WORKING MEMORY TRAINING INCREASES GENERAL LEARNING ABILITIES IN CD-1 OUTBRED MICE

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ABSTRACT OF THE DISSERTATION

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General intelligence is a cognitive trait that is purported to influence most domain-specific learning abilities in humans. Like humans, CD-1 outbred mice express individual differences in their "general" cognitive abilities, such that performance across diverse batteries of learning tasks tend to be positively correlated, and this general learning factor accounts for 32-48% of the variance of individual animals performance in cognitive test batteries. It has been demonstrated that in both humans and mice, the efficacy of working memory capacity correlates highly with measures of general cognitive ability. In three experiments, here we demonstrate that in genetically heterogeneous mice, repetitive working memory training promotes an increase in selective attention and has a commensurately positive effect on the animals' aggregate performance on a battery of five learning tasks. The enhancement of general cognitive

performance by working memory exercise was attenuated if the selective attention demands of that exercise were reduced. Finally, because much of the human research conducted on working memory training is done in pre-pubescent children, we trained a group of mice beginning in pre-pubescence and found no difference between that group and one trained at our typical young-adult age. In total, these results provide initial evidence that the efficacy of working memory capacity and selective attention are causally related to an animal's general cognitive performance, and suggest behavioral strategies to promote those abilities.

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General Introduction

In humans, general intelligence, or "g," has been called the "single most dominant cognitive trait ever discovered" (Plomin, 1999). General intelligence is one single dominant factor that is purported to influence all domain-specific learning abilities in some, as of yet, unknown manner. While the psychometric properties of general intelligence have been studied extensively in humans, the elucidation of a neurological substrate for this trait has been greatly restricted by the ethical and logistical constraints inherent in human research.

Like humans, CD-1 outbred mice express individual differences in their "general" cognitive abilities, such that performance across batteries of five to eight learning tasks tend to be positively correlated (Matzel et al, 2003; Kolata et al, 2008). Furthermore, via the application of principal component analysis, a general learning factor has been identified that accounts for 32-48% of the variance of individual animals. A mouse model of human intelligence can then provide a way to circumvent the ethical restrictions of human research and thus open the brain to research and thereby develop a more complete understanding of this trait.

In order to verify that this primary factor extracted from mice's performances across a battery of learning tasks truly a general learning factor, other possible mediators of this performance need to be assessed. First, because mice are generally stressed by learning tasks and the handling and novel food rewards associated with them, it was plausible that stress responses or emotionality, rather than learning abilities, could have been primarily responsible for performances across all tasks in the learning battery. This possibility was examined in two ways. The first was to examine various measures of

emotionality and stress responsivity and their relation to the primary factor (Matzel et al, 2006). Here it was found that stress reactivity, as measured by stress-induced corticosterone levels, was related only to measures of emotionality, but not exploration. Importantly, measures of emotionality were found to be unrelated to general learning abilities. Second, stress was manipulated through the use of the anxiolytic drug chlordiazepoxide (Grossman et al, 2007). Here manipulations of stress levels by the drug did not impact general learning abilities. That is, mice which experienced a reduction of stress hormone did not perform better than their controls across all tasks in our learning battery.

Another potential mediator of performances across the battery of learning tasks is the propensity for exploration. In prior work it was determined that native exploratory tendencies were found to correlate with general learning abilities (Matzel et al 2003, 2006). Therefore it was possible that our primary factor represented an exploratory factor rather than a true learning factor, as mice which explored their environments more might learn within them more efficiently due to their heightened exploration rather than a more effective learning mechanism. To evaluate this possibility, exploration was up-regulated. To achieve an up-regulation of exploration, mice were exposed to either a series of twelve novel environments or a single novel environment twelve times (Light et al, 2008). Both of these manipulations succeeded in producing more exploratory mice, but failed to produce mice which outperformed mice which did not receive novel environment adaptation across all tasks in the learning battery. Thus we concluded that the primary factor extracted from all learning tasks was not mediated by mice's exploratory tendencies. While the relationship between exploration and general learning

abilities has not been definitively explained, unpublished research points to rates of habituation (learning) as its primary determinant. That is, mice which habituate faster to the less stressful portions of the environment (i.e., walled quadrants in an open field or walled arms of an elevated plus maze) tend to explore the more stressful portions of that apparatus at an earlier time point and thereby spend more time in stressful portions of the apparatus than their more slowly habituating cohorts.

All of this previous research has led us to the assertion that the primary factor extracted from mice's performances across a battery of learning tasks trait is, in fact, a general learning factor, and therefore possibly a mouse analogue of "g" (Blinkhorn, 2003; Kolata et al, 2008). It is thus reasonable to ask what processes *do* mediate or modulate this latent factor. To this end, it has been determined that performance on tasks that heavily tax the working memory system tend to correlate very highly with independent measures of general intelligence (Kolata et al, 2005). To examine this, animals were first assessed in the learning battery and then were tested on a complex working memory task that consisted of simultaneous performance in two radial arm mazes with overlapping visual cues. The extent to which the animal was affected by the interference created by performing in two mazes simultaneously was correlated to their general learning abilities. That is, mice which were affected less by the interference tended to be perform better across all learning tasks. This was contrasted by the effect of a delay between arm choices in a single radial arm maze. In this case, where time induced a decay of the memory rather than competing information, the rate of decay was *not* correlated with performance in the learning battery. Thus resistance to interference from competing cues was more indicative of general learning abilities than the short term memory demands

created by a delay.

Working memory is thought to be comprised of both storage and processing components, the latter of which includes selective attention (Baddeley, 1986, 2003; Jarrold & Towse, 2006). In a series of experiments, we have found that the component of working memory which correlates most highly with general intelligence is selective attention (Kolata et al, 2007). That is, the ability to direct one's attention to the target, while simultaneously disregarding distracting stimuli, appears to be the component of working memory most relevant to its relation to general intelligence. Here, subsequent to the learning battery, animals were tested on three working memory tasks designed to maximally task one of three components of working memory (selective attention, short-term memory duration, and short-term memory capacity) while minimizing demands on the other two. Specifically, a mouse-adapted Stroop task maximally taxed selective attention, a delayed reinforced alternation task maximally taxed short-term memory duration, and a non-spatial radial arm maze (in which animals maintained memory of multiple choices) maximally taxed short-term memory capacity. Here it was shown that the largest relationship was between “Stroop” test performance and general learning abilities, indicating that selective attention was the component of working memory most responsible for the relationship between working memory and general intelligence.

