Figure 2**). Fewer errors were made on test session 2 (mean = 0.25 ± 0.25) as compared to test session 1 (mean = 1.13 ± 0.44) on the 4on8 VM ($t(7) = 1.99$, $p = 0.087$). In addition, a significant difference between the number of total errors made in part 2 of all trials (1-3 & 5) was also found (**Figure 3**) where there were fewer errors on test session 2 (mean = 0.38 ± 1.83) as compared to test session 1 (mean = 1.88 ± 0.64) ($t(7) = 2.39$, $p = 0.048$). A significant difference between total time to complete all trials between test sessions was also found (**Figure 4**). Participants took less time to complete

all trials administered on test session 2 (mean = 1157.96 ± 196.35) as compared to test session 1 (mean = 1299.41 ± 190.10) ($t(7) = 6.34$, $p < 0.0001$).

1.3 Side Effects & Effects of Mood: Study 1

There were significantly higher ratings of anxiety on test session 2 (Mean: 2.38, SD: 1.41) compared to the first test session (Mean: 1.50, SD: 1.07; $F(1,7)= 8.80$, $p= 0.021$). Ratings of anxiety on test session 1 did not correlate with measures of probe absolute errors ($p= 0.69$), errors on part two of all trial ($p= 0.82$), trials to criteria ($p= 0.90$) or time taken to complete all trials ($p= 0.47$). Ratings of anxiety also did not correlate with these measures on test session 2 ($p= 0.79$, $p= 0.45$, $p= 0.54$, $p= 0.45$, respectively). There were no other differences in ratings of total adverse effects ($p= 0.89$) between test sessions. There was also no difference between test sessions ($F(1,7)= 0.008$, $p= 0.930$), test day x timepoint interaction ($F(2,14)= 1.65$ $p= 0.23$) for total POMS *t*-scores or any subscale of the POMS.

1.4 Discussion: Study 1

Study 1 indicated that there were significant practice effects on the second testing session, and no effects of AA mixture on the 4/8 VM navigation variables. Given these results it was hypothesized that a more difficult version of the 4/8VM would be less susceptible to practice effects, and consequently provide a more sensitive indicator of performance differences following AA mixtures.

2. **Pilot Study 2**: Within subjects design with more challenging 4/8VM task

Study 2 served to test whether a repeated measures design could be feasible with a more challenging version of the 4/8VM. A total of 11 subjects took part with a mean age of 23.91 ± 4.08 years. The proportion of male (6) and female (5) participants was 54.5% and 45.5% respectively. Participants had an average number of years of education at 13.91 ± 6.71 and a non-verbal intelligence quotient of 110.45 ± 9.07. Additional demographic and neuropsychological measures for Study 2 participants are in **Table 5.**

2.1 Study 2 Methods: Modified Four on Eight Virtual Maze Task (4/8VM)

4/8VM task difficulty was increased in Study 2 by using three different learning environments as opposed to the single learning environment used in Study 1. The learning environment constitutes the surrounding 3-D foreground and background along with the distal and proximal landmarks of the 8-arm radial maze. In the more challenging protocol, participants needed to remember which pathways they visited with reference to a new environment from one trial to the next. In comparison, the virtual environment and its landmarks remained fixed between learning trials in the original 4/8VM protocol.

Table 5. Demographics and baseline neurocognitive measures in Study 2

Characteristic	Study 2 (*n* = 11) Mean ± SD
Age	23.91 ± 4.08
Years of Education	13.91 ± 6.71
Weight (kg)	61.77 ± 22.91
Height (cm)	173.41 ± 10.46
Sex	M: 6, F: 5
Non-Verbal IQ-TONI III	
Raw Score	35.91 ± 3.88
Quotient	110.45 ± 9.07
Percentile	72.55 ± 16.39
Rey Auditory Verbal Learning Test (RAVLT)	
Total recall score for trials 1-5 (List A) (Maximum score: 75)	57.90 ± 7.17
Interference score (List B). (Maximum score: 15)	7.30 ± 2.05
Recall score after interference (List A). (Maximum score: 15)	12.10 ± 1.19
Delayed recall score (List A). (Maximum score: 15)	12.20 ± 1.19
Recognition (List A)	14.60 ± 0.51
Recognition (List B)	12.30 ± 1.49
Rey Osterrieth Complex Figure (ROCF)	
Copy drawing score (Maximum score: 36)	34.73 ± 1.19
Immediate drawing score	24.73 ± 4.73
Delay drawing score	24.82 ± 4.74
Wechsler Memory Scale- Story Recall	
Story A Recall Score	13.36. ± 3.41
Story A Thematic Score	5.27 ± 1.27
Story B 2nd Recall Score	14.80 ± 6.31
Story B 2nd Recall Thematic Score	6.50± 1.58
Story A Delayed Recall	10.82 ± 3.62
Story B Delayed Recall	14.82 ± 3.65
Learning Slope Story Recall	5.22 ± 2.64
Yes/No Story Recall	26.18 ± 2.27
Percent Retention	97.54 ± 23.15
Trail Making Errors: Trail A	0.00 ± 0.00
Trail Making Errors: Trail B	0.09 ± 0.30
Digit Span (Backward & Forward)	21.55 ± 2.97

Abbreviations: TONI, Test of Nonverbal Intelligence.

2.2 *Effect of AA Mixture on 4on8 VM Navigation Measures in Study 2*

There was no effect of AA mixture using repeated measures analyses of variance with all primary dependent navigation measures for the more challenging 4/8VM. These are summarized in **Table 6** where all *p*-values for primary dependent measures were above 0.35.

Table 6. Analyses of 4/8VM variables across AA mixtures in Pilot Study 2.

		Statistic	
Analysis	Measure		
AA Mixture x Learning Trials Repeated Measures ANOVA	Errors	Main effect of AA Mixture	$p = 0.51$
		AA Mixture x Learning Trials Interaction	$p = 0.29$
	Time to Complete Trials	Main effect of AA mixture	$p = 0.96$
		AA Mixture x Learning Trials Interaction	$p = 0.60$
Probe Errors Across AA Mixtures	Absolute Errors	Mean- APTD: 1.67 ± 1.50; BAL: 1.44 ± 1.24	$p = 0.71$
	Rotational Errors	Mean- APTD: 0.33 ± 0.50; BAL: 0.11 ± 0.33	$p = 0.35$
Trials to Criteria Across AA Mixtures	Number of Trials to Reach Criterion	Means: APTD: 1.44 ± 0.53; BAL: 1.67 ± 0.71	$p = 0.45$

2.3 *Practice Effects in Study 2: 4on8 VM Test Session 1 vs. Test Session 2 Irrespective of AA Mixture*

To determine whether there were practice effects with a more challenging 4/8 VM, Repeated Measures ANOVAs across test days 1 and 2 were performed to determine effects over time across test sessions. A Main effect of test session for total length of time taken to complete trials was found ($F(1, 8) = 23.27$, $p = .001$). A summary of post-hoc paired *t*-tests for trial completion times and performance errors is tabulated in **Table 7**.

Table 7. Post-hoc paired samples *t*-tests comparing 4/8VM performance across test sessions irrespective of AA Mixture.

		Statistic		P-Value
Variable	Test Day	Mean	SD	
Total Time on Probe Trial	1	229.65	10.61	*(t(8) = 5.64, p < 0.000)*
	2	205.00	6.82	
Rotational Errors on Probe Trial	1	0.44	0.18	*t(8) = 2.53, p = 0.035)*
	2	0.00	0.00	
Total Errors: Part 2 of All Trials	1	2.20	0.76	*t(8) = 2.14, p = 0.065*
	2	0.89	0.39	
Total Time, All Trials	1	1306.60	60.48	*t(8) = 4.95, p = 0.001*
	2	1114.20	47.68	

Participants in Study 2 were faster to complete the 4on8 VM probe trial on test session 2 (mean = 205 ± 6.82) than on session 1 (mean = 229.65 ± 10.61) ($t(8) = 5.64$, $p < 0.000$). In addition, a significant difference between the mean of rotational errors made was found across the two test sessions irrespective of AA Mixture. There were fewer errors on test session 2 (mean = 0.0 ± 0.0) as compared to test session 1 (mean = 0.44 ± 0.18) on the 4on8 VM ($t(8) = 2.53$, $p = 0.035$; **Figure 5**). A trend was observed for the number of total errors on part 2 of all trials (**Figure 6**) where there were fewer errors on test session 2 (mean = 0.89 ± 0.39) compared to test session 1 (mean = 2.2 ± 0.76; $t(8) = 2.14$, $p = 0.065$). A significant difference in time to complete all trials was found (**Figure 7**). Shorter lengths of time to complete all trials were observed on test session 2 (mean = 1114.2 ± 47.68) as compared to test session 1 (mean = 1306.6 ± 60.48) on the 4on8 VM ($t(8) = 4.95$, $p = 0.001$).

2.4 Side Effects & Effects of Mood: Study 2

There was no significant difference between test sessions 1 and 2 on ratings of total adverse effects ($F(1,5)= 0.002$, $p= 0.97$). There were also no differences between test sessions ($F(1,5)= 0.70$, $p= 0.44$) or a test day x timepoint interaction ($F(2,10)= 0.10$ $p= 0.91$) for total POMS *t*-score. A significant main effect of timepoint for the Elated-Depressed subscale of the POMS ($F(2,10)= 5.03$, $p= 0.03$) was found where ratings decreased steadily from baseline to the end of the study day. However there was no timepoint x AA Mixture interaction ($F(2,10)= 0.76$, $p= 0.49$) and this response was not altered by the AA mixtures ($F(1,5)= 0.14$, $p= 0.73$).

2.5 Discussion for Study 2

In the modified more challenging version of the 4/8VM, participants were significantly faster in completing all trials and continued to make fewer errors on the second testing session.

For both Studies 1 and 2, there were main effects of test session on completion time and error measures across trials confirming that strong practice effects continued to occur. These results suggest that a within-subjects design is not optimal with the 4/8 VM task.

3. **Pilot Study 3**: Between-groups design with original 4/8VM task

Given the marked carryover effects described in Study 2 with the more challenging version of the 4/8VM task, we switched to a between-groups design for pilot Study 3 using the original version of the 4/8 VM. All other methods and experimental procedures were identical to those of Study 1. To increase statistical power, data from subjects in the between-groups design were combined with test session 1 data from Study 1 (N= 9) for a total of 20 subjects. These study populations did not differ significantly from each other with regards to demographic variables including mean age ($t(18) = 1.30$, $p= 0.21$) and years of education ($t(18) = .747$, $p= 0.465$) among other characteristics (**Table 8)** and participants in the APTD mixture group did not differ significantly from participants in the BAL mixture group on these same demographic variables and characteristics either (**Table 9).**

Table 8: Comparison of Baseline Demographic & Neurocognitive Measures Across Studies

Characteristic	All Studies (*n* = 31) Mean ± SD	Study 1 (*n* = 9) Mean ± SD	Study 2 (*n* = 11) Mean ± SD	Study 3 (*n* = 11) Mean ± SD
Age	23.61 ± 4.57	25.00 ± 4.90	23.91 ± 4.08	22.18 ± 4.75
Years of Education	14.71 ± 4.67	14.56 ± 4.28	13.91 ± 6.71	15.64 ± 2.01
Weight (kg)	63.11 ± 15.22	61.56 ± 8.57	61.77 ± 22.91	65.71 ± 9.99
Height (cm)	173.2 ± 9.96	173.06 ± 12.23	173.41 ± 10.46	173.12 ± 8.27
Sex	M: 14, F: 17	M: 3, F: 6	M: 6, F: 5	M: 5, F: 6
Non-Verbal IQ-TONI III				
Raw Score	33.32 ± 6.71	36.11 ± 7.18	35.91 ± 3.88	28.45 ± 6.17
Quotient	106.35 ± 13.44	113.00 ± 15.76	110.45 ± 9.07	96.82 ± 4.75
Percentile	62.42 ± 26.53	73.78 ± 28.12	72.55 ± 16.39	43.00 ± 4.75
Rey Auditory Verbal Learning Test (RAVLT)				
Total recall score for trials 1-5 (List A) (Maximum score: 75)	58.93 ± 6.85	60.00 ± 5.55	57.90 ± 7.17	59 ± 7.94
Interference score (List B). (Maximum score: 15)	7.27 ± 1.80	6.89 ± 1.90	7.30 ± 2.05	7.55 ± 1.57
Recall score after interference (List A). (Maximum score: 15)	12.43 ± 2.00	12.56 ± 2.46	12.10 ± 1.19	12.64 ± 2.29
Delayed recall score (List A). (Maximum score: 15)	12.43 ± 2.45	13.11 ± 1.69	12.20 ± 1.19	12.09 ± 3.56
Recognition (List A)	14.23 ± 1.22	14.44 ± 1.24	14.60 ± 0.51	13.73 ± 1.56
Recognition (List B)	10.23 ± 2.62	8.78 ± 2.22	12.30 ± 1.49	9.55 ± 2.70
Rey Osterrieth Complex Figure (ROCF)				
Copy drawing score (Maximum score: 36)	34.69 ± 1.55	34.72 ± 2.11	34.73 ± 1.19	34.64 ± 1.50
Immediate drawing score	24.36 ± 6.21	26.833 ± 7.30	24.73 ± 4.73	21.96 ± 6.21
Delay drawing score	24.05 ± 5.71	25.72 ± 6.30	24.82 ± 4.74	21.91 ± 5.94
Wechsler Memory Scale- Story Recall				
Story A Recall Score	13.35 ± 3.84	11.78 ± 3.84	13.36. ± 3.41	13.82 ± 4.54
Story A Thematic Score	5.26± 1.39	5.78 ± 1.39	5.27 ± 1.27	5.09 ± 1.64
Story B 2nd Recall Score	16.03 ± 3.93	17.00 ± 2.40	14.80 ± 6.31	16.36 ± 4.20
Story B 2nd Recall Thematic Score	6.83± 1.32	7.00± 1.00	6.50± 1.58	7 ± 1.34
Story A Delayed Recall	11.00 ± 3.88	11.56 ± 2.60	10.82 ± 3.62	10.73 ± 5.12
Story B Delayed Recall	14.97 ± 4.00	15.67 ± 3.67	14.82 ± 3.65	14.55 ± 4.80
Learning Slope Story Recall	3.00 ± 4.93	5.22 ± 2.64	5.22 ± 2.64	4.27 ± 3.69
Yes/No Story Recall	25.87 ± 6.79	25.56 ± 1.51	26.18 ± 2.27	25.82 ± 3.82
Percent Retention	90.60 ± 19.87	91.80 ± 13.00	97.54 ± 23.15	82.68 ± 19.74
Trail Making Errors: Trail A	0.10 ± 0.30	0.11 ± 0.333	0.00 ± 0.00	0.18 ± 0.30
Trail Making Errors: Trail B	0.19 ± 0.40	0.22 ± 0.44	0.09 ± 0.30	0.27 ± 0.41
Digit Span (Backward & Forward)	20.1 ± 3.46	20.62 ± 4.49	21.55 ± 2.97	19.09 ± 2.98

Abbreviations: TONI, Test of Nonverbal Intelligence.