This set of findings in mice corroborated and extended work that had previously been reported in the human intelligence literature. For the past decade, studies have found a correlation between the construct of general fluid intelligence and the construct of working memory (Colom et al, 2004; Conway et al, 2002; Engle et al, 1999; Jarrold & Townse, 2006; Sub et al, 2002; for review, see Conway, Kane & Engle, 2003). That is,

when latent variables are extracted from both tests of intelligence and tests of working memory, the two latent variables tend to correlate rather extensively. Estimates of the magnitude of the relation vary widely, however. The problem here is that in the human literature the line between a “working memory” task and those tasks which constitute the intelligence test used tend to be highly related. Any overlap between the two sets of tests would clearly tend to enhance the relationship between the two constructs extracted from those sets. Therefore estimates of the magnitude of the relation between intelligence and working memory tend to depend highly on the precision with which experimenters choose their "working memory" tasks, but a significant positive correlation persists in a robust manner nonetheless.

In order to determine whether or not the relationship between working memory and general intelligence is causal in nature such that better working memory abilities directly lead to higher intelligence, a working memory training regimen is necessary, a theme reviewed by Tang and Posner (2009). One early attempt to this end was by Klingberg, Forssberg, and Westerberg (2002). Participants were trained on a computerized regimen of working memory tasks which contained a visuo-spatial working memory task, backwards digit span, letter span with an n-back component, and choice reaction time. In their first experiment, children diagnosed with attention deficit hyperactivity disorder (ADHD) received this training for 20 minutes a day, 4-6 days a week, for 6 weeks. For this group, the training got progressively more difficult over the course of the 6-week time period. A second group received similar training, for only 10 minutes a day, 4-6 days a week for 6 weeks. For this group, the tasks did not get more difficult over the course of the 6 weeks. The authors considered this a “placebo” training

condition. The most striking result of the progressive working memory training regimen was a 26% increase in the groups' score on Raven's progressive matrices, compared to a 2% increase seen in the placebo training group. Additionally, the progressive working memory training group showed larger pre-training to post-training differences in both the video-spatial working memory task that was used in a training and a second visuo-spatial working memory task that was not used in training.

In addition to having larger increases in both working memory and intelligence test performances, the children in the training group showed improvements on measures typically deficient in children with ADHD. Children with this disorder are typically impaired in a "Stroop" test, a phenomenon typically attributed to their impulsivity. After progressive working memory training, Stroop test performance was enhanced as measured by accuracy, but not time spent to complete the task. It appears from these results, then, that these children were less impulsive as a result of the training. Additionally, a very large (62%) reduction in head movements was observed in the post-test compared to the pre-test in the children trained on the progressive working memory regimen. The placebo training group demonstrated an increase in head movements on the post-test compared to the pre-test, though this does not appear to be significant (statistics not reported, SEM's overlap).

In their second experiment, conducted on mentally healthy college students, Klingberg et al (2002) found consistent results. No placebo control group was implemented here, but participants demonstrated improved performances in both the visuo-spatial working memory task used in the training regimen and a second one, decreased latencies in the Stroop task (no accuracy difference, presumably because it was

98.25% in the pre-test), and improved scores on Raven's Advanced Progressive Matrices. The very small sample sizes used in this study, however, and the lack of any sort of control group in the second experiment, make the results intriguing, but far from conclusive.

A subsequent study improved on those very small samples. Diamond *et al* (2007) trained pre-school students with a skill set they referred to as "Tools" which included training on approximately 40 tasks which comprised 80% of the students' school day, including vocalizing what one should do, dramatic play, and "aids to facilitate memory and attention" (Diamond et al, 2007). The researchers subsequently measured outcome on measures of executive function. They found dramatic increases in executive function, indicating the potential for better IQ scores, but IQ measures were not taken. While the training appeared effective, a problem with this study is that the curriculum included many different training tasks and therefore it cannot here be determined which were responsible for subsequent increases in executive function and which were not. Thus, while the results are generally encouraging for applications to children, and possibly adults, they are experimentally weak because there is no way to identify what caused the observed improvements.

Interestingly, when Diamond et al (2002) asked teachers about the overall behavior and obedience of the children trained on Tools, they were significantly better behaved than students trained on the control curriculum. Therefore while the results may be of little use to one trying to understand the nature of the relation between working memory and intelligence, they do provide intriguing evidence that working memory training regimens improve more than just intelligence. Presumably through an

improvement in executive function, this training regimen appears to have provided the children with a higher level of what one might call “self control.” When combined with the more empirical, less anecdotal evidence from Klingberg et al (2002) regarding Stroop test accuracy and head movements, the possibility arises that working memory training may serve as effective treatment for ADHD.

Posner conducted a study of the effects of working memory training as well. There they trained children, 4 and 6-years old, on an elaborate and extensive training regimen given over only five training sessions spread out across two to three weeks’ time. Questionably, the tests used to quantify outcome measures were identical to those used to train subjects. Therefore it is impossible to distinguish between practice effects of the training and a more general increase in the intelligence of the subjects. Curiously, the controls seem to have improved *more* than experimental children on all training measures even though they did not complete the training. Statistics for this comparison are not reported so there is no way to know if the increase is significant. All criticisms aside, though, it was found that children trained on the working memory task improved their IQ scores to a higher level than their untrained controls, albeit a very modest increase of 4.7 points, roughly a third of a standard deviation.

Finally, Jaeggi et al (2008) trained subjects on two n-back working memory tasks simultaneously, one visual and one auditory, for 8, 12, 17, or 19 days. Training on these working memory tests effectively increased general fluid intelligence, as measured by Raven's Advanced Progressive Matrices (Raven, 1990) or Bochumer Matrizen-Test (Hossiep, 1999). More extensive training led to larger improvements in general fluid intelligence in a fairly linear fashion (Jaeggi et al, 2008).

While this is the best evidence of improved IQ through working memory training to date, some researchers are still skeptical, citing important alterations to testing procedures as a possible confound to the study's findings (Moody, 2009). In the study, the group tested on a traditional IQ test (Raven's progressive matrices) was both given the least amount of working memory training and showed the smallest improvement. Groups given more training were given both a different test (BOMAT) and a format of that test which eliminates its progressive nature. The progressive nature of both Raven's and BOMAT are what make the results interpretable because it necessitates that higher scores are earned by solving more difficult items. The researchers reduced the time allowed to complete the BOMAT from 45 minutes to 10, which eliminates the possibility that any subject progressed to the most difficult items. Here higher scores would presumably have been earned by subjects who solved the easier items faster rather than subjects with the ability to solve more difficult items. (Moody, 2009) The effect of that alteration on the validity of the test is debatable. However, more importantly, it adds a second variable between the group that received the least training and all other experimental groups, which showed greater improvement. Therefore one cannot definitively determine whether the longer training time or the different test format created the apparent larger increase in intelligence.

Many of the problems seen in the human literature can be reduced or eliminated by using a mouse model. First, mice are trained by simpler tasks than humans, therefore the extensive training regimens of the human literature can be reduced to simpler elements and one can discern with a great deal more certainty which component or components of training are responsible for the effects of the regimen. Second, the

learning battery used in our laboratory can be used effectively and consistently to test the results of a training regimen, therefore the testing confounds of Jaeggi et al can be eliminated.

To this end, we have modified the radial arm maze task used by Kolata et al (2005). Rather than using relatively few trials to assess working memory, here we trained animals for twelve trials so that most, if not all, mice were able to learn the task and in so doing overcome the interference presented by overlapping cues. Specifically, mice were trained in two radial arm mazes (an elevated maze with 8 arms around a central hub that the mice navigates by extra-maze visual cues to find food rewards at the end of each arm), one at a time, which were situated in the same room and therefore shared a set of overlapping visual cues. The mice were then trained in such a way that they could perform asymptotically in both mazes before they were given 12 days of working memory training with a high selective attention load. In the relevant training, mice alternated choices between the two mazes *within* a trial, one trial a day for 12 days, over a period of 14 days (two days were designated as "off" days).

Using this working memory training regimen, we are able to assess its effects on several measures, including individual learning tasks in the learning battery, selective attention (as measured by performance on the Stroop test) and, most importantly, general learning abilities (as measured by performance across tasks in the learning battery). Demonstration an increase in general learning abilities as a result of working memory training would have two major implications. First, it would further verify that the general learning abilities studied in our laboratory are a mouse analogue of general intelligence. Thus far, we have demonstrated that our primary factor is a learning factor and that it

shares relationships common to general intelligence such as a relationship to exploration and a relationship to working memory. However, we would here have evidence that, general learning ability is manipulatable through mechanisms similar to those mechanisms through which general intelligence is manipulated, further verifying its similarity to the human construct. Further, this would indicate the possibility that there is an evolutionarily conserved mechanism at work here that could be studied in mice and extrapolated to human beings.

Further, we sought to extend the work done in the human literature by attempting to isolate the component of the training most relevant to its ability to increase general intelligence. Both previous research in our laboratory and human research on the relationship between general intelligence and various aspects of working memory seem to point to selective attention as the primary determinant of the relationship. Therefore we subsequently designed a version of the working memory training which minimized the selective attention demands of the task. To achieve this, we minimized the shared visual cues the mouse must use to navigate the two mazes by placing a curtain (with different patterns on each side) between the mazes. Should we significantly reduce or eliminate the effect of training with this lesser version of the manipulation we would, first, provide strong evidence that the relationship between working memory and general intelligence is heavily dependent on selective attention. Secondarily, we would be able to then suggest that human researchers focus on making working memory regimens maximally demanding on the abilities of their subjects to selectively attend to relevant stimuli in order to make them as effective as possible.

Finally, many human studies use very young, prepubescent, children as subjects

in experiments like those described above (Klingberg et al, 2002; Diamond et al, 2007) as it is assumed that working memory might be particularly labile during this stage of development. The age at which we typically experiment on our animals is one more analogous to young-adulthood in human subjects. Therefore we felt it necessary to examine the impact that beginning training at a pre-pubescent age would have on its effect on intelligence. Should we find that these mice outperform mice trained in young-adulthood, it would be strong evidence to support early intervention in pre-school and kindergarten classroom. Should the mice perform indistinguishably from their young-adult trained cohorts, it would indicate that training at any developmental time period should be effective in humans.

Experiment I

Methods:

This experiment was designed to assess the effects of working memory training in young adult mice on selective attention as measured by performance on a mouse-adapted Stroop test, domain-specific learning abilities as measured by performance on individual learning tasks, and general learning abilities as measured by an aggregate performance measure across the battery of individual learning tasks.

One difference between our work here and working memory training conducted in the human literature is that were are limited to only post-training tests of general learning abilities and selective attention. This is necessary for a number of reasons. Among them, it is advantageous to use naïve mice rather than mice which have already gone through a battery of testing to maximize our ability to discern effects of working memory training on general intelligence. Second, testing on a learning battery for a pre-test measure would span a great deal of the mouse's lifespan. That is, the few weeks necessary to conduct pre-tests would be analogous to a few years of a human's lifespan. Thus we had no direct measure of intelligence or selective attention prior to the start of working memory training. In lieu of a direct measure, we used a correlate of intelligence to match groups at the start of the experiment. To that end, here we have measured animals' activity in an elevated plus maze and matched groups for percent of entries into open arms, a measure previously demonstrated to be highly correlated with general learning abilities (Matzel et al, 2006).

Subjects:

Thirty outbred CD-1 mice were obtained at 45 days of age, an age typical of previous experiments in our laboratory. They were housed individually and maintained on ad libitum food and water (unless noted otherwise) in a temperature-controlled vivarium on a 12-hour light/dark cycle. They were allowed to acclimate to the vivarium for one week and were subsequently handled (removed from the home cage and held by the experimenter for 60s/day) for one week prior to behavioral testing.

Apparatus and Procedure:

This experiment was a two-group, between subjects design. All measures (e.g. exploration, general learning, and specific learning abilities) were tested statistically using analysis of variance. The two groups were matched for native exploratory tendencies based on the percentage of crossings they made into the open arms of an elevated plus maze when tested at 60 days of age (prior to working memory training and subsequent tests of learning). Native exploratory tendencies are a good predictor of general learning abilities (Matzel et al, 2003; Matzel et al, 2006) so in this manner we can equalize groups and match individual animals for general learning abilities without the complications that would arise from directly assessing these abilities before training.

Elevated Plus Maze

This maze had four arms constructed of grey Plexiglas in the shape of a “plus”. Each arm of the maze was 28 cm long and 6 cm wide, and the maze was elevated 30 cm above a white floor. Two opposing arms of the maze were enclosed in 8 cm high, grey Plexiglas walls, and the other two of the arms were unwalled (open). The maze was located in a 300 Lux environment.

Animals were placed in the center of the maze facing a closed arm, and their

behavior in the maze was recorded for 4 minutes. Of interest was the time to exit the first closed arm entered, total number of arm entries, percent time in closed and open arms, latency to enter the first open arm, percent of entries into open arms and the percent of re-entries into an arm previously exited. Generally, entries into open arms are considered to be stressful to animals, thus measures in the open arms provide indices of exploratory tendencies similar in nature to that of exploration of the open quadrants of an open field, which was used to assess exploration after radial arm maze training.