Table 9. Comparison of Baseline Demographic & Neuropsychological Measures Across AA Mixture Groups.

	AA Mixture Randomly Assigned		
	APTD (*n* = 10)	BAL (*n* = 10)	
Characteristic	Mean ± SD	Mean ± SD	*p*-value
Age	23.80 ± 3.36	23.00 ± 6.26	*p* = 0.18
Years of Education	16.20 ± 1.40	14.10 ± 4.12	*p* = 0.08
Weight (kg)	66.54 ± 9.58	61.14 ± 8.81	*p* = 0.37
Height (cm)	176.27 ± 10.40	169.91 ± 8.83	*p* = 0.34
Sex	M: 5, F: 5	M: 3, F: 7	
Non-Verbal IQ-TONI III			
Raw Score	33.20 ± 7.04	30.60 ± 8.20	*p* = 0.33
Quotient	106.40 ± 14.66	101.80 ± 15.87	*p* = 0.55
Percentile	61.80 ± 27.60	51.90 ± 32.21	*p* = 0.34
Rey Auditory Verbal Learning Test (RAVLT)			
Total recall score for trials 1-5 (List A) (Maximum score: 75	60.80 ± 6.41	58.10 ± 7.26	*p* = 0.65
Interference score (List B). (Maximum score: 15)	6.90 ± 1.45	7.60 ± 1.96	*p* = 0.11
Recall score after interference (List A). (Maximum score: 15)	13.00 ± 2.00	12.20 ± 2.62	*p* = 0.31
Delayed recall score (List A). (Maximum score: 15)	13.20 ± 1.81	11.90 ± 3.60	*p* = 0.13
Recognition (List A)	14.40 ± 0.97	13.70 ± 1.77	*p* = 0.08
Recognition (List B)	9.70 ± 2.83	8.70 ± 2.06	*p* = 0.10
Rey Osterrieth Complex Figure (ROCF)			
Copy drawing score (Maximum score: 36)	34.55 ± 1.95	34.80 ± 1.62	*p* = 0.80
Immediate drawing score	24.75 ± 7.25	23.55 ± 7.07	*p* = 0.75
Delay drawing score	25.20 ± 5.85	22.05 ± 6.54	*p* = 0.52
Wechsler Memory Scale- Story Recall			
Story A Recall Score	12.20 ± 4.34	14.50 ± 3.81	*p* = 0.67
Story A Thematic Score	5.00 ± 1.33	5.50 ± 1.65	*p* = 0.45
Story B 2nd Recall Score	16.80 ± 2.66	16.50 ± 4.22	*p* = 0.70
Story B 2nd Recall Thematic Score	7.30 ± 0.95	6.70 ± 1.34	*p* = 0.29
Story A Delayed Recall	11.10 ± 3.57	11.10 ± 4.77	*p* = 0.19
Story B Delayed Recall	13.90 ± 2.28	16.20 ± 5.49	*p* = 0.05
Learning Slope Story Recall	4.70 ± 2.71	4.70 ± 3.80	*p* = 0.31
Yes/No Story Recall	25.50 ± 2.46	25.90 ± 3.48	*p* = 0.70
Percent Retention	87.49 ± 11.14	86.07 ± 22.44	*p* = 0.14
Trail Making Errors: Trail A	0.20 ± 0.42	0.10 ± 0.32	*p* = 0.23
Trail Making Errors: Trail B	0.10 ± 0.10	0.40 ± 0.52	*p* = 0.003
Digit Span (Backward & Forward)	19.70 ± 4.11	18.90 ± 3.00	*p* = 0.16

Abbreviations: TONI, Test of Nonverbal Intelligence.

3.1 *Navigation Measures in Study 3*

3.2 4/8 VM Performance: Time Taken to Complete Trials & Errors in Learning Phase

Measures of navigation in the APTD and BAL AA Mixtures groups were assessed. A significant main effect of Trial for total time taken to complete trial ($F(2, 36) = 6.59$ $p= 0.004$) and a trend for error measure ($F(2, 36) = 3.12$, $p= 0.056$) were found reflecting a progressive decrease in the number of errors and time taken to complete learning trials from one trial to the next in the learning phase (**Figures 8 and 9,** respectively). However, significant main effects of AA Mixture were not found for time to complete trial ($F(1, 18) = 0.018$, $p= 0.895$) or errors ($F(1, 18) = 0.200$, $p= 0.660$), nor were there significant AA Mixture x Trial interactions for these measures ($F(2, 36) = 0.183$, $p= 0.833$), $F(2, 36) = 0.034$, $p= 0.926$), respectively). This shows that the two groups did not differ significantly in the learning phase of the 4/8VM. Additionally, multivariate analysis of variance revealed that there were no differences in the number of trials needed to reach criteria before the probe (100% accuracy) in the APTD and BAL mixture groups ($F(1,19) = 0.973$, $p= 0.336$), the number of total errors made on part two of all 4/8VM trials ($F(1,19) = 0.339$, $p= 0.568$) or total time taken to complete all trials ($F(1,19) = 0.002$, $p= 0.568$).

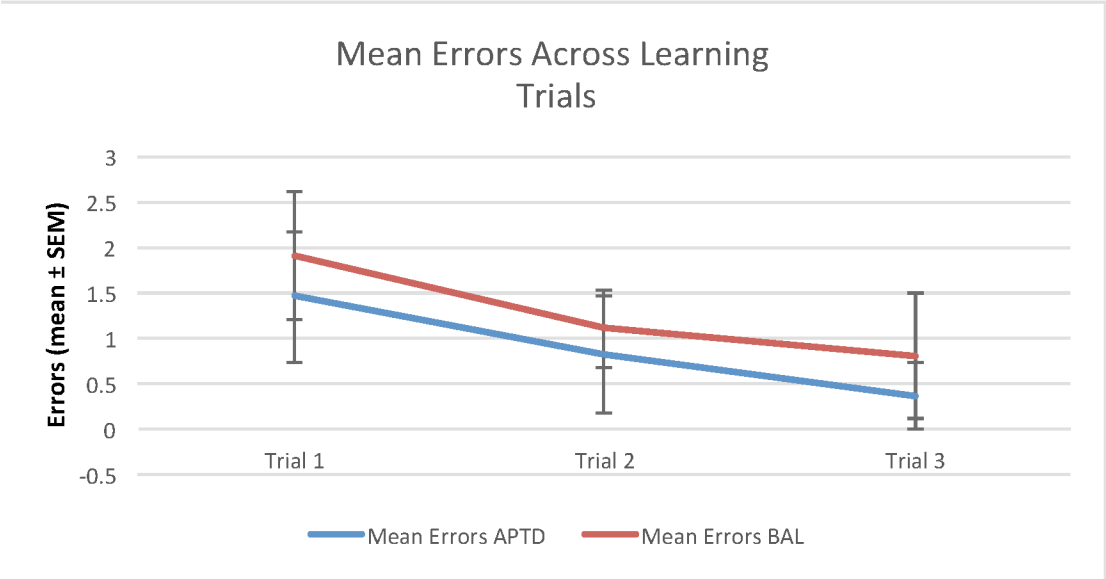


Figure 9: Errors made in the three learning trials of the 4/8VM. There was a uniform effect of trial over learning phase trials across APTD and BAL mixture groups. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

3.3 4/8 VM Performance on Probe Trial

Univariate analyses of variance comparing measures of probe completion time and probe errors (absolute and rotational errors) across AA Mixture groups (APTD *vs.* BAL) were performed. No significant main effects of AA Mixture group on absolute errors ($F(1, 18) = 0.626$, $p = 0.439$) or rotational errors ($F(1, 18) = 0.200$, $p = 0.660$) were found. In addition, there was no main effect of AA Mixture group on probe completion time ($F(1, 18) = 0.020$, $p = 0.89$).

3.4 Navigation Measures Across Groups and Strategies on Probe Trial in Study 3

To compare 4/8 VM measure differences across AA Mixture groups for spatial and response learners in the probe trial, a 2x2 (AA Mixture group x Strategy) between-subjects univariate ANOVA was performed for probe errors. No main effect of AA mixture group for absolute errors made on the probe trial in spatial and response learners was found ($F(1,16) = 0.031$, $p = 0.863$). However, there was a trend for a significant AA Mixture by initial strategy interaction for probe errors ($F(1,16) = 3.93$, $p = 0.065$). Whereas in the BAL AA Mixture group spatial learners (Mean: 1.67, SD: 0.58) made more absolute probe errors than response learners (Mean: 0.57, SD: 0.98), in the APTD Mixture group this trend was reversed, wherein response learners (Mean: 1.67, SD: 1.37) made more absolute errors than spatial learners (Mean: 0.75, SD: 0.96) (**Figure 10**). Post-hoc comparisons show that these differences between spatial and response learners did not reach significance for either the BAL ($t(8) = 1.79$, $p = 0.11$) or APTD ($t(8) = 1.16$, $p = 0.28$) mixture groups. Post-hoc comparison of response learners ($t(11) = 1.69$, $p = 0.12$) and spatial learners ($t(5) = 1.46$, $p = 0.21$) across the APTD *vs.* BAL mixture groups also did not show significance in this sample.

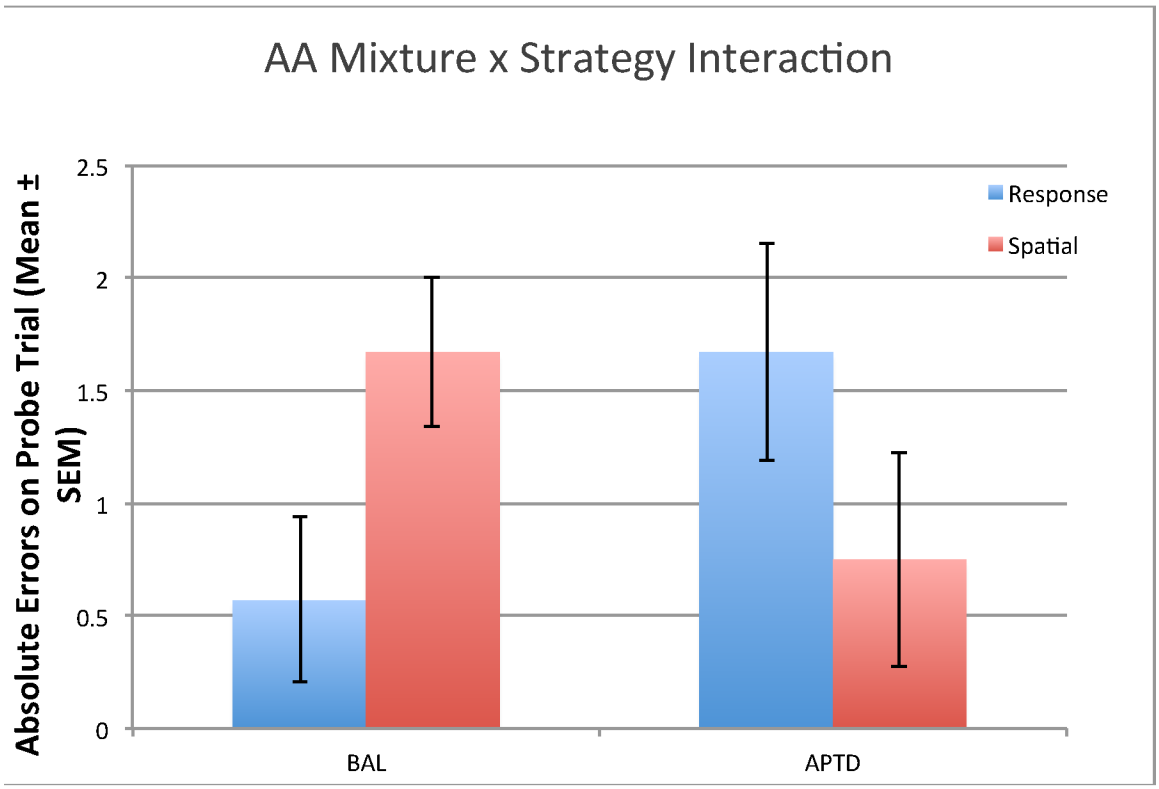


Figure 10: Absolute Errors on probe trial of the 4/8VM in spatial and response learners across AA Mixture groups. There was an Initial Strategy x AA Mixture interaction trend for errors made on probe (p = 0.065). Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

3.5 *Navigation Measures Across Groups and Strategies in 4/8VM Learning phase*

Performance on learning trials across both AA Mixture groups and strategies was analyzed. A 2 x 2 x 3, AA Mixture group x Initial Strategy x Trial, 3-way analysis of variance was carried out with Trials as the repeated measure and AA Mixture group and Initial Strategy as the fixed factors with dependent measures of errors and time to complete the learning trials. No interaction between AA Mixture group and Initial Strategy were found for trial completion time (F(1, 16) = 0.153, p= 0.70) or error

measures *(F*(1, 16) = 0.041, *p*= 0.84). A statistically significant 3-way, AA Mixture group x Initial Strategy x Trial interaction was found for errors (*F*(2, 32) = 4.12, *p*= 0.044; **Figure 11**) and a trend was observed for completion time (*F*(2, 32) = 3.23, *p*= 0.053**)**. Further investigation of the interaction indicated that in trial 1, spatial learners compared to response learners made more errors in both APTD and BAL groups and about two times more errors in the BAL group (**Table 10**). This is not the case for response learners who make about the same number of errors in both AA Mixture groups. On trial 2 of the learning phase, spatial learners in the APTD group make five times more errors than response learners while both response and spatial learners in the BAL group make about the same number of errors. On trial 3, spatial learners in the APTD group make more errors while response learners make none. As well, the opposite trend is observed in the BAL group where response learners make three times more errors as compared to spatial learners.

Table 10. Errors made in learning trials by spatial and response learners across AA Mixture groups.

		Strategy			
		Response Learners		Spatial Learners	
Trial	AA Mixture	Mean	SD	Mean	SD
Trial 1	APTD	0.83	1.17	2.50	3.79
	BAL	0.71	0.76	4.67	2.08
Trial 2	APTD	0.33	0.82	1.75	3.50
	BAL	1.14	1.35	1.00	1.73
Trial 3	APTD	0.00	0.00	1.00	2.00
	BAL	1.00	2.65	0.33	0.58

Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

An additional univariate 2x2 (AA Mixture x strategy) analysis of variance with the average number of errors made on learning trials does not reveal an interaction between AA Mixture and strategy ($F(1,16) = 0.047$, $p = 0.831$), however. This could suggest that the 3-way interaction noted above might be occurring at single or multiple individual levels of the trials factor, for example within trial 2 and/or trial 3. Additionally, multivariate analysis of variance revealed that there was no difference in the number of trials needed to reach criteria before the probe (100% accuracy) ($F(1,16) = 0.047$, $p = 0.831$), the number of total errors made on part two of all 4/8VM trials ($F(1,16) = 0.051$, $p = 0.825$) and total time taken to complete all trials ($F(1,16) = 0.483$, $p = 0.496$) in spatial and response learners across both AA Mixture groups.