As noted earlier, mice were then divided into two groups, matched on percent of entries into open arms in the elevated plus maze. One group received working memory training as described below, while the other was kept in their home cages and received only handling equivalent to that received by the trained group.

Radial Arm Maze Training

Two radial arm mazes were constructed of Plexiglas, one black and one gray. They both consisted of an 18 cm diameter central hub surrounded by eight 40 x 5 cm arms. The arms had a .75 cm lip above the floor of the arms. The entire maze was raised 26 cm above its base. At the end of each arm was a 1 cm food cup. Each food cup had a hole at the bottom through which the scent of unavailable food was detectable to prevent mice from navigating by the scent of food.

The black maze was distinct from the gray maze in two ways other than color. First, the maze had a clear Plexiglas apparatus built onto its central hub, capable of raising and lowering clear Plexiglas doors between the hub and each arm. The apparatus was 18 cm tall and the entrances to the arms were arches 5 cm wide and 4 cm tall at their peaks. Second, shields made of clear Plexiglas were placed between arms to discourage

animals from moving between arms without passing through the central hub. These were 10 cm tall and extended 14 cm from the central hub. The two mazes were placed side by side in the same testing room and thus shared an overlapping set of extra-maze visual cues with which the animals navigated the mazes.

At the start of each trial, the animal was placed in the central hub of the maze and allowed to navigate freely until it successfully found and ate all eight Bio-Serv 14 mg dustless precision pellets, one located at the end of each arm. Mice were judged as choosing an arm when their hind paws crossed the edge of the clear Plexiglas shield in the black maze or an equivalent distance (marked on the side of the arms, out of view of the mice) on the gray maze. Errors were scored as choosing an arm from which food has already been obtained. In the black maze, navigation was minimally impeded by the operation of the doors to the central hub. They were opened immediately upon the start of a trial and closed when the animal selected an arm. The door to the selected arm was then re-opened to allow the mouse to re-enter the central hub, and the rest of the arms were opened immediately upon the mouse's re-entry to the central hub, producing a nominal "zero second" delay. That is, a delay that is less than a second, but one which could potentially discourage animals from choosing the next arm impulsively.

Training in these mazes occurred over approximately seven weeks and had six phases, with two days off between phases. During all radial arm maze training, animals were food deprived with only 90 min/day access to food. In this first experiment, all mice experienced the black maze as its primary (first) maze. The first phase was acclimation. On the first day of acclimation, animals were placed on each of the eight arms of the primary maze for 90s with no access to the central hub. One piece of food

was in each of the food cups and the number eaten by each mouse was recorded. On the second day, they were placed into the central hub of the primary maze with the entrance to one arm open and allowed to emerge from this hub onto that arm, which contained two food pellets, one in the cup and one outside of it. After emergence, animals were confined to the arm for 20 min or until food pellets were eaten. Both latency to emerge from the central hub and latency to eat the pellets (or time out) were recorded.

In phase two, animals received training in the primary(black) maze, one trial per day, for five days. In phase three, animals received training in the secondary (gray) maze, one trial per day, for five days. In phase four, animals received training in both mazes on alternating days. They were trained in the primary maze the first and third of four days, and the secondary maze the second and fourth of four days. In phase five, animals received training in both mazes on the same day. They were trained in the primary maze in the morning and the secondary maze in the afternoon with a four hour inter-trial interval (ITI).

All of the above training sessions were intended to have mice trained to asymptotic performance on both mazes at the same time. This allowed mice to perform in phase six, in which animals received 12 trials over the course of 16 days (with four "rest" days). During these trials, animals essentially performed in both mazes simultaneously (a procedure intended to heavily tax working memory and specifically selective attention). They began each trial in the primary maze, where they were allowed to make three correct choices (plus any errors). They were then moved by the experimenter into the secondary maze where they were allowed to make their first three correct choices (plus any errors). They were then moved back to the primary maze for

choices four through six, moved back to the secondary maze for choices four through six, moved back to the primary maze for the final two choices, and moved back to the secondary maze for the final two choices. Thus by the end of a trial, mice had obtained all 16 food rewards from both mazes. Mice tended to make more errors towards the end of a trial and comparatively few errors in the first 9-12 correct choices.

Open Field

After working memory training or equivalent handling, animals were tested in the open field after a day of rest. The open field was a square field (46 x 46 cm) with 13 cm high walls, constructed of white Plexiglas and located in a brightly-lit room (400 Lux) with a background noise of 65 dB_c. The field was divided into a grid comprised of 6 x 6 7.65 cm quadrants, where 20 of the quadrants abutted the outer walls of the field (i.e., "wall" quadrants), and 16 quadrants were displaced from the walls and comprised the interior (i.e., "open" quadrants) of the field.

To start the test, animals were placed in the center of the open field. After 20 sec had elapsed (during which the animals self-selected a starting location, typically a walled quadrant), the animals' behavior was monitored for 4-min. Throughout this time the animal's entries into walled and open quadrants were recorded. An entry was recorded whenever both front paws crossed the border of a quadrant. Both total activity (i.e., quadrant entries regardless of category) as well as the percentage of entries into unwalled (open) quadrants of the field were recorded.

Learning Tasks

After one day of rest following the open field test, animals began testing in the

learning battery at 112 days of age. While we have utilized batteries of as many as eight tasks, here we used five tasks to limit the amount of training (which could in principal facilitate working memory) received by control animals. The order of testing was designed to provide a temporal separation between any two tasks that are motivated by either food or water deprivation (to prevent excessive physical strain and to minimize any potential cross-task influences due to motivational factors). In addition, the testing order was designed to separate tasks based on similar processes or motor requirements (e.g. mazes of a similar nature, activity vs. passivity), again so as to minimize any potential transfer between tasks. All animals were tested in the following order: Water maze, Lashley maze, fear conditioning, odor discrimination, and passive avoidance.

The possibility exists that, despite a control group which receives no training, one could make the argument that repeated exposure to anything outside of the home cage could lead to an enhancement in learning abilities across tasks. That is, mice could become acclimated to performing in tasks outside of their home cages. This experiment was previously conducted (Light et al, 2008), with mice exposed to a series of novel environments or repeatedly exposed to a single novel environment. In that study, mice with repeated exposure to environments outside their home cages did not outperform controls in the learning battery. However, we altered our typical learning battery in that experiment to reduce the exposure of control mice to novel environments during battery testing.

To allow better comparisons between the present study and that previous study, the modifications to the learning battery made in that study were replicated here. Specifically, the acclimation days for both odor discrimination and passive avoidance

were eliminated, the acclimation time for fear conditioning was cut from 20 min to 10 min, and mouse's home cages were used in place of holding chambers in the spatial water maze. These modifications were made in that prior experiment to reduce the amount of exposure to novel environments received by the controls because that was the manipulation being tested. Full descriptions of the learning battery can be found below.

Spatial Water Maze

For this task, animals were immersed in a round pool of opaque water from which they were able to escape onto a hidden (i.e., submerged) platform. The latency for animals to find the platform decreased across successive trials. In this task, performance of animals can improve across trials despite the animals beginning each trial from a new start location. Such a procedure mitigates against egocentric navigation and promotes the animals' dependence on extra-maze spatial landmarks. As demonstrated by Morris (Morris, 1981), rats performance in the water maze does not rely on fixed motor patterns (i.e., performance improves despite the animal's irregular starting location) or the presence of discernable cues within the maze (e.g., visual, tactile, or olfactory signals). Instead, performance is dependent on the stability of extra-maze cues, or "landmarks", and is said to reflect the animals' representation of its environment as a "cognitive map".

We have developed a protocol in which mice exhibit significant reductions in their latency to locate the escape platform within ten training trials. In our protocol, animals were confined in a clear Plexiglas cylinder on the safe platform for 5 min on the day prior to training. Second, a considerably longer ITI (10 min) was used than is typical (c.f., 90 sec). Lastly, the maze, surround, and water were black and visual cues were constructed of patterns of lights.

A round black pool (140 cm diameter, 56 cm deep) was filled to within 24 cm of the top with water made opaque by the addition of a nontoxic, water soluble, black paint. A hidden 11 cm diameter perforated black platform was in a fixed location 1.5 cm below the surface of the water midway between the center and perimeter of the pool. The pool was enclosed in a ceiling-high black curtain on which five different shapes (landmark cues) were variously positioned at heights (relative to water surface) ranging from 24-150 cm. Four of these shapes were constructed of strings of white LEDs (spaced at 2.5 cm intervals) and included an "X" (66 cm arms crossing at angles 40^{o} from the pool surface), a vertical "spiral" (80 cm long, 7 cm diameter, 11 cm revolutions), a vertical line (31 cm) and a horizontal line (31 cm). The fifth cue was constructed of two adjacent 7 W light bulbs (each 4 cm diameter). A video camera was mounted 180 cm above the center of the water surface. These cues provided the only illumination of the maze, totaling 16 fc at the water surface.

On the day prior to training, each animal was confined to the escape platform for 360 sec. Training was conducted on the two subsequent days. On Day 1 of training, animals were started from one of three unique locations on each of six trials. (The pool was conceptually divided into four quadrants, and one starting point was be located in each of the three quadrants that did not contain the escape platform. The starting point on each trial alternated between the three available quadrants). An animal was judged to have escaped from the water (i.e., located the platform) at the moment at which four paws were situated on the platform, provided that the animal remained on the platform for at least 5 sec. Each animal was left on the platform for a total of 20 sec, after which the trial was terminated and the animal was returned to its home cage for a 10 min ITI. On each

trial, a 90 sec limit on swimming was imposed, at which time any animal that had not located the escape platform was placed by the experimenter onto the platform, where it remained for 20 sec.

Animals were observed from a remote (outside of the pool's enclosure) video monitor, and animals' performance was recorded on video tape for subsequent analysis. Day 2 of training proceeded as did Day 1, albeit with four trials only. After the last training trial, a 90 min retention period began, after which animals were tested with a "probe" trial. On the probe test, the escape platform was removed from the pool, and all animals were started from the first position for that day. A 90 sec test was conducted in which the animals' time searching in the target quadrant (that in which the escape platform will be previously located) and non-target quadrants were recorded.

Lashley III Maze

The Lashley III maze consisted of a start box, four interconnected alleys, and a goal box containing a food reward. Over trials, the latency of rats to locate the goal box decreased, as did their errors (i.e., wrong turns or retracing). Under certain conditions, such as the ones used here, mice rely heavily on fixed motor patterns rather than spatial navigation, which differentiates this task from the spatial water maze, described above. Here, the Lashley III maze was scaled for mice, and parameters were developed that support rapid acquisition. The maze was constructed of black Plexiglas. A 2 cm wide x 0.1 cm deep white cup was located in the rear portion of the goal box, and 45 mg BioServe (rodent grain) pellets served as reinforcers. Illumination was 80 Lux at the

floor of the maze. The maze was isolated behind a shield of white Plexiglas to prevent the use of extra-maze landmark cues.

Food-deprived animals were acclimated and trained on two successive days. On the day prior to acclimation, all animals were provided with two food pellets in their home cages to familiarize them with the novel reinforcer. On the acclimation day, each mouse was placed in the four alleys of the maze, but the openings between the alleys were blocked so that the animals were not able to navigate the maze. Each animal was confined to the start and subsequent two alleys for 4 min, and for 6 min in the last (goal) alley, where three food pellets were present in the food cup. This acclimation period promotes stable and high levels of activity on the subsequent training day. On the training day, each animal was placed in the start box and allowed to traverse the maze until it reached the goal box and consumed the single food pellet present in the cup. Errors were defined was making a wrong turn or retracing back to a previous choice points. Upon consuming the food, the animal was returned to its home cage for a 20 min inter-trial interval (ITI), during which the apparatus was cleaned. After the ITI, the mouse was returned to the start box to begin the next trial, and the sequence was repeated for five trials. Both the latency and errors to enter the goal box were recorded on each trial.

Associative Fear Conditioning

Two distinct experimental chambers (i.e., contexts; 32 x 28 x 28 cm, l x w x h) were used, each of which was contained in a sound- and light-attenuating enclosure. These boxes were designated as “training” and “testing” contexts, and differ as follows: The training context was brightly illuminated (100 Lux), had clear Plexiglas walls, no

lick tube, and parallel stainless-steel rods (5 mm, 10 mm spacing) formed the floor. The test context was dimly illuminated (6 Lux), the walls were covered with an opaque pattern of alternating black and white vertical stripes (3 cm wide), and the floor was formed from stainless 1.5 mm rods arranged at right-angles to form a grid of 8 mm squares. A water-filled lick tube protruded through a small hole in one wall of the test chamber, such that the tube's tip was flush with the interior surface of the wall at a point 3 cm above the floor. Upon contacting the tube, the animal completed a circuit such that the number of licks/sec were able to be recorded. This circuit was designed so that if an animal made continuous contact with the tube (i.e., "mouthed" the tip), the circuit recorded 8 licks/sec, a rate that approximates continuous licking.