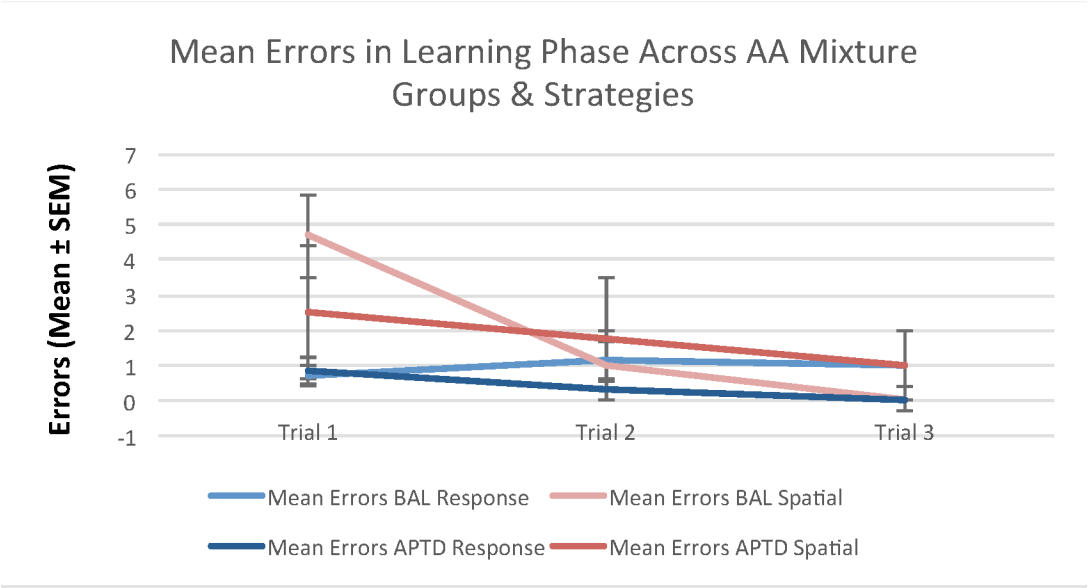


Figure 11: Mean Errors in Learning Phase Across AA Mixture Groups & Strategies. There was a AA Mixture x Strategy x Trial Interaction. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine/Tyrosine Depletion mixture.

3.6 Side Effects & Effects of Mood in Study 3

There was a significant AA Mixture x Time point interaction for the Elated-Depressed ($F(2,52) = 2.17$, $p= 0.034$) and the Agreeable-Hostile ($F(2,52) = 3.65$, $p= 0.0043$) subscales of the POMS. Post-hoc comparisons showed that the Agreeable-Hostile scores were lower for APTD than for BAL at both the morning baseline (time 1; $t(13)= 2.40$, $p= 0.032$) and at time 4.5 hours post-ingestion of drink & pre-testing (time 2; $t(13)= 1.83$, $p= 0.09$) but were similar post-testing of the 4/8VM (time 3; $t(13)= 0.09$, $p= 0.93$). The Elated-Depressed scores were lower on the APTD day only for baseline measures ($t(13)= 3.13$, $p= 0.008$) but not at time 2 or time 3 ($p= 0.67$, $p= 0.12$, respectively). Since there were significant baseline differences in these POMS subscales, Delta POMS (Δ POMS) scores relative to baseline scores were compared across AA mixtures for all subscales. There was a main effect of AA Mixture for Δ POMS scores for the Elated-Depressed subscale where scores were higher in the APTD mixture group compared to the BAL mixture ($F(2,26) = 5.30$, $p= 0.03$). There was also a significant AA Mixture x timepoint interaction for the Elated-Depressed Subscale **(Figure 12**) and the Agreeable-Hostile subscale (**Figure 13**) where scores at time 2 increased after baseline for APTD but decreased from baseline for BAL. Post-hoc comparisons however did not show that time 2 mean Δ POMS score significantly changed from baseline in the APTD group for the Elated-Depressed subscale ($t(9) = 1.19$, $p= 0.27$) or the Agreeable- Hostile subscale ($t(9) = 0.62$, $p= 0.55$). Post-hoc comparisons also did not show that time 2 mean Δ POMS scores significantly decreased from baseline for the BAL mixture ($t(9) = 0.85$, $p= 0.42$), $t(9) = 0.85$, $p= 0.42$), respectively). The Elated-Depressed scores did not correlate with errors in the learning phase of the 4/8VM at time 1 ($p= 0.51$), time 2 ($p= 0.54$) or time 3 ($p= 0.73$). These scores also did not correlate with absolute errors on

probe at any time point (p= 0.68, p= 0.38, p= 0.93, respectively). The Agreeable-Hostile subscale of the POMS did not correlate with errors in the learning phase of the 4/8VM at time 1 (p= 0.91), time 2 (p= 0.27) or time 3 (p= 0.27). These scores also did not correlate with absolute errors on probe (p = 0.71, p= 0.24, p= 0.50, respectively).

There was a significant main effect of time for the Composed-Anxious subscale of the POMS (F(2,52) = 3.76, p= 0.035) where participants had significantly higher Composed-Anxious score before the start of the 4/8VM testing compared to post-testing (t(17)= 2.43, p= 0.027).

There was no significant difference in ratings of nausea (p=0.66), lethargy (p= 0.40), significantly lowered energy (p= 0.79) or bloated feeling (p= 0.086) across AA Mixture groups.

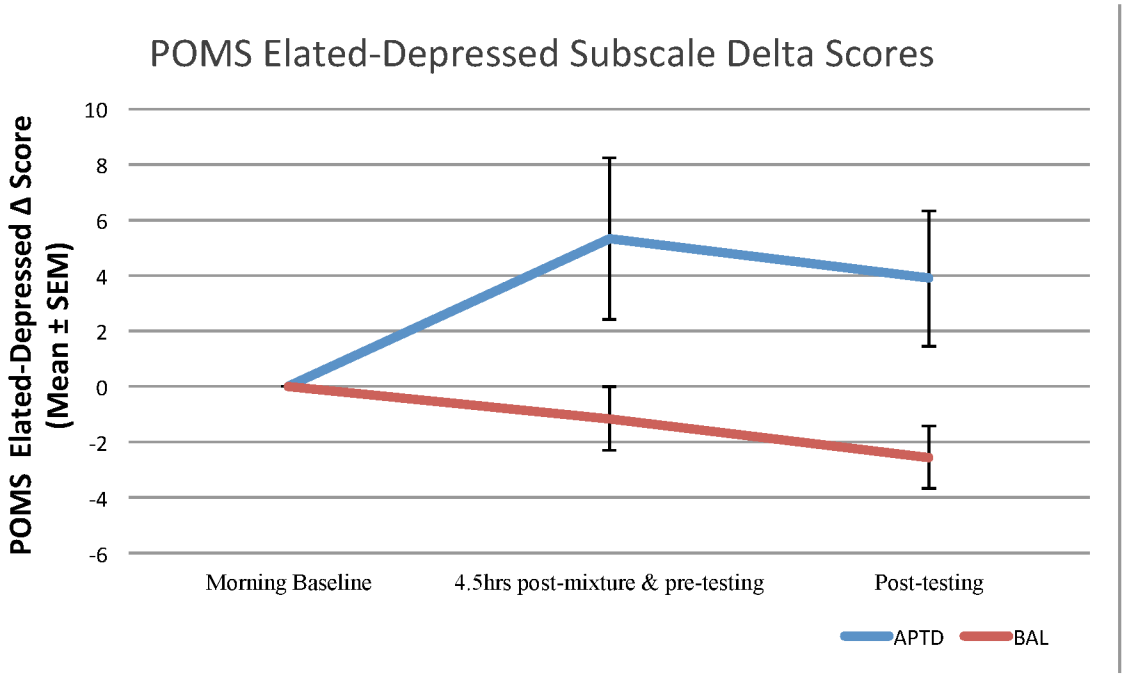


Figure 12: POMS Elated-Depressed subscale delta scores relative to morning baseline for each AA Mixture Group. Values shown are mean Δ POMS score ± SEM. There was a

main effect of AA Mixture where scores were higher in the APTD mixture group compared to the BAL mixture. There was also a significant AA Mixture x timepoint interaction. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

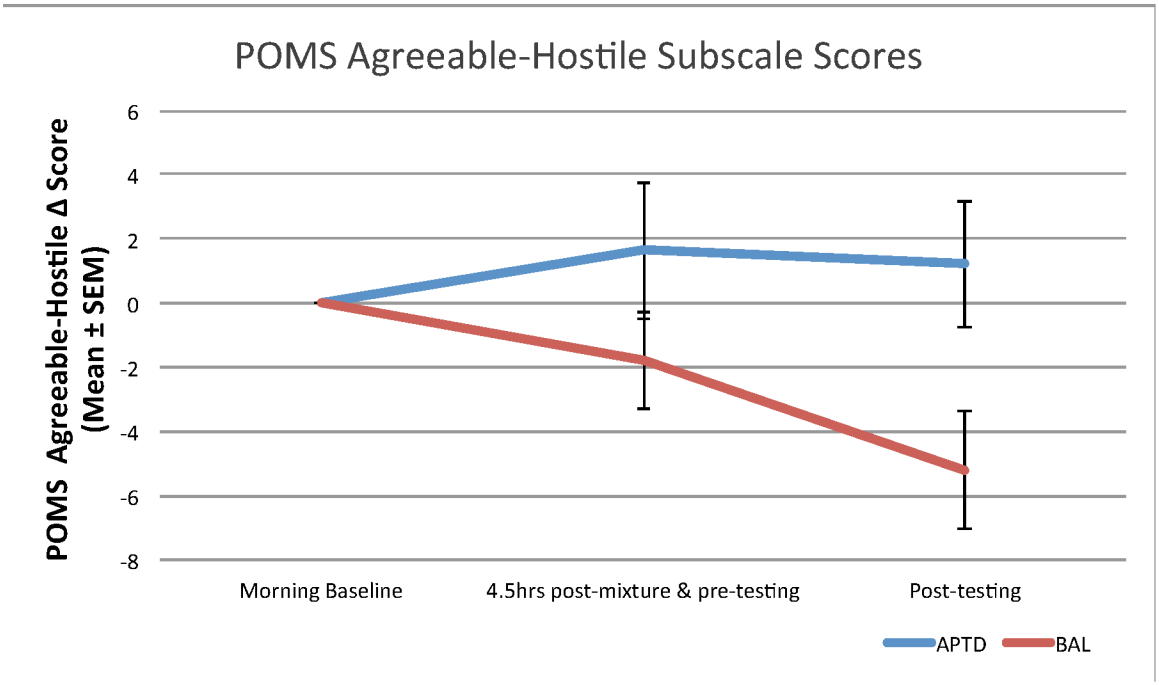


Figure 13: POMS Agreeable-Hostile subscale delta scores relative to morning baseline for each AA Mixture Group. Values shown are mean Δ POMS score ± SEM. There was a significant AA Mixture x time point interaction where scores at time 2 increased after baseline for APTD but decreased from baseline for BAL, though not significantly. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

4.0 Relative Proportion of Spatial and Response Strategy Users Across AA Mixture Groups In Studies 1, 2 & 3

4.1 Demographic Variables & Neuropsychological Measures

For increased statistical power, verbal reports from the first test session of all three pilot studies were used to determine the proportion of spatial and response learners in APTD and BAL AA groups. Verbal report recordings were transcribed and assessed by two individual raters for strategy use. These assessments were unaffected by the practice effects observed on the second test session in Studies 1 and 2, all participants were recruited using identical procedures and demographic variables or baseline neurocognitive measures did not differ significantly as shown in **Table 8**. The age range for all participants in Studies 1, 2 and 3 was between 18 and 35 years with a mean age of 23.61 ± 4.57 years. The proportion of male (14) and female (17) participants consisted of 45% and 55% respectively. Participants had an average number of years of education at 14.71 ± 4.67 and a non-verbal intelligence quotient of 106.35 ± 13.44.

4.2 Proportion of Response and Spatial Strategy Users and Differences Across AA Mixture Groups

Assessments of verbal reports by two independent raters following completion of the 4/8VM revealed that 19 participants (61.3%) used an initial response strategy whereas 12 participants (38.7%) used an initial spatial strategy.

To examine the relation between the use of spontaneous navigation strategy and experimental dopamine manipulation, chi-square analyses were performed to compare the nominal variables of AA Mixture Group (APTD vs. BAL) and initial strategy (response vs. spatial). We found no significant difference in proportions of the number of response and spatial learners between the experimental and control mixture groups (**Figure 14**) $X^2(1, N= 31) = 0.020$, $p= 0.886$.

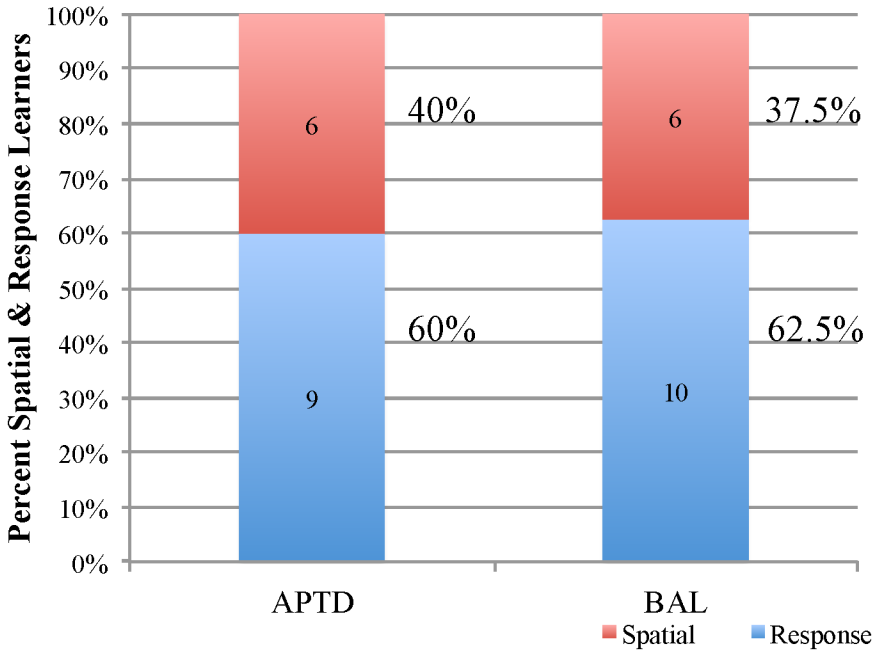


Figure 14: Proportion of response and spatial learners between AA Mixture groups. No significant difference in proportions of the number of response and spatial learners between the experimental and control mixture groups was found. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

5. Word Frequency Mirror Effect Task

Twenty healthy young adults (mean age = 24 years, demographics of Studies 1 and 2 in **Table 8**) were tested in a within-subject, double-blind, counter-balanced, placebo-controlled experiment using the APTD method. Participants took part in two testing days one month apart where they ingested an amino acid mixture that was i) nutritionally balanced (BAL), or ii) devoid of the dopamine precursors, phenylalanine and tyrosine (APTD). Three participants were lost to follow-up and one participant's data on the second testing session were unable to be recovered following a crash in the E-

Prime software used to administer this task. A total of 16 participants completed the word frequency mirror effect task on both testing sessions. Performance on the word frequency mirror effect task was assessed on testing sessions following presentation of 48 words to memorize (half high frequency (HF), half low frequency (LF)). After a delay participants classified 96 words (half old, half new) as "Remember" (conscious recollection), "Know" (familiar: no specific contextual recollection) or "New".

Questions that were of interest with respect to the word frequency mirror effect task included: i) whether there is a replication of the standard word frequency mirror effect that has been previously reported in healthy populations (Glanzer & Adams, 1985; *i.e.,* More hits to LF words compared to HF words and more false positives to HF words compared to LF words), and ii) whether dopamine influences the word frequency mirror effect in a way that has been seen in Parkinson's patients (*i.e.,* an elevated rate of false positives for HF foils in patients compared to healthy controls in the word frequency mirror effect task). To examine these questions, 2-way repeated measures ANOVA tested for main effects of AA Mixture (APTD *vs.* BAL) and Word Frequency (High *vs*. Low) and AA Mixture by Word Frequency interactions. A recognition memory discrimination score, P_r (hit rate minus false alarm rate), was calculated (Snodgrass & Corwin, 1988). 2 x 2 (AA Mixture x Word Frequency) ANOVAs were performed separately for $P_{r,}$, hit rate and false alarm rate irrespective of subjective Remember/Know judgments. For estimates of recollection and familiarity, *d*' measures were computed for Remember and Know judgment responses followed by a 2 x 2 (AA Mixture x Judgment) ANOVA comparing these scores. Results are shown in **Table 11**.

There was a main effect of Word Frequency for P_r where there was better discrimination for low- than high frequency words ($F(1,15) = 29.33$, $p < 0.0001$). No main effect of AA mixture ($F(1,15) = 0.11$, $p = 0.75$) or interaction of AA Mixture by Word Frequency ($F(1,15) = 2.06$, $p = 0.17$) were found for discrimination meaning that recognition memory was not significantly different under APTD and BAL. No main effect of AA Mixture was found for hit rates or false alarm rates. There were similar hit rates ($F(1,15) = 0.34$ $p = 0.57$) and false alarm rates ($F(1,15) = 0.79$ $p = 0.39$) under both AA Mixtures. A main effect of word frequency for hits ($F(1,15) = 9.82$, $p = 0.007$) and false alarms ($F(1,15) = 25.54$, $p < 0.0001$) were found. There was no interaction between AA Mixture and Word Frequency for hit rate and false alarm rate ($p = 0.18$, $p = 0.93$, respectively). The latter signify that the word frequency mirror effect occurred under both AA Mixtures, where there were more hits to low frequency words compared to high frequency words and more false alarms to high frequency words than to low frequency words (**Figure 15**).

Table 11. Recognition Memory Performance in APTD and BAL AA Mixtures.

	AA Mixture	
	APTD	BAL
	Mean ± SD	Mean ± SD
Word Frequency		
Hit Rate		
Low Frequency	0.89 ± 0.05	0.91 ± 0.08
High Frequency	0.84 ± 0.11	0.80 ± 0.15
False Alarm Rate		
Low Frequency	0.13 ± 0.13	0.10 ± 0.07
High Frequency	0.31 ± 0.21	0.29 ± 0.19
P_r Discrimination Index		
Low Frequency	0.76 ± 0.16	0.80 ± 0.11
High Frequency	0.53 ± 0.26	0.51 ± 0.26
Subjective Remember/Know Judgments		
d' Sensitivity Measure		
Remember	1.90 ± 0.94	2.14 ± 0.68
Know	0.53 ± 1.13	0.26 ± 0.55

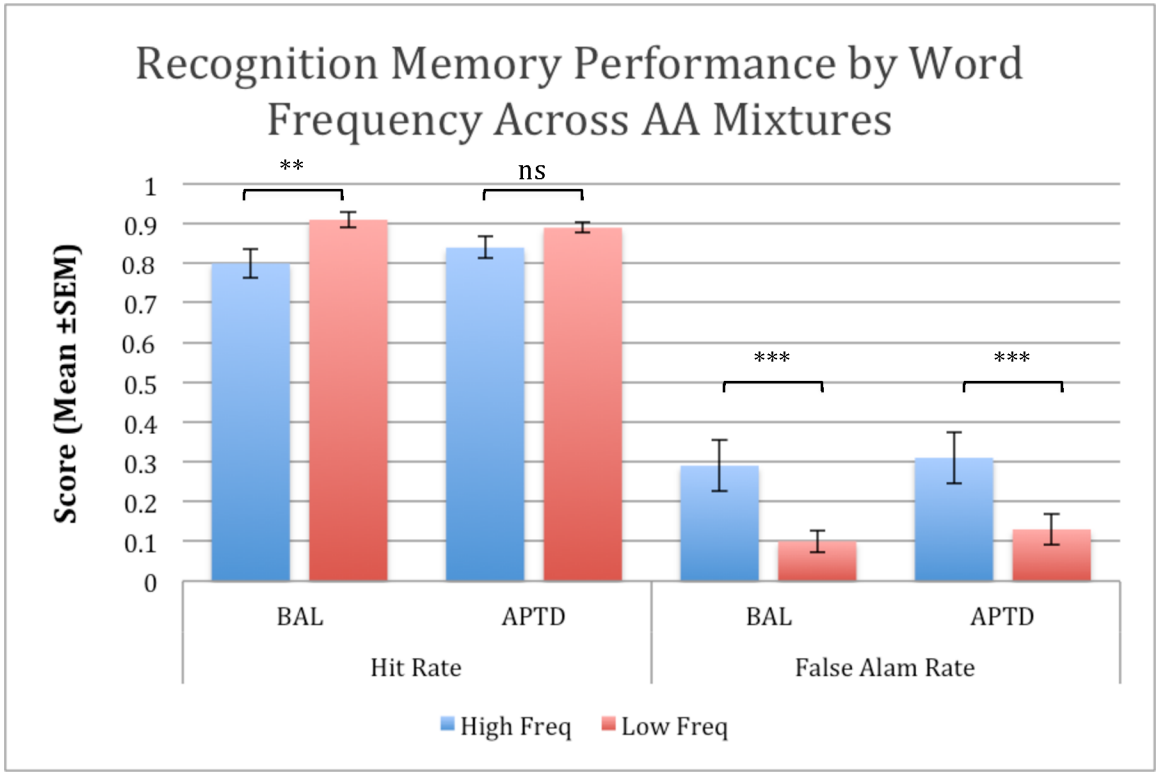


Figure 15: The rate of hits and false positives for high and low frequency words across AA Mixtures. A main effect of word frequency for hit rate and false alarm rate was observed. The word frequency mirror effect occurred under both AA Mixtures, where there were more hits to LF words compared to HF words and more false positives to HF words than to LF words. Abbreviations: HF, High Frequency; LF, Low Frequency; BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

5.2 Subjective Remember versus Know Judgments Across AA Mixtures

Rates of recollection and familiarity were estimated using *d'* sensitivity measure. This was computed for Remember and Know responses using standard Signal Detection Theory (SDT) calculations (**Table 11**). A repeated measures 2 x 2 AA Mixture x Judgment (Remembering *vs* Knowing) was then performed with *d'* sensitivity measures. Remembering was uniformly better across both AA Mixtures ($F(1,15) = 55.99$ $p<$

0.0001). No significant difference in Remembering (p= 0.40) or Knowing (p= 0.38) was found across AA mixtures (**Figure 16**).

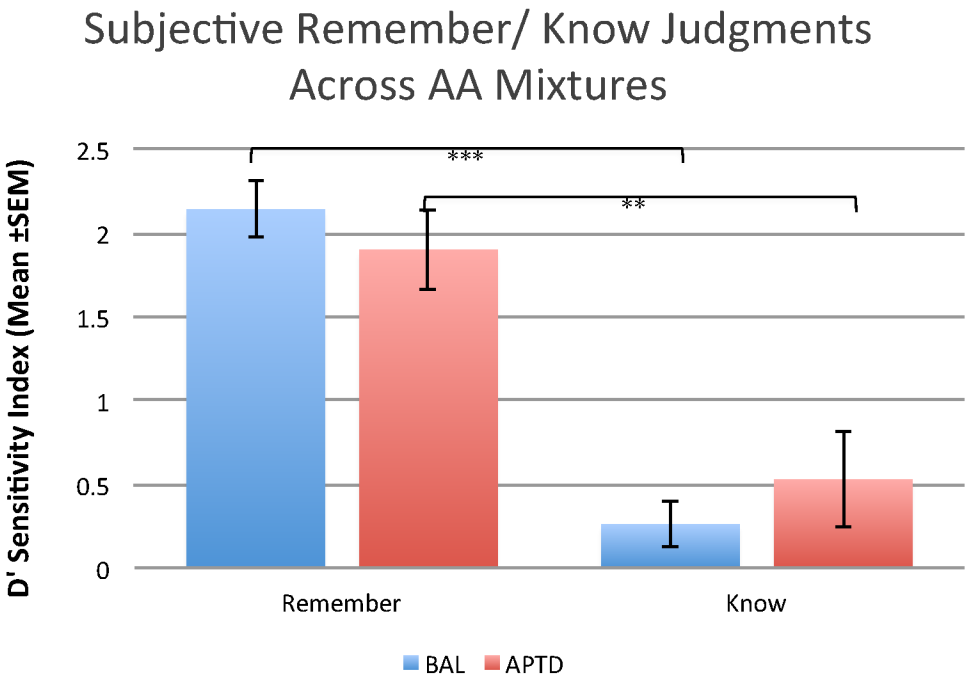


Figure 16. An estimation of the rates of recollection and familiarity using *d'* measures for Remember and Know judgments across AA Mixtures. A main effect of Judgment was observed where remembering was better across both AA Mixtures. No significant difference in Remembering or Knowing was found across AA mixtures. Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

5.3 Recognition Memory Across APTD and BAL AA Mixtures vs. Recognition Memory Across PD patient and Control Groups

Previous studies have used the same dual process recognition memory task to compare recognition memory performance across PD patients and healthy controls (Davidson et al., 2006; Weiermann et al., 2010). In these studies, the word frequency mirror effect occurred in both groups. However, elevated false alarm rates for high

frequency words in PD patients were reported compared to healthy controls. In addition, though there was no difference across these groups in Remembering, PD patients showed impairments in Knowing. Further analyses to obtain an estimate of recollection and familiarity revealed higher reliance on familiarity in PD groups compared to control groups in these studies. In the current study, we also showed that the word frequency mirror effect occurred under both AA Mixtures (APTD *vs.* BAL) and no difference in Remembering across mixtures. However, other findings were not analogous in comparing AA Mixture sessions including the following: 1) We do not report higher false alarm rates under APTD compared to BAL and 2) we do not show impairments in Know judgments under APTD compared to BAL. .

5.4 Word Frequency Mirror Effect Task Across Navigation Strategies: Between-Groups Analyses

Whether spontaneous navigation strategy use may be related to effects and interactions in this verbal memory task was an exploratory question. Between-subjects analyses (N= 31) rather than within-subject analyses were carried out for this exploratory question since spontaneous strategy was hypothesized to be subject to influence from the effects of AA mixture and also from practice with the navigation task. Mixed analyses of variance were performed separately for hit rate and false alarm rate with AA Mixture group and Initial Strategy as the fixed between-group factors and word frequency as the repeated measure.

A mixed analysis of variance revealed that there was no significant 3-way strategy (spatial *vs.* response) x AA Mixture (APTD *vs.* BAL) x Word Frequency (high *vs.* low)

for hit rate ($F(1,27) = 0.29$, $p = 0.60$) or false alarm rate ($F(1,27) = 0.04$, $p = 0.84$). As well, there was no 2-way interaction between strategy and word frequency for hits ($F(1,27) = 0.53$, $p = 0.47$) or false alarms ($F(1,27) = 0.02$, $p = 0.90$). Together this suggests that spontaneous strategy use did not modulate the word frequency mirror effect nor did it modulate overall scores across AA Mixture groups.

Chapter Four: Discussion

The studies in this dissertation sought to determine the role of dopamine in human navigational processes both generally and in distinct memory systems that are involved in navigation. Specifically, the APTD method was used to experimentally deplete the precursors of dopamine, tyrosine and phenylalanine, and participants were tested in a virtual radial maze task that dissociated spatial learners who used a hippocampus-dependent spatial strategy from response learners who used a caudate-nucleus dependent stimulus-response strategy. In the verbal memory domain the effect of a dual-process recognition memory task was also explored.

In studies 1 and 2 we found that when participants returned to the lab to complete a second test day approximately one month following their first visit, there were significant practice effects in terms of accuracy and time to complete the 4/8VM regardless of which mixture they had ingested. These pilot repeated-measures design studies were deemed suboptimal since they would potentially obscure the subtle effects that tyrosine depletion could have on navigational processes compared to the control condition. There is electrophysiological evidence of practice effects using measurements of evoked potentials (EP) in subjects who repeatedly completed a spatial n-back working memory task. This validates the notion that there are changes in the functional neural networks in a spatial working memory task as a result of practice. This included shorter latencies and improved accuracy in performance, increase in pre-stimulus preparatory slow wave amplitude, practice-dependent effects in task specific peaks (McEvoy, Smith

and Gevins, 1998) and diminished cortical activation (Gevins, Smith, McEvoy & Yu, 1997). It is plausible that corresponding changes in widespread neural networks would have occurred in the more complex spatial memory task in the present studies.