In this procedure, animals were exposed to a stimulus (i.e., a CS; white noise) that terminated at the onset of a mild footshock (i.e., a US). These tone-shock (CS-US) pairings came to elicit conditioned fear responses when animals were subsequently presented with the tone. This learned fear can be assessed in various ways. In the present studies, fear was indexed by CS-elicited suppression of ongoing drinking, as this measure is easily and precisely quantified. "Lick suppression" is conceptually analogous to the more commonly used measure of CS-elicited generalized "freezing" (i.e., during that time in which an animal freezes it necessarily is not capable of drinking from a lick tube). To avoid any interaction of the training context, which itself acquires an association with shock, with the CS at the time of testing, training and testing were conducted in separate distinct contexts.

In the training chamber, a 0.6 mA constant-current scrambled footshock (US) was delivered through the grid floor. In both the training and test chambers, a 40 dB above

background white noise (the CS) was presented through speakers mounted at the center of the chambers ceiling.

Water-deprived animals were acclimated to the training and test chambers by placing them each in both contexts for 10 min on the day prior to training. Within several minutes of their first placement in the test context, water-deprived mice exhibit stable licking. When subsequently placed in the chamber, these animals initiate licking within 5-10 sec and lick at relatively stable rates for the subsequent 3-5 min. Training occurred in the training context in a single 20 min session during which each animal was administered a noise-shock pairing 7 and 14 min after entering the chamber. Each 10 sec noise terminated with the onset of a 500 msec footshock. Asymptotic performance (as evident in group means) has been observed with these parameters after 4-6 such pairings. Thus two pairings, in most instances, supports sub-asymptotic conditioned responding.

At the end of the training session, animals were returned to their home cages for 60 min, after which they were re-acclimated to the test context for 10 min where they were allowed free access to the lick tubes. On the subsequent day (23-25 hours post training), animals were tested. Each animal was placed in the test context whereupon, after making 25 licks, the noise CS was presented continuously until the animal completed an additional 25 licks. The latencies to complete the last 25 licks during the pre-tone interval and in the presence of the tone were recorded, with a 600 sec limit imposed on the second 25 licks, a limit not reached by any animal described here. With these measures, the ratio of latency to complete 25 licks in the presence of the tone CS and the latency to complete 25 licks prior to CS onset served as our index of learned fear.

Odor Discrimination and Choice

Rodents rapidly learn to use odors to guide appetitively-reinforced behaviors. In a procedure based on one designed by Sara (Sara, Roullet, & Przybyslawski, 2001) for rats, mice learned to navigate a square field in which unique odor-marked (e.g., almond, lemon, mint) food cups were located in three corners. Although food were present in each cup, it was accessible to the animals in only one cup, the one marked by mint odor. An animal was placed in the empty corner of the field, after which it explored the field and eventually retrieved the single piece of available food. On subsequent trials, the location of the food cups was changed (and therefore the starting location of the mouse), but the accessible food was consistently marked by the same odor, mint. On successive trials, animals required less time to retrieve the food and made fewer approaches (i.e., "errors") to those food cups in which food was not available. Using this procedure, errorless performance is typically be observed within 3-4 training trials.

A black Plexiglas 60 cm square field with 30 cm high walls was located in a dimly lit (10 fc) testing room with a high ventilation rate (3 min volume exchange). Three 4 x 4 x 2.0 cm (l, w, h) aluminum food cups were placed in three corners of the field. A food reinforcer (30 mg portions of chocolate flavored puffed rice) were placed in a 1.6 cm deep, 1 cm diameter depression in the center of each cup. The food in two of the cups was covered (1.0 cm below the surface of the cup) with a wire mesh so that it was not accessible to the animal, while in the third cup (the "target" cup), the food was able to be retrieved and consumed. A cotton-tipped laboratory swab, located between the center and rear corner of each cup, extended vertically 3 cm from the cups' surface.

Immediately prior to each trial, fresh swabs were loaded with 25 ul of either

lemon, almond, or mint odorants (McCormick flavor extracts). The mint odor was always associated with the target food cup. It should be noted that, in pilot studies, the odor associated with food was counterbalanced across animals, and no discernible differences in performance were detected in response to the different odors.

On what would normally be the acclimation day, animals were given 60 minutes of free feeding time at the same time of day they would have received it had they been acclimated. On the subsequent test day, animals received four training trials in the field with three food cups present. On each trial, an animal was placed in the empty corner of the field. On Trial 1, the reinforcing food was available to the animal in the cup marked by mint odor. An additional portion of food was placed on the top surface of the same cup for the first trial only. The trial continued until the animal retrieved and consumed the food from the target cup or reached a 300s time out, after which the animal was left in the chamber for an additional 20 sec and then returned to its home cage to begin a 6 min ITI. On Trials 2-4, the location of the food cups was re-arranged, but the baited cup remained consistently marked by the mint odor. Both the corner location of the mint odor and its position relative to the remaining odors were changed on each trial. On each trial, the latency to retrieve the food and errors were recorded. An error was recorded any time an animal made contact with an incorrect cup, or its nose crossesed a plane parallel to the perimeter of an incorrect cup. Similarly, an error was recorded when an animal sampled (as above) the target cup but did not retrieve the available food.

<u>One-Trial Passive Avoidance</u>

Animals learn to suppress movement to avoid contact with aversive stimuli. This "passive avoidance" response is exemplified in step-down avoidance procedures, where

commonly, an animal is placed on a platform, whereupon stepping off of the platform it encounters a footshock. Following just a single encounter with shock, animals are subsequently reluctant to step off of the safe platform. The animals' reluctance to leave the platform is believed to *not* reflect fear, because typical fear responses are not expressed in animals engaged in the avoidance response (Morris, 1974; Bolles, 1969). Upon stepping off the platform, animals here were exposed to a compound of bright light and loud oscillating noise rather than shock, so as not to duplicate stimuli between tasks (see fear conditioning, above). Like more common procedures, our variant of this task supports learning after only a single trial (i.e., subsequent step-down latencies are markedly increased).