In study 3, participants in both AA Mixture groups were able to learn the 4/8VM task. We observed a uniform effect of trial such that accuracy increased and time to completion decreased over trials with very similar learning slopes for both AA Mixture groups (**Figures 11, 13**). Thus, the learning phase was not observed to be influenced by decreased dopamine synthesis resulting from APTD. Dopamine depletion studies in laboratory animals and tyrosine depletion studies in human subjects are also in line with this finding. In the learning phase of their study, de Wit et al (2012) employed an instrumental learning task where participants learned that certain responses to stimuli led to rewarding outcomes (stimulus-response learning). In an outcome-devaluation task, some of these outcomes were then devalued (no longer associated with points) and participants needed to remember response-outcome associations. In this study, there was no impairment in learning S-R relationships under tyrosine and phenylalanine depletion (TPD) and no impairment in learning R-O relationships. The final stage of their experiment, the performance phase, is when more specific effects of depletion were observed. This is consistent with the incentive salience hypothesis of dopamine that suggests its necessity specifically for seeking or incentive motivation for goal-directed behaviour rather than learning per se (Berridge & Robinson, 1998; Robinson et al., 2005; Hnasko et al., 2005; Leyton et al. 2005; Berridge, 2012). Other tasks in humans have also demonstrated dissociation between the role of dopamine for learning and performance on

reinforcement learning and instrumental learning tasks (Frank et al., 2004, Pessiglione et al., 2006). Indeed, studies in genetically engineered dopamine deficient mice indicate that spatial learning can still occur following dopamine depletions sufficiently severe that the animals are unable to move without pharmacological rescue with L-DOPA (Denenberg, Kim & Palmiter, 2004). This is also similar to an earlier finding by Zhou & Palmiter (1995) that dopamine seems to influence motivation to initiate drinking, eating or moving in the otherwise aphagic, adipsic and hypoactive dopamine-deficient mice, but that the formation of the neural circuitry for these behaviours is not dopamine-dependent. Genetically engineered dopamine deficient mice can also learn the location of food in a T-maze following a) administration of caffeine without which they do not show exploratory behavior and b) exhibit learning when this group of caffeine-treated mice is then given L-DOPA to restore dopamine levels (Palmiter, 2008). This was also true for learning of the conditioned place preference task for drugs like morphine and cocaine where animals learned the association between pleasurable effects and context (Palmiter, 2008). Taken together, the evidence suggests no direct influences of dopamine in learning per se and that these are dissociable from other effects such as in performance or motivation.

In the current study we find a trend for stimulus-response learners to be more affected following depletion. This was shown by an interaction between strategy use and AA Mixture in the probe trial of the 4/8VM for mean errors. Following the BAL mixture, spatial learners made more probe errors compared to response learners as would be predicted under standard conditions. However, under APTD, response learners were seen

to make more probe errors compared to spatial learners. Though this was a modest effect given that post-hoc comparisons did not reveal significant differences between these groups, it is worth considering with respect to earlier findings in the literature. Lesion to the dorsolateral striatum and lesions of the nigrostriatal dopaminergic pathway disrupted stimulus-response habit formation (Yin et al, 2004, Faure et al., 2005). As well, when phasic dopamine was disrupted by genetic inactivation of NMDA-type, ionotropic glutamate receptors in dopamine neurons, knockout mice were slower in learning the cued version of the Morris water maze task (Zweifel et al., 2009). In the present study, the preliminary evidence that response learners tended to increase reliance on landmarks under APTD compared to BAL might be explained by the three following changes. First, response landmark users may be driving this effect due to weaker associations between the stimulus used and the associated responses under APTD, which exhibited poor performance when these stimuli were removed from the environment. Since we have reviewed that S-R learning can proceed notwithstanding diminished dopamine state, this is improbable. Alternatively, given the literature reviewed earlier, it is possible that response landmark users successfully acquired the task using a stimulus-response strategy starting with a landmark that acted as a cue, but were unable to express this learning in the probe phase under APTD. To test this hypothesis, the subgroup of response users who used landmarks to remember a pattern or sequence (exclusion of response learners who used starting position in their sequence pattern) was assessed. The interaction between AA Mixture group and strategy in this case no longer held though it is worth noting that this could be due to low power to detect differences given small subgroup samples. This could suggest that the AA Mixture x Strategy trend we observed is likely due to other

processes that could be occurring. The third more plausible explanation could be that under APTD, response learners are shifting away from the use of stimulus-response strategies altogether and employing instead a strategy similar to those of spatial learners. This could be occurring following parallel acquisition of spatial relationships at a subconscious level since verbal reports do not suggest this while errors made on probe do so. Evidence for parallel acquisition of navigation response strategies has been shown in Igloi et al (2009) where bidirectional shifts between strategies were observed. Under certain circumstances, such as diminished dopamine synthesis, release and transmission, otherwise competing memory systems may actually be encoding information non-competitively in a cooperative fashion (*i.e.* between hippocampus and striatal memory systems among others). Non-competitive encoding between multiple parallel systems, which produces a larger variety of spatial cognition behaviours, has validity and has increasingly been suggested in the literature (Burgess, 2006). This includes cooperation between the hippocampus and dorsal striatum in non-incremental episodic learning for memory of remembered items (Sadeh et al., 2011). As well, when one structure of a memory system is impaired, the other memory system compensates for the impairment (Hartley & Burgess, 2005). For example, in early-stage Huntington's disease patients where there is atrophy in the striatum, severity of disease symptom was reflected in differential fMRI activity in a virtual navigation task. Low severity of symptoms was related to activity in the striatum whereas high severity was linked to increased hippocampal activity reflecting gradual compensation to preserve normal behavior (Voermans et al., 2004). A similar interaction between the two memory systems where hippocampal activity is enhanced has also been demonstrated in Parkinson's disease

patients (Moody, Bookheimer, Vanek, & Knowlton, 2004; Dagher, Owen, Boecker, & Brooks, 2001). Though this does not suggest that the nature of these interactions is direct, neither does it preclude the possibility. There have in fact been reports of an anatomically direct connection between the hippocampus and the striatum (Sorensen & Witter, 1983). The suggestion that certain circumstances may substantiate cooperation between memory systems to preserve compatible behavior has a clear basis in previous studies but warrants further investigation about whether a dopamine diminished state is one such instance.

When comparing the proportion of response to spatial learners under both AA Mixtures, we found similar distributions. These findings are not in line with those of a previous study by de Wit et al. (2012) who also used a tyrosine and phenylalanine depletion paradigm. In their study, participants were tested on a "slips of action test" where habitual and goal-directed control were assessed in direct competition with each other using stimuli from a learning phase where they learned both S-R associations as well as outcome-response associations. In this task participants had to refrain from responding (no-go) when the outcome of the stimulus no longer had value and to respond (go) when the outcome of the stimulus still had value. Slips of action indicated strong habitual control from reliance on S-R associations while successful inhibition/selective responding by remembering outcome value indicated goal-directed control. The depletion compared to the control drink led to stronger habitual control in females where they were more reliant on S-R associations suggesting that the balance between the two responses was shifted towards habitual control. In the current study, we saw an increase in probe errors in response learners following APTD, however when considering verbal reports,

we did not observe favoured habitual control under APTD at the expense of goal-directed behavior. Since comparing proportions of strategy users requires large samples, it is possible that we might observe a different distribution of response and spatial learners with the addition of subjects in both mixture conditions. For instance, in a previous virtual navigation study employing a between-subjects design that also looked at the proportion of response and spatial learners across the stages of the menstrual cycle, a larger sample size of about 25 participants per group was needed to see the subtle differences in performance in virtual navigation (Hussain et al., 2015, under review). In another study that looked at the proportion of spatial and response-learning strategies in stressed and control subjects, 80 subjects were needed to see effects of the experimental manipulation (Schwabe et al., 2007). Still, even with an additional sample the direction of the distribution remains disputable and favouring of stimulus-response strategies unlikely given the other findings we report pertaining to probe errors, which may be a more sensitive measure to changes in dopamine transmission.

Though we found poorer performance in response learners on the probe trial of a dual-solution maze task, we do not observe deficits in spatial memory. To date, there are mixed findings on the role of dopamine in spatial working memory. Whereas Harmer et al (2001) and Gijsman et al. (2002) found impaired spatial recognition and spatial working memory under tyrosine and phenylalanine depletion, McLean et al. (2004) were unable to replicate these findings. A study by Mehta, Gumaste, Montgomery, McTavish and Grasby (2005) also found no impairment following TPD in a spatial delayed response task. To the contrary, this group actually found response latencies to be faster

following TPD on the test of spatial delayed response. Though they did not find that performance on the delayed response task was impaired by TPD, they did find that in the subgroup of participants that underwent positron emission tomography (PET), changes in [11C]raclopride binding were correlated with a measure of accuracy on this task. There was an inverse correlation between the two such that increased binding in the dorsal striatum was correlated to poor performance on the delayed response task. This meant that only those with the highest depletion showed performance deficits. The authors conclude that since changes in performance correlated to some changes in dopamine levels, that the variability with which TPD reduces dopamine may not produce reliable effects on spatial working memory. Following the study by Mehta et al (2005), a follow-up study was conducted to determine whether regional cerebral blood flow (rCBF) following tyrosine depletion would confer a similar pattern of findings with respect to performance. This was not found for any brain regions (Ellis, Mehta, Murthy, McTavish, Nathan, Grasby, 2007). Interestingly, the authors found that following tyrosine and phenylalanine depletion, there was widespread overall increase in blood flow with maximal rCBF to parahippocampal gyrus. They take these findings to mean that compensatory mechanisms may be taking place to increase catecholamine synthesis and that there could be complex interactions between other neurotransmitter systems occurring following TPD. This also supports the hypothesis that the activity between the medial temporal lobe and the striatum are inversely correlated, though the authors do not discuss this. Nonetheless, it suggests that a complex network of systems is involved in learning, all of which may not be directly affected by dopamine levels.

The word frequency mirror effect task using the APTD method provided insight for the first time into the possible role of dopamine in recognition memory in a healthy population. Recognition memory can be based on two separate processes, either familiarity or recollection that rely on different neural correlates. Both deficits are seen in at least some patients with Parkinson's disease (PD), but familiarity deficits develop earlier than recollection problems and when dual process models of recognition were used, Parkinson's patients were seen to perform normally on recollection but were impaired primarily on familiarity (Davidson et al., 2006, Weiermann et al., 2010). It remains unclear whether either deficit reflects loss of dopamine neurons. To investigate the role of dopamine in recognition memory more directly, we tested whether decreasing dopamine synthesis in healthy volunteers would produce the pattern of performance deficits that is seen in patients with PD using a dual-process model recognition task.

We showed that the word frequency mirror effect occurred under both APTD and control conditions and was not shown to be modulated by decreased dopamine synthesis. Specifically, we did not find an AA Mixture x Word frequency interaction for either hits or false positives and these scores were similar across both AA mixtures. An interaction between group (PD patient *vs*. age- & education-matched controls) and word frequency for scores was found in an earlier study using the same recognition memory task, where PD patients made more false alarms to high frequency items (Davidson et al., 2006). It is possible that we did not see an analogous performance pattern between PD patients and the APTD condition due to the dissimilar extents to which dopamine is lowered in both (≥ 80% in patients *vs*. 30 to 50% with APTD). Alternatively, dopamine might not directly

influence recognition memory lacking motivational significance (Shohamy & Adcock, 2010, Flagel et al., 2011). For example, familiarity recognition memory deficits are usually apparent only in those with additional non-dopamine related symptoms (Dujardin et al, 2013). This would suggest that other mechanisms might contribute to this impairment in PD patients including the role of acetylcholine given reported deficiencies in cholinergic systems (Dubois et al., 1990; Bedard et al., 1999) and medial temporal lobe atrophy (Tam et al., 2005). Eleven studies of dopaminergic stimulation to date have been reviewed to determine effects in verbal declarative memory including single doses of *d*-amphetamine, methylphenidate, modafinil, L-DOPA and a COMT inhibitor drug (Riedel & Blokland, 2015). The majority of these did show at least some positive enhancing effects in both healthy young and older adults and these effects were also best measured when word lists were long. However, other mechanisms of cognitive enhancing effects on declarative memory reviewed including those of acetylcholine, serotonin and polyunsaturated fatty acids among others (**Table 1**) demonstrated that these effects were not exclusive to dopamine enhancing substances. Our results are also in line with the literature demonstrating that dopamine is not essential to all forms of learning but rather to specific processes. These include incremental feedback-based learning processes, those that are motivated by rewards or that have high motivational significance (Robinson & Palmiter, 2005; Shohamy & Adcock, 2010; Flagel et al., 2011). Given this, it is likely that previously reported non-declarative recognition memory impairments in PD patients may not be due to decreased striatal dopamine input. It is important to note that certain limitations of the APTD method render it difficult to draw definitive conclusions. This includes differences in severity of dopamine depletion between APTD and PD patients as

well as the time course over which these depletions are present. Chronic depletion may exhibit different behavioural symptoms than acute decreases.

Conclusions and Future Directions

To summarize, our pilot studies provided two important findings. First, consistent with the literature, we found that learning per se in both spatial and response learners was not affected by dopamine precursor depletion. This was also true in the verbal memory domain as we did not observe differences in familiarity and recollection processes as a result of APTD. Second, we provide preliminary evidence that stimulus-response strategies may be more susceptible to performance deficits following global reduction in dopamine function. Specifically we report increased reliance on landmarks under APTD selectively in response learners while the opposite was observed in spatial learners. This provides support to counter the hypothesis that global reduction in dopamine release and transmission would cause a general effect in navigation and suggests instead that determining the specific nature of navigation deficits following APTD is plausibly related to spontaneous strategies.

Though the APTD method has been validated to produce lowered dopamine release in humans (Leyton et al., 2004; Montgomery, McTavish, Cowen, & Grasby, 2003) and in animal studies (Fernstrom & Fernstrom, 1995; McTavish, Cowen, & Sharp, 1999), these central effects are global. Since the question we aimed to address is one involving distinct memory systems, the APTD method alone is not ideal for determining

specific changes within distinct brain networks. Future studies could benefit from an imaging component to follow up on the explicit findings, for instance those of impaired performance in response learners, perhaps using fMRI. This would allow us to detect time-specific changes as participants are engaging in the navigation task and allow comparison of brain states (*i.e.* APTD *vs*. BAL) to determine whether dopamine changes are related to activity changes in the striatum and the hippocampus. This could also be used to determine whether familiarity and recollection performance in a dual-process verbal memory task are related to changes in dopamine levels to better assess whether these are important to recognition memory deficits. In all, this would also help to elucidate the role of dopamine more finely in what appears to be a complex network of systems involved in learning and memory.

Methods Appendices

Appendix A. Inclusion & Exclusion Criteria

Inclusion Criteria		
	Age 18 - 35	
	10+ years of education	
	Good vision or optical correction (cannot exceed +6/-6)	
Exclusion Criteria		
	Pregnancy or breastfeeding	
	Substance abuse	
		> 10 Cigarettes/day
		> 10 Drinks/week
		Drugs
	Cholesterol medication	
	Cardiovascular diseases or other medical conditions	
		Myocardial infarction
		Heart block
		Slow cardiac conduction
		Heart failure
		Hypotension
		Hypertension
		Diabetes
		High cholesterol
		Kidney disease
		Asthma, respiratory disease
		Infectious illness
		Thyroid dysfunction
		Adrenal dysfunction
		Lupus
		Arthritis in Hands
		Inflammatory Bowel Disease
		Chrohn's disease
	History of psychological or neurological illnesses	
		Epilepsy
		Stroke
		Multiple Sclerosis
		Head trauma
		Depression
		Bipolar disorder
		Anxiety disorder
		Schizophrenia
		Alcohol or drug abuse
	Eye diseases	
		Cataract
		Glaucoma
		Age-related maculopathy
	Color-blindness	
	Thyroid medication	
	Hormone medication	

Appendix B. Randomization of Battery Order: Session 1

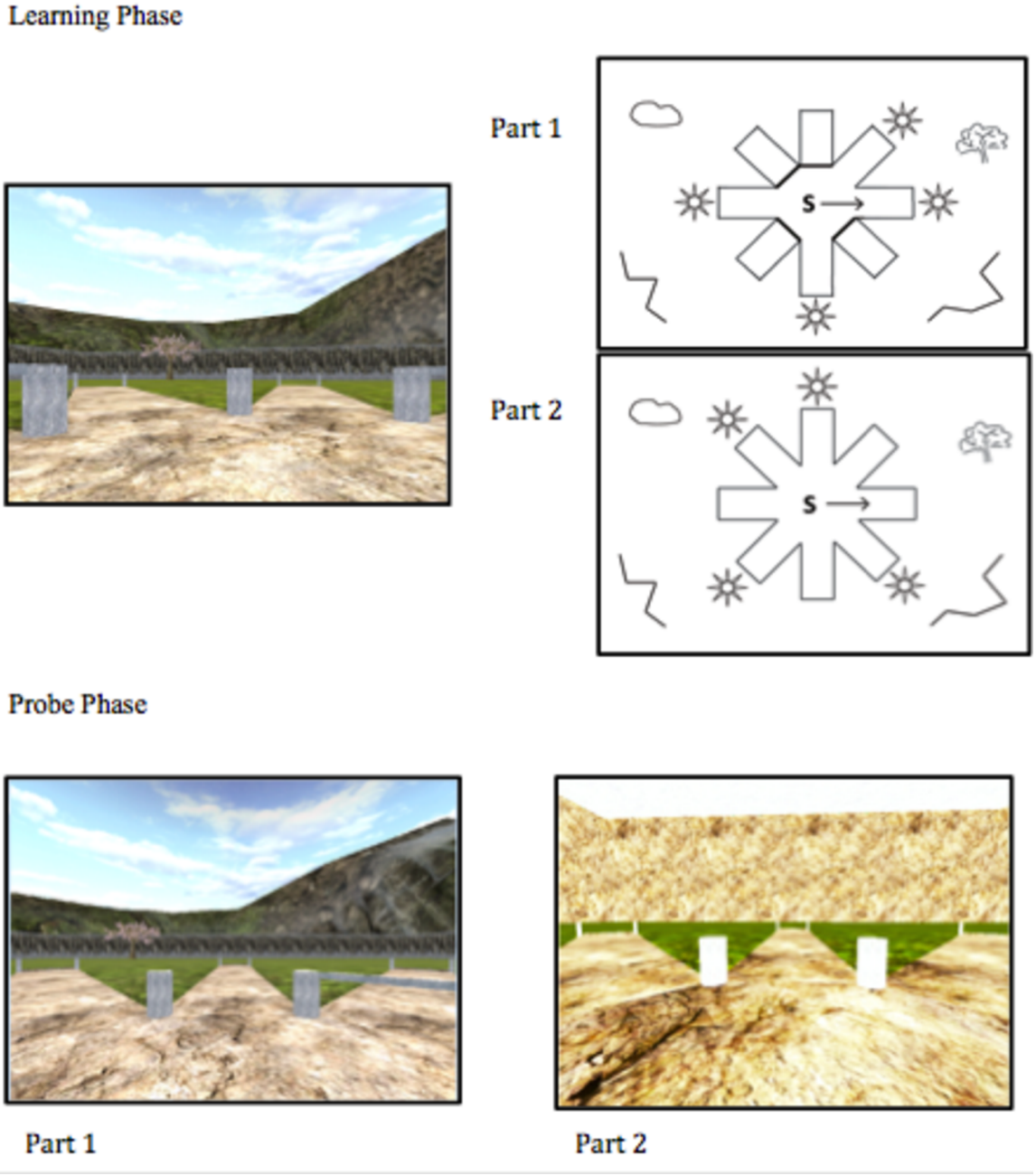
Group 1	Group 2
General Cognition/Questionnaires	
Story Recall	Drug Use Questionnaire
TONI-3	Trail Making Test
Digit Span	Digit Span
Trail Making Test	Story Recall
Drug Use Questionnaire	TONI-3
Rey- Osterrieth Complex Figure	Rey-Auditory Verbal Learning
Rey Auditory Verbal Learning	Rey- Osterrieth Complex Figure
NEO-PI Personality Inventory	BIS
SURPS	SPRSQ
TPQ	TPQ
SPSRQ	SURPS
BIS	NEO-PI Personality Inventory

Appendix C. Balanced and APTD Amino Acid Mixtures

	Balanced Mixture	APTD Mixture	Women
L-Alanine	5.5	5.5	4.58
L-Arginine	**4.9**	**4.9**	**4.08**
L-Cysteine	**2.7**	**2.7**	**2.25**
Glycine	3.2	3.2	2.67
L-Histidine	3.2	3.2	2.67
L-Isoleucine	8.0	8.0	6.67
L-Leucine	13.5	13.5	11.25
L-Lysine monohydrochloride	11.0	11.0	9.17
L-Methionine	**3.0**	**3.0**	**2.5**
L-Phenylalanine	5.7	0.0	4.75
L-Proline	12.2	12.2	10.17
L-Serine	6.9	6.9	5.75
L-Threonine	6.5	6.5	5.42
L-Tryptophan	2.3	2.3	1.92
L-Tyrosine	6.9	0.0	5.75
L-Valine	8.9	8.9	7.42

The amino acids listed in bold are given in capsule form.
A slightly smaller version of the mixture (83.3%) is administered to women due to their average lower body weight.

Appendix D. Original 4/8VM Task



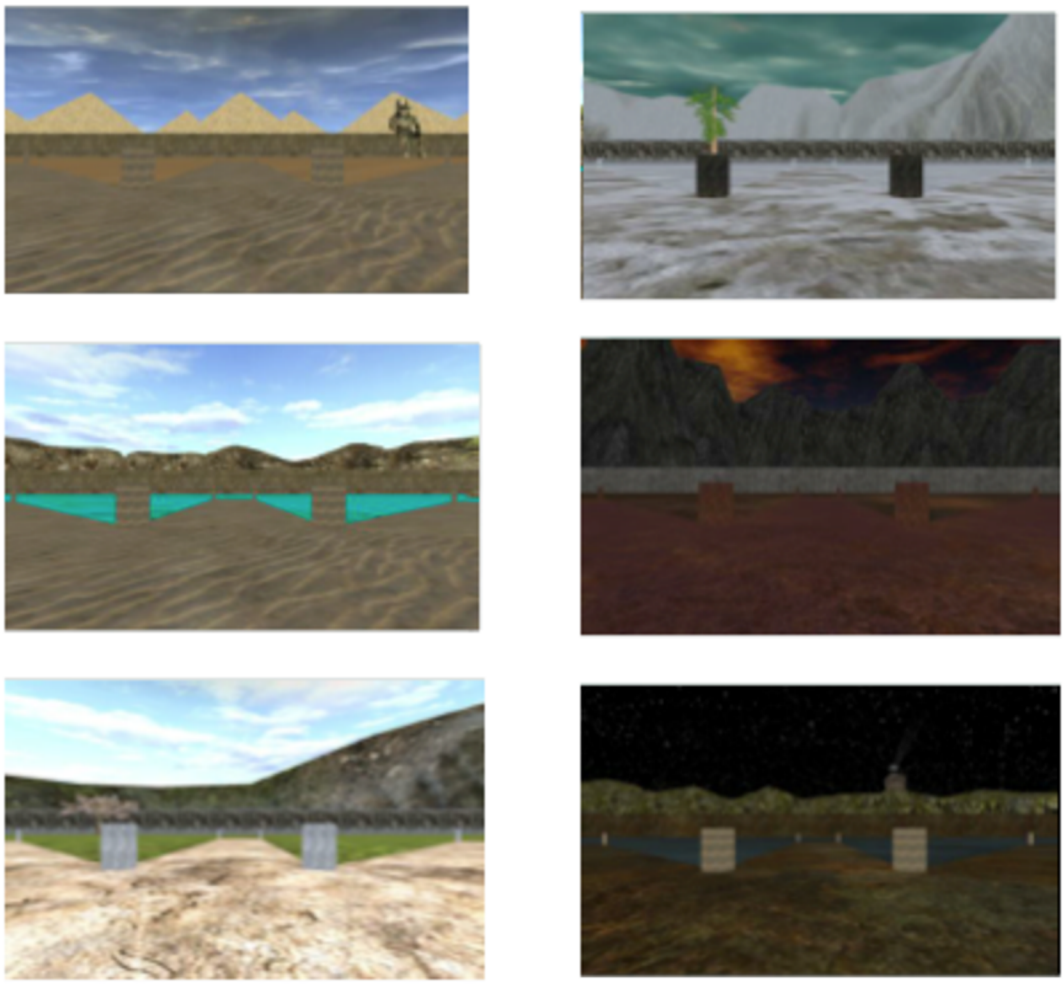
Learning Phase- Part 1: Participants must retrieve 4 objects from the 4 unblocked arms. The 4 rewarded arms are indicated with stars. S indicates the start position and the arrow indicates the direction of gaze at starting position. Part 2: Participants must retrieve objects in the previously blocked locations out of the 8 unblocked arms

Probe Phase- Following acquisition, in part 2, a wall is erected around the radial maze blocking the participant's view of environmental landmarks. This trial dissociates spatial learners who used landmarks from response learners who did not use them in the same way.

Appendix E. Challenging 4/8VM Task

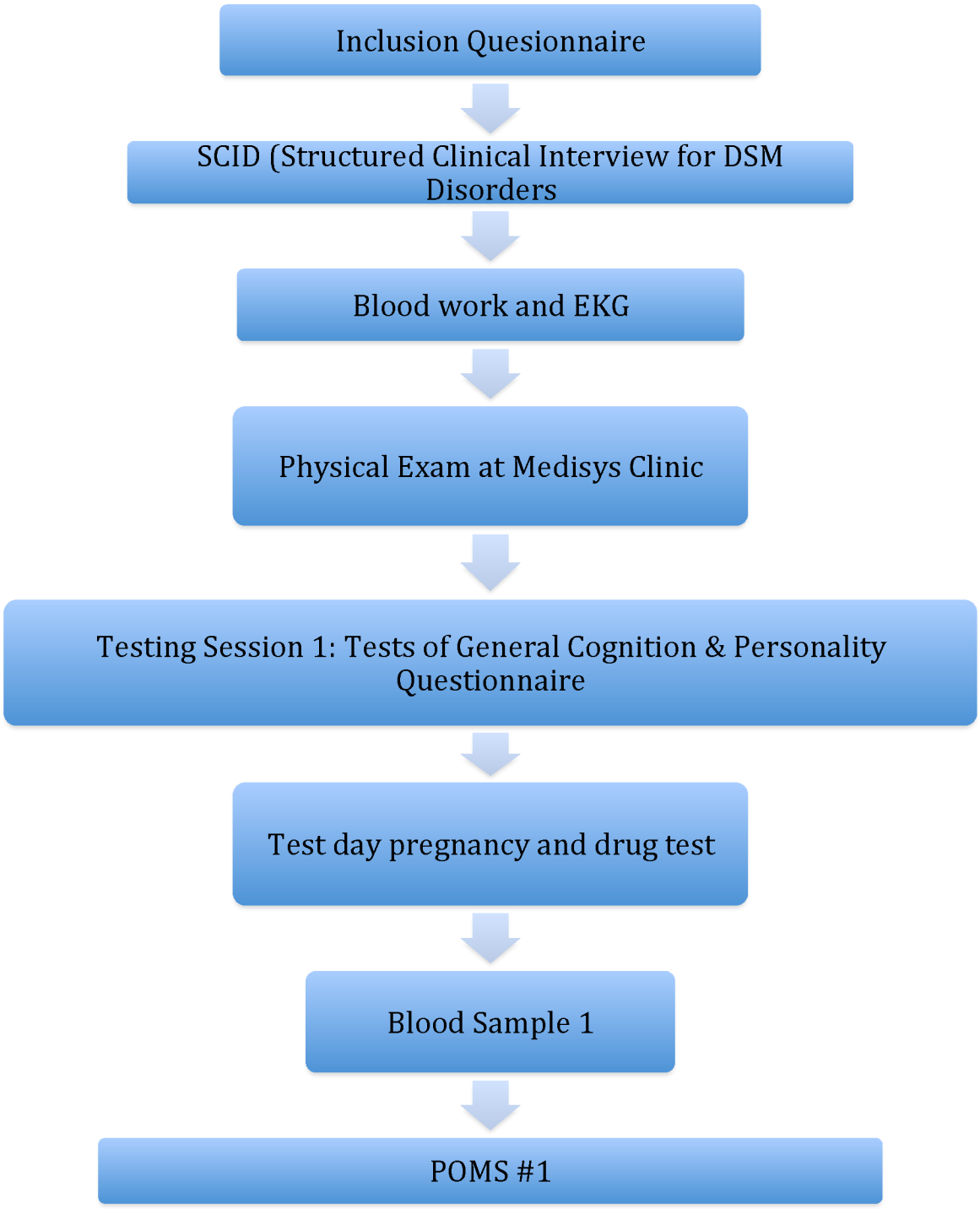
Challenging 4/8 VM Protocol Learning Phase Environments

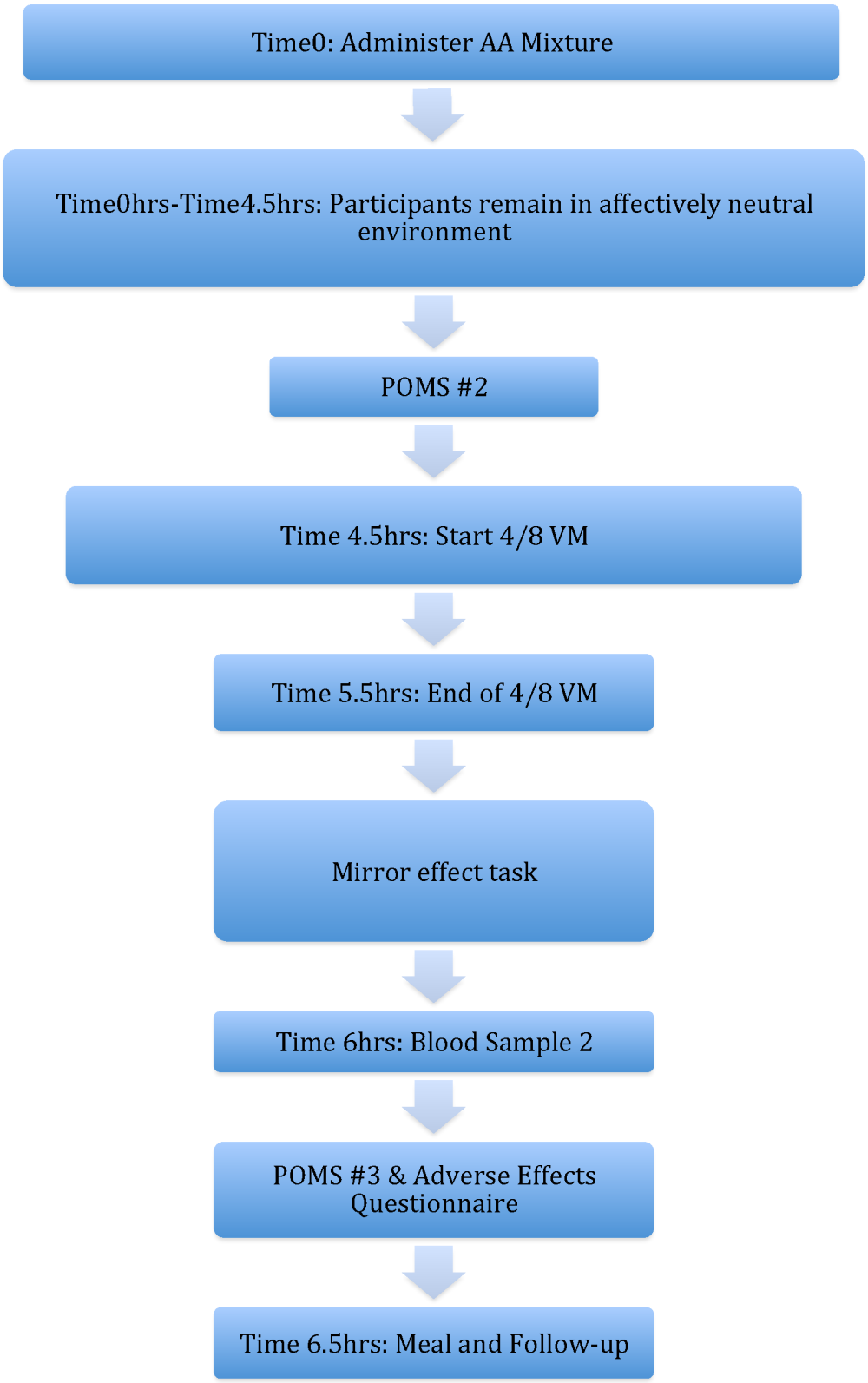
Version 1 Version 2



In the more challenging version of the 4/8 VM, participants completed the learning phase in three novel environments on each testing session.