A chamber illuminated by dim (< 5 fc) red light was used for training and testing. Animals were confined to circular ("safe") chamber (10 cm diameter, 8 cm high). The walls and floor of this chamber were white, and the ceiling was translucent orange. The floor was comprised of plastic rods (2 mm diameter) arranged to form a pattern of 1 cm square grids. A clear exit door (3 CM square) was flush with the floor of the safe compartment, and the door was able to slide horizontally to open or close the compartment. The bottom of the exit door was located 4 cm above the floor of a second circular chamber (20 cm diameter, 12 cm high). This "unsafe" chamber had a clear ceiling and a floor comprised of 4 mm wide aluminum planks that formed a pattern of 1.5 cm square grids oriented at a 45^{o} angle relative to the grids in the safe compartment. When an animal stepped from the safe compartment through the exit door onto the floor of the unsafe compartment, a compound aversive stimulus comprised of a bright (550 Lux) white light and "siren" (60 dBc above the 50 dB background) was initiated.

Animals were placed on the platform behind the exit blocked by the Plexiglas door. After 4 min of confinement, the door was retracted and the latency of the animal to leave the platform and make contact with the grid floor was recorded. Prior to training, step-down latencies typically range from 8-20 sec. Upon contact with the floor, the door to the platform was closed and the aversive stimulus (light, noise, and vibration) was presented for 4 sec, at which time the platform door was opened to allow animals to return to the platform, where they were again confined for 5 min. At the end of this interval, the door was opened and the latency of the animal to exit the platform and step onto the grid floor (with no aversive stimulation) was recorded. The ratio of post-training to pre-training step-down latencies was calculated for each animal and serve to index learning. It has previously been determined that asymptotic performance is apparent in group averages following 2-3 training trials; thus performance after a single trial reflects, in most instances, sub-asymptotic learning.

Mouse-Adapted Stroop Test

This task was conducted in two apparati. The first was the same black Plexiglas box used in the odor-guided discrimination task. The second was an identical box in all respects except it had white stripes on the inside walls. The odor discrimination apparatus was further modified with interchangeable walls that fit over the corners of the square boxes. Also made of black Plexiglass, these attachments fit over the existing corner walls of the apparatus, creating flat surfaces and placing cups on the floor. These flat surfaces were capable of being backlit by a single white led and had holes drilled into them in three shapes: a circle, an x and two horizontal lines. The food cups contained cotton and a metal grate. The starting location of the mouse did not contain a corner

attachment and thus was still a corner of the box. All parameters such as lighting conditions, ITI, and deprivation schedule were identical to those used in odor discrimination.

In the box previously used for odor discrimination, the cotton in the food cups contained the previously described odors and mice were trained to learn to associate the mint odor with food in the previously described manner. To eliminate the possibility that mice could smell the food reward, chocolate flakes were placed under the cotton in the two distracter cups. Though the lights behind the flat surfaces were not lit and the lighting in the room was dim, the three patterns of holes were associated with the odors in a random manner in order to assure these remain neutral components of the apparatus.

In the second phase of the task, mice began testing in the box with white stripes. Here the three flat surfaces were lit, creating visual cues for the mice to follow. There were no odorants on the cotton in the food cups. Chocolate flakes were again placed under the cotton in distracter cups. The mice were trained to associate the circle visual cue with food in this box and navigate the apparatus accordingly.

Once asymptotic performance was reached in visual discrimination, mice began alternating between days followed by two days of training where they experienced odor discrimination in the morning and visual discrimination in the afternoon, separated by approximately 4 hours. This assured that animals were at asymptotic performance in both tasks before experiencing the relevant testing day. On this final day of training, animals first entered the odor discrimination box with both sets of cues present. To perform effectively in the task, the animals were required to inhibit their association between visual cues and food to selectively attend to odor cues and find the food. Next

they were placed in the visual discrimination apparatus with both sets of cues present and therefore had to inhibit their associations between odor cues and food to selectively attend to visual cues and find the food. Animals then continued to alternate between boxes in this manner until they had experienced each box three times.

Results:

Prior to radial arm maze training, there were no differences between groups in the elevated plus maze for percent of open entries (Figure 1A), $F(1, 25) = .01$, n.s. After radial arm maze training, mice trained on the radial arm maze explored the open quadrants of the open field significantly more than mice which did not receive working memory training (Figure 1B), $F(1, 25) = 12.22$, $p < .001$.

When performing in both radial arm mazes simultaneously, mice's performances improved over trials as indicated by the number of errors made to find all sixteen food rewards (Figure 2A). Subsequently, in the mouse-adapted Stroop test, mice that received working memory training made significantly fewer errors than mice which did not receive working memory training (Figure 2B), $F(1, 25) = 8.20$, $p < .01$. That is, mice which received working memory training were significantly less affected by the presence of distracter cues. There was no interaction between group and trial, $F(2, 50) = .18$, n.s.

In the water maze, mice learned the task, as evidenced by a significant reduction in latency to find the hidden platform across trials, $F(9, 225) = 5.16$, $p < .00001$. However, there was no statistical difference between groups (Figure 3A), $F(1, 25) = 1.56$, n.s, nor was there an interaction between group and trial, $F(9, 255) = 1.65$, n.s. Importantly, there was a consistent trend towards shorter latencies for mice which received working memory training. Consequently, during the probe trail, mice which

received working memory training spent significantly more time in the target quadrant (Figure 3B), $F(1, 26) = 5.38$, $p < .05$. This is indicative of better learning and/or retention by the end of the test.

In the Lashley III maze, mice learned the task, as evidenced by a significant reduction of errors across trials (Figure 4A), $F(4, 100) = 11.25$, $p < .000001$. However, there was no difference between groups, $F(1, 25) = 2.66$, n.s. nor was there an interaction between group and trial, $F(4, 100) = .63$, n.s. Again, however, mice which received working memory training consistently made fewer errors across trials, although it did not reach significance.

In fear conditioning, there was no statistical difference between groups (Figure 4B), $F(1, 25) = 2.44$, n.s.

In odor discrimination, mice learned the task, as evidenced by a significant reduction of errors across trials (Figure 5A), $F(3, 75) = 37.42$, $p < .000001$. However, there was no difference between groups, $F(1, 25) = 1.02$, n.s. nor was there an interaction between group and trial, $F(3, 75) = .28$, n.s. However, mice which received working memory training made consistently fewer errors than mice which did not receive working memory training across all four trials, although this nominal difference did not reach significance.

In passive avoidance, there was a trend towards greater step-down latency ratios for mice that were trained in the radial arm maze (Figure 5B), $F(1, 25) = 3.25$, $p = .08$.