Appendix E. Summary/Overview/Timeline of Experimental Procedures


Inclusion Quesionnaire
SCID (Structured Clinical Interview for DSM Disorders
Blood work and EKG
Physical Exam at Medisys Clinic
Testing Session 1: Tests of General Cognition & Personality Questionnaire
Test day pregnancy and drug test
Blood Sample 1
POMS #1


Time0: Administer AA Mixture
Time0hrs-Time4.5hrs: Participants remain in affectively neutral environment
POMS #2
Time 4.5hrs: Start 4/8 VM
Time 5.5hrs: End of 4/8 VM
Mirror effect task
Time 6hrs: Blood Sample 2
POMS #3 & Adverse Effects Questionnaire
Time 6.5hrs: Meal and Follow-up

Results Appendices

Figure F1: Number of trials needed to reach criteria across test day 1 and test day 2 irrespective of condition in Study 1, N=9. There were fewer trials needed to reach criteria on the second testing session reflecting practice effects ($t(7) = 2.38$, $p = 0.049$).

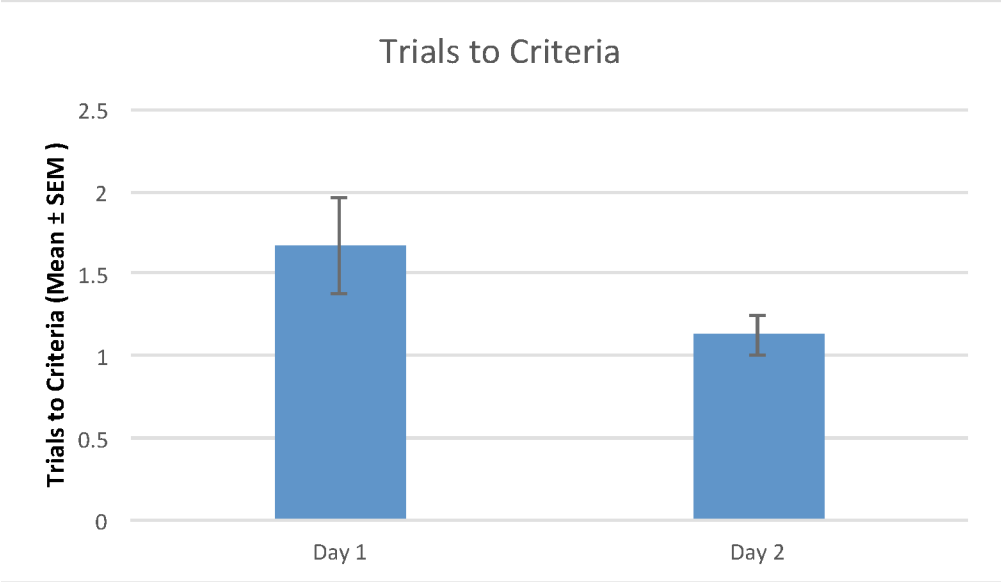


Figure F2: Average absolute errors made on probe trial across the two test days irrespective of condition in Study, N=9. There were fewer absolute probe errors made on the second testing session compared to the first testing session reflecting practice effects ($t(7) = 1.99$, $p = 0.087$).

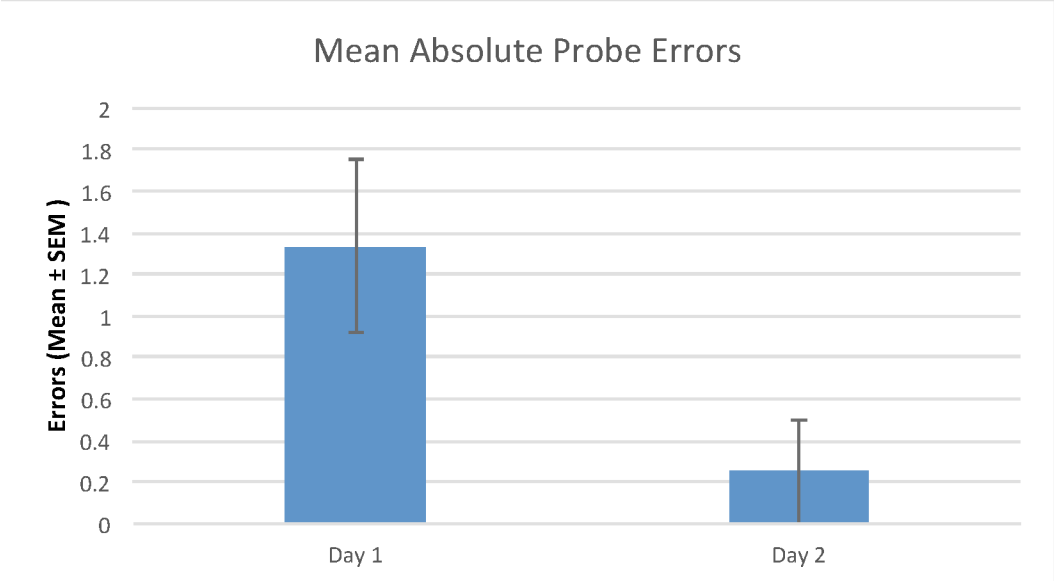


Figure F3: Mean total errors made in part 2 across all trials (1-3 & 5) irrespective of condition in Study 1, N=9. There were fewer errors made on the second test session compared to the first ($t(7) = 2.39$, $p = 0.048$).

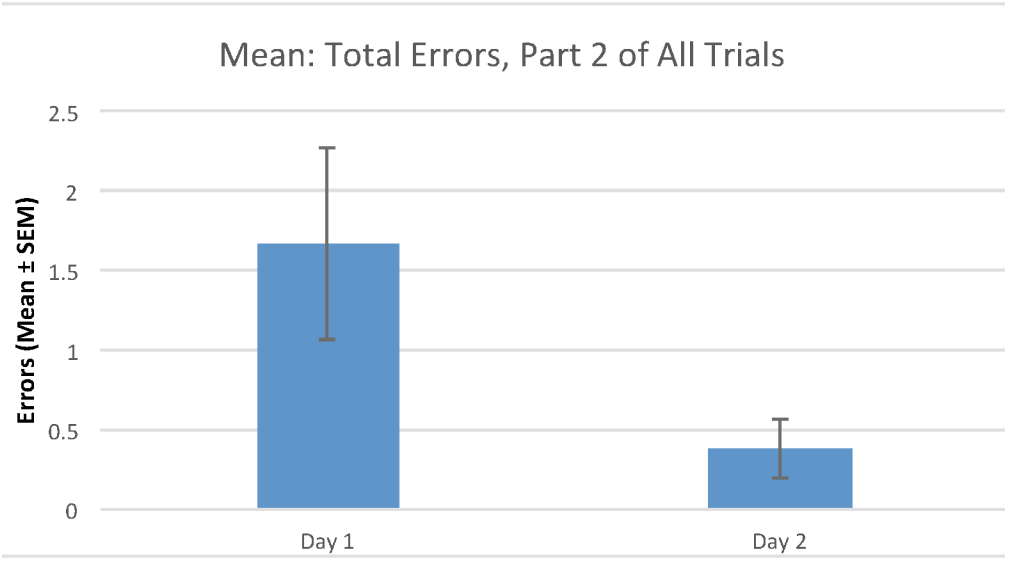


Figure F4: Mean total time across test day 1 and test day 2 irrespective of condition in Study 2, N=11. There were shorter completion times on trials on the second testing session as compared to the first ($t(7) = 6.34$, $p < 0.0001$).

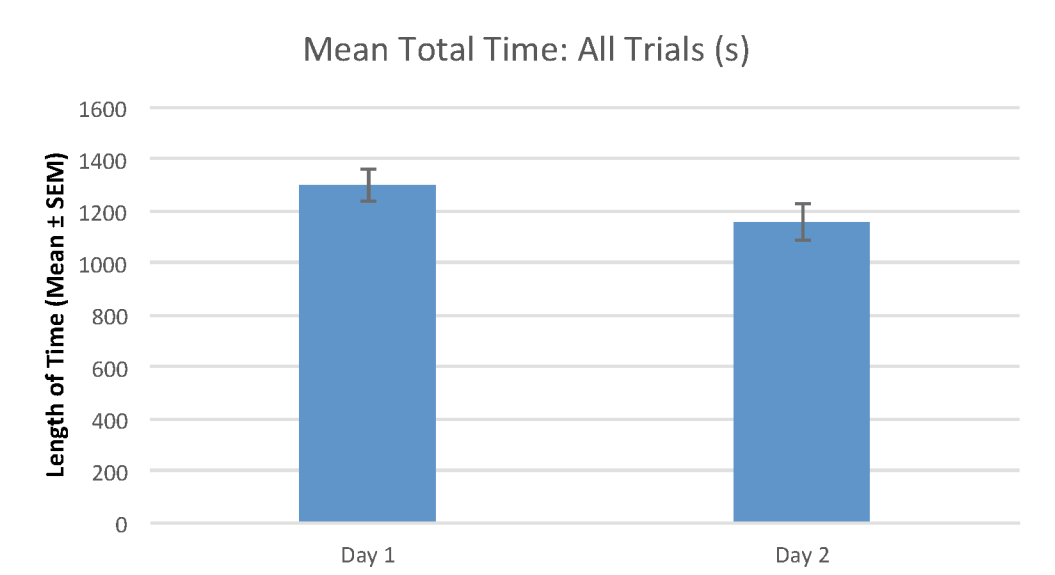


Figure F5: Mean rotational errors on probe time across test day1 and test day 2 irrespective of condition in Study 2, N=11. There were more errors made on test session one and none made on the second test session (*t(8) = 2.53, p = 0.035).*

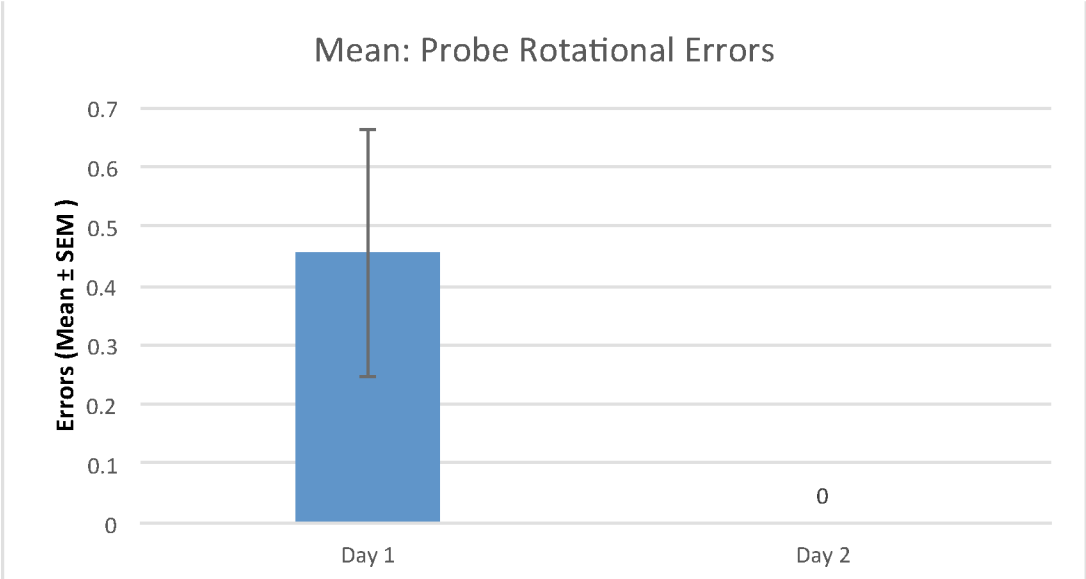


Figure F6: Mean total errors on part two of all trials across Test Day1 and Test Day 2 irrespective of condition in Study 2, N=11. More errors were made on the first testing session whereas none were made on the second testing session (*t(8) = 2.14, p = 0.065).*

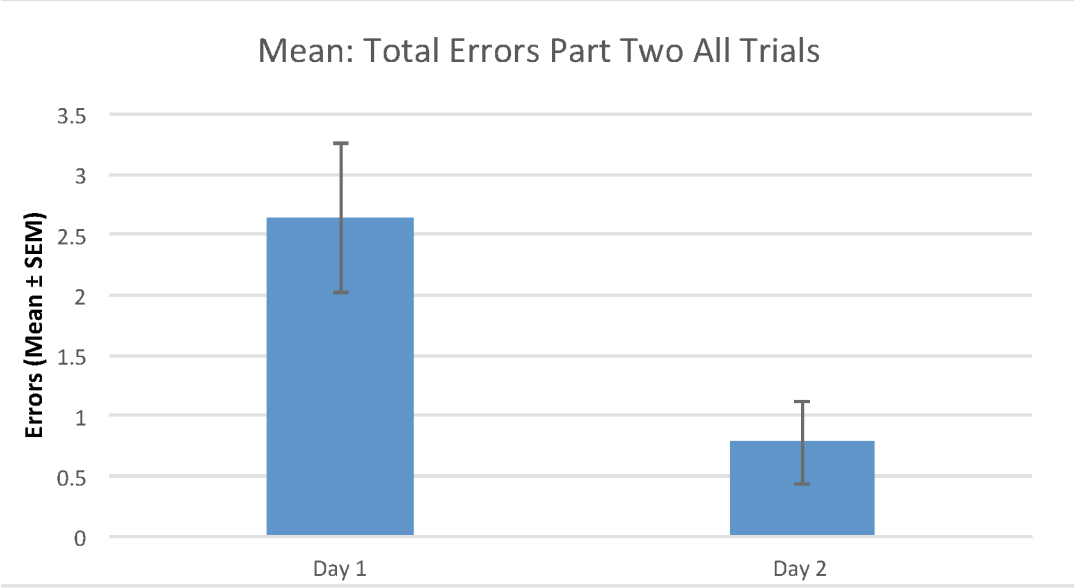


Figure F7: Mean total time across test day 1 and test day 2 irrespective of condition in Study 2, N=11. There were shorter completion times on trials on the second testing session as compared to the first (*t(8) = 4.95, p = 0.001).*

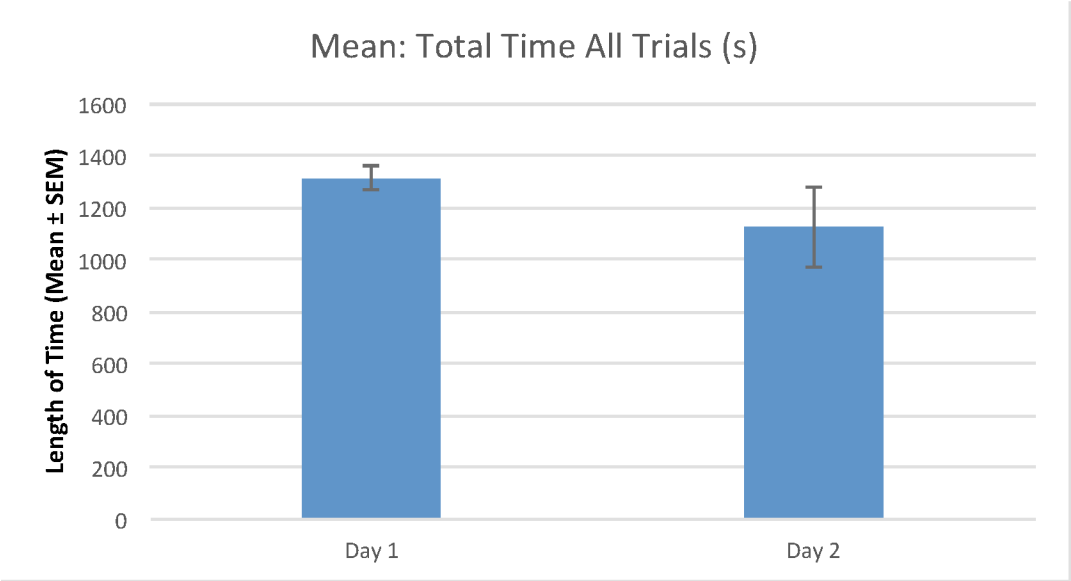


Figure F8: Time taken to complete learning trials in seconds. There was a uniform main effect of Trial for time taken to complete trials across AA Mixture groups in Study 3 ($F(2, 36) = 6.59$ $p= 0.004$). Abbreviations: BAL, Balanced amino acid mixture; APTD, Acute Phenylalanine Tyrosine Depletion mixture.

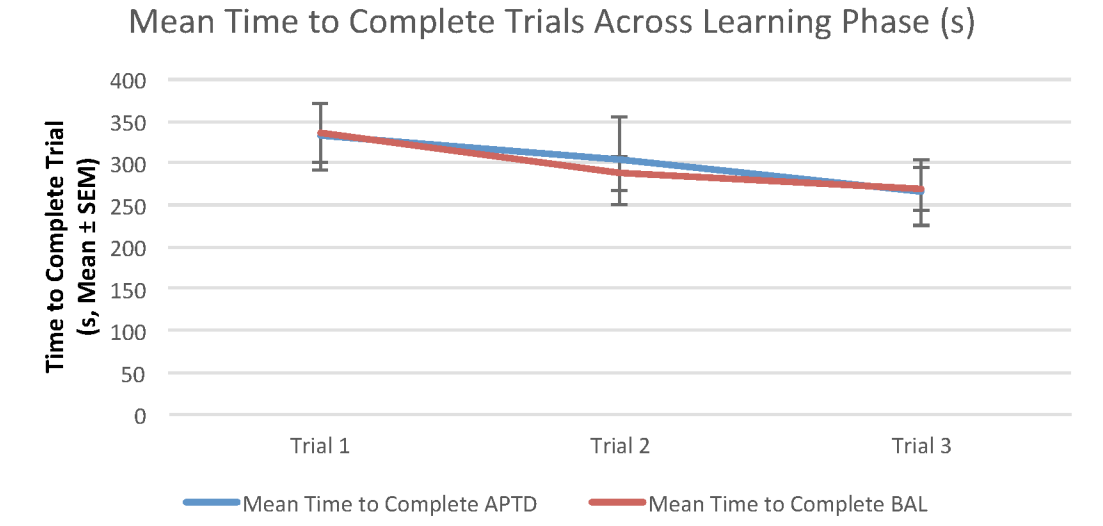


Table F3: Description of 4/8VM Variables

4/8 VM Variable Name	**Description**
Trials to Criteria	Number of trials needed until performance in part 2 had 100% accuracy
Total time on trial	Time taken for participant to complete both parts of a given trial in seconds.
Working Memory Errors	Revisiting either a rewarded or non-reward pathway in a given trial.
Reference Memory Errors	Visiting a rewarded pathway that had an object in part 1 of a trial but was no longer rewarded (empty) in part 2 of the same trial.
Absolute Errors (Probe Only)	Visiting an incorrect pathway (reference memory error) and/or revisiting any pathway (working memory error) in the probe.
Rotational Errors (Probe Only)	Considering what the least number of errors would be if the goal arms in absolute space were rotated around the radial maze. This reveals knowledge of the learnt relationship between goal arms.
Total Errors: Part Two of All Trials	The number of errors made in the second part of each trial administered, excluding the probe trial. This includes errors made in part two of trials 1-3 and 5.

Discussion Appendix

Table G1: Overview of acute and subchronic double-blind placebo-controlled studies describing cognition enhancing drug- or nutrition effects on tests of episodic memory in humans. (From Riedel and Blokland, 2015 and reprinted with permission from Springer)

Table 1 Overview of acute and subchronic double-blind placebo-controlled studies describing cognition enhancing drug- or nutrition effects on tests of episodic memory in humans.

Study	Population sample	*n* (M/F)	Age	Tx	Mechanism	Regimen	Design	Doses	Task	Measure	Effect	Clinical area
Theunissen et al. (2013)	Healthy	11/13	31, 0	Vortioxetine	5-HT	16 d	db-co	10 mg	30 w	Recall	No drug-placebo difference	
Wingen et al. (2006)	Healthy	9/9	31, 4	Escitalopram	5-HT	15 d	db-co	10–20 mg	30 w	Recall	No drug-placebo difference	
Schmitt et al. (2005)	Healthy with PMS Sx	0/16	18–45	Alpha-lactalbumin	5-HT	Acute	db-co	20 g	PRM	Recog	Alfa-lactalbumin improved delayed pattern recognition	PMS
Sambeth et al. (2014)	Healthy	7/9	19–34	Citalopram	5-HT	Acute	db-co	20 mg	30 w	Recall	No drug-placebo difference	
Gron et al. (2005)	Healthy	30/0	23, 9	Donepezil	Ach	30 d	db-pg	5 mg	15 w	Recall	Donepezil improved immediate recall	
Gron et al. (2006)	MCI	42/0	69, 3	Galantamine	Ach	7 d	ca-ctrl	4 mg bid	16 w	Recall	Galantamine improved immediate and delayed recall	Aging, dementia
Stough et al. (2009)	Healthy	43	22–66	Procera AVH	Ach	30 d	db-pg	1,500 mg +15 mg +150 mg	QoM	Accuracy	The combination product improved episodic memory accuracy	Aging, dementia
Balsters et al. (2011)	Healthy	6/14	59–77	Donepezil	Ach	4 w	db-pg	5 mg	PAL	Accuracy	Donepezil impaired accuracy	Aging, dementia
Theunissen et al. (2014)	Healthy cannabis preTx	9/6	21, 23	Rivastigmine, vardenafil	Ach, PDE5	Acute	db-co	3 mg (riv), 20 mg (var)	30 w	Recall	Rivastigmine attenuated cannabis-induced impairment of delayed recall	
Izquierdo et al. (2008)	Healthy	50/55	16–82	Methylphenidate	DA	Acute	db-pg	10 mg	LM	Recall	MPH improved consolidation	Aging, dementia

Table 1 (continued)

Study	Population sample	*n* (M/F)	Age	Tx	Mechanism	Regimen	Design	Doses	Task	Measure	Effect	Clinical area
Zeeuws and Soetens (2007)	Healthy	36/0	18–25	d-amphetamine	DA	Acute	db-co	10 mg	20 w	Recall	Improved delayed recall (consolidation)	
Zeeuws et al. (2010b)	Healthy	40/0	18–25	d-amphetamine	DA	Acute	db-co	10 mg	70 w	Recog	Improved delayed recognition (consolidation)	
Linssen et al. (2014)	Healthy	10/10	18–28	Levo/carbi-dopa	DA	Acute	db-co	125 mg	30 w	Recall	No drug-placebo difference	
Hermens et al. (2007)	Healthy	32/0	18–30	Methylphenidate	DA	Acute	db-co	5, 15, 45 mg	12 w	Recall	No drug-placebo difference	
Zeeuws et al. (2010a)	Healthy	17/0	18–30	d-amphetamine	DA	Acute	db-co	10 mg	70 w	Recog	Improved delayed recognition (consolidation)	
Apud et al. (2007)	Healthy by COMT genotype	24/23	18–55	Tolcapone	DA	1 w 6 d	db-co	100–200 tid	15 w	Recall	Trend drug x Gx, val/val Gx improved; met/met Gx worsened	
Randall et al. (2005)	Healthy	29/31	19–22	Modafinil	DA	Acute	db-pg	100, 200 mg	PRM	Recog	Modafinil both doses improved pattern recognition	
Muller et al. (2013)	Healthy	31/33	19–36	Modafinil	DA	Acute	db-pg	200 mg	PRM	Recog	Modafinil improved delayed pattern recognition	
Linssen et al. (2012)	Healthy	19/0	19–37	Methylphenidate	DA	Acute	db-co	10, 20, 40 mg	30 w	Recall	Methylphenidate improved delayed recall	
Kuypers and Ramaekers (2005)	Healthy	9/9	20–39	Methylphenidate	DA	Acute	db-co	20 mg	15 w	Recall	No drug-placebo difference	

Riby et al. (2006)	Healthy	27	20–80	Glucose	Glucose	Acute	db-co	25 g	PAL	Recall	Greater immediate recall in glucose vs. placebo condition	Aging, dementia
Christmas et al. (2014)	Healthy	32/0	18–55	Org25935	GlyTRI	Acute	db-pg	12 mg	20 w	Recall	No drug-placebo difference	
Liem-Moolenaar et al. (2010)	Healthy scopolamine preTx	43/0	18–55	R213129	GlyTRI	Acute	db-co	3, 10, 30 mg	30 w	Recall	No drug-placebo difference	
van Ruitenbeek and Mehta (2013)	Healthy	8/8	18–50	Betahistine	Histamine	Acute	db-co	2 × 48 mg	PAL	Accuracy	No drug-placebo difference	
Reneerkens et al. (2013)	Healthy	5/19	18–25	Vardenafil	PDE5	Acute	db-co	10 mg, 20 mg	30 w	Recall	No drug-placebo difference	
Benton et al. (2013)	Healthy	0/285	21, 8	DHA	PUFAs	50 d	db-pg	400 mg	30 w	Recall	DHA impaired recall at 50 days end of treatment	
Jackson et al. (2012)	Healthy	46/94	18–35	EPA, DHA	PUFAs	12 w	db-pg	90 + 450 mg; 300 + 200 mg	15 w	Recall	No drug-placebo difference	
Karr et al. (2012)	Healthy	12/29	18–35	EPA, DHA	PUFAs	4 w	db-pg	720 mg + 480 mg	15 w	Recall	Minor improvement of delayed recall after PUFA	Aging, dementia
Stonehouse et al. (2013)	Healthy low DHA in diet	83/145	18–45	EPA + DHA	PUFAs	6 mo	db-pg	170 mg + 1,160 mg	15 w	Recall	Improved speed of word recognition and improved recall in females	
Stough et al. (2012)	Healthy	74	45–80	EPA + DHA	PUFAs	90 d	db-pg	60 mg + 252 mg	QoM	Accuracy	No drug-placebo difference	Aging, dementia
Vakhapova et al. (2010)	Elderly with memory complaints	62/60	50–90	PS + EPA + DHA	PUFAs	15 w	db-pg	300 mg + 20 mg + 60 mg	15 w	Recall	PS + DHA only improved 1st trial immediate recall	Aging, dementia

Table 1 (continued)

Study	Population sample	*n* (M/F)	Age	Tx	Mechanism	Regimen	Design	Doses	Task	Measure	Effect	Clinical area
Yurko-Mauro et al. (2010)	Healthy with ARCD	485	>=55	DHA	PUFAs	24 w	db-pg	900 mg	PAL	Accuracy	Better PAL performance after DHA supplementation	Aging, dementia
Lee et al. (2013)	MCI	8/27	>=60	EPA + DHA	PUFAs	12 mo	db-pg	150 mg + 430 mg	15 w	Recall	DHA improved immediate and delayed recall	Aging, dementia
Dangour et al. (2010)	Healthy	411/337	70–79	EPA + DHA	PUFAs	24 mo	db-pg	200 mg + 500 mg	16 w	Recall	No drug-placebo difference	Aging, dementia

MCI mild cognitive impairment, *ARCD* age-related cognitive decline, *Pre Tx* pretreatment, *Tx* treatment, *Gx* genotype, *PMS* premenstrual syndrome, *Procera AVH* acetyl-L-carnitine + vinpocetine + huperzine A, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *PS* phosphatidylserine, *PUFA* polyunsaturated fatty acid, *GlyTRI* glycine transporter reuptake inhibitor, *DA* dopamine, *Ach* acetylcholine, *5-HT* serotonin, *PDE5* phosphodiesterase-5 inhibitor, *w* weeks, *d* days, *mo* months, *db* double-blind, *pg* parallel groups, *co* crossover, *ca-ctrl* case-control, *mg* milligrams, *tid* three times daily, *bid* twice daily, *μg* micrograms, *N-w* N word list, *PAL* paired associates learning, *LM* logical memory, *PRM* pattern recognition memory

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