To obtain general learning scores for all mice, a Pearson's product-moment correlation matrix was created from the data (Table 1A), which was then subjected to principal components analysis. A primary factor was extracted with an eiganvalue of

1.51, which accounted for 30 percent of the variance (Table 1B). From this primary factor, factor scores were extracted to represent animals' general learning scores. Mice trained on the radial arm mazes had significantly higher general learning scores than mice which received no working memory training (Figure 6A), $F(1, 25) = 4.48$, $p < .05$. Because it was possible that the manipulation differentially improved general learning scores in a subset of mice (e.g., either more intelligent or less intelligent mice), factor scores were compared for the top and bottom half of each group (Figure 6B). The top half mice trained on the working memory task had significantly higher general learning abilities than the top half of mice not trained on the working memory task, $F(1, 11) = 6.88$, $p < .05$. This effect was replicated in the comparison between the bottom halves of both groups, $F(1, 11) = 13.92$, $p < .005$.

Discussion:

In this experiment, animals that were trained on two radial arm mazes and extensively trained to perform in them simultaneously performed significantly better on a test of selective attention (the mouse-adapted Stroop test) than mice not trained on the working memory task. This increase in selective attention abilities led to an increase in general intelligence scores compared to equivalently handled mice. Further, this effect was evident in comparisons within the faster learning subset of each group as well as the slower learning subset of each group. In other words, working memory training appeared to improve the general learning abilities of all mice regardless of innate learning ability levels. Interestingly, their performances on individual learning tasks were not

significantly better than those of mice which did not receive working memory training, though the trend towards better performances was consistent throughout the learning battery.

The finding that general learning differences were significantly different between groups despite only trends toward significant differences across individual learning tasks might not be as surprising as it first seems. While general learning abilities, and in humans general intelligence, has an influence over domain-specific abilities, it is not necessarily the primary determinant of performance in individual learning tasks. Overall, general learning abilities here only account for 30% of the variance across all tasks in the learning battery, and its greatest influence on an individual task is the water maze, and in this case only accounts for 50% of the variance between animals. Presumably due to the large influence of general learning abilities on the water maze task, mice given working memory training performed significantly better than mice which did not receive working memory training on the probe test. One could imagine, then, that the impact of enhanced general learning abilities creates effects on the rest of our individual tasks too small to reach statistical significance in our small sample. It is only when this factor is extracted from each learning task in the battery and then quantified in a *general* learning score, then, that we are able to examine the effect of our manipulation on this *general* process.

The factor analytic approach, then, has the ability to discern general learning effects from domain-specific (such as spatial navigation) or task-specific (such as food neophobia) effects. This was demonstrated in a previous study (Light et al, 2008). Throughout the learning battery of that study, some trends towards significant learning effects were observed. However, when our general learning factor was extracted from

the data and factor scores were obtained, the groups were nearly identical on their general learning abilities. Here again the factor analytic approach was able to discern general learning effects from task-specific effects. Therefore beyond providing good evidence that our working memory training improves the general learning abilities of our mice, when one combines this set of results to our previous set, we see conclusively that our factor analytic approach is uniquely able to discern real learning effects from domain and task-specific effects.

Importantly, using the factor analytic approach, Light et al (2008) determined that mice which were free to explore either one or many novel environments outside of their home cages demonstrated no subsequent increase in their general learning abilities. This is important because one might imagine a scenario where *any* experience where a mouse leaves its home cage could promote a subsequent increase in general learning abilities. Additionally, unpublished results from our laboratory indicate that mice extensively trained in a Hebb-Williams maze (briefly, a negatively reinforced maze where the path the mouse must navigate changes daily) do not show a subsequent increase in general learning abilities. Therefore we can conclude here that there is something unique to this working memory training task (i.e. working memory or selective attention demands) which is responsible for the observation that mice trained on the working memory task demonstrate an increase in general learning abilities subsequent to training.

Experiment II

We have previously broken down working memory into three major component parts: selective attention, short-term memory duration, and short-term memory capacity (Kolata et al, 2007). That study suggested that, of those three, selective attention is most related to general learning abilities. Thus, reducing the selective attention component of working memory training should reduce or even eliminate the effect of working memory training on the subsequent expression of general learning abilities. This experiment was designed to test this possibility by manipulating the level of selective attention demand the working memory training regimen placed on the animal.

It is likely that the overlap of cues between mazes used in the previous experiment placed the largest demand on selective attention. Presumably, mice must inhibit memories involving overlapping cues in one maze while performing in the other. In other words, if a single cue is used by the mouse to navigate to arms in both mazes, the mouse must attend to whether or not it has navigated to that cue in the current maze and simultaneously block out whether or not it has navigated to that cue in the other maze.

In this experiment we employed a three-group, between subjects design. One group of mice received working memory training in two mazes with overlapping cues, as described in the previous experiment. This group was intended to receive the full selective attention load of the task. A second group was intended to receive a reduced selective attention load. To that end, this group received working memory training in two mazes without overlapping cues. A third group was intended to receive only task-irrelevant components of training. This group received handling equivalent to the first two for the duration of working memory training, were food deprived on the same

schedule, and were given food rewards equal to those received by the groups trained on working memory regimens, in their home cage.

Subjects:

Forty-five outbred CD-1 mice were obtained at 45 days of age, an age typical of previous experiments in our laboratory. They were housed individually and maintained on ad libitum food and water unless noted otherwise in a temperature-controlled vivarium on a 12-hour light/dark cycle. They were allowed to acclimate to the vivarium for one week and were subsequently handled (removed from the home cage and held by the experimenter for 60s/day) for one week prior to behavioral testing.

Apparatus and Procedure:

All apparati have previously been described, but there was one procedural change. In the fear conditioning task, mice were not water deprived in this experiment, the measure obtained was freezing rather than lick suppression, and the animals received 3 pairings of a pulsed (changed from continuous) white noise with shock on the training day. This new procedure allowed us to obtain a measure of learned fear on all four trials (3 on the training day in the "training" context and one 24 hours later in the novel, "testing" context).

In this experiment, 45 mice arrived at 45 days of age. As in Experiment I, they were all assessed in the elevated plus maze at 60 days of age. They were then assigned to one of three groups, matched for exploration in the elevated plus maze: one group received radial arm maze training as described in Experiment I and two new groups.

One of the two new groups received radial arm maze training as described in Experiment I with only one alteration. A curtain with different designs on each side was

placed between the two radial arm mazes. This curtain effectively split the room in half, leaving one maze in each half of the room. The relevant effect of this alteration was to have mice trained on two radial arm mazes without overlapping cues, in the same manner as they were trained in Experiment I. In humans, cue overlap has been shown by Conway and Engle (1994) to be highly relevant to the relationship between working memory and general fluid intelligence. In our laboratory, measures of selective attention (heavily taxed by cue overlap) is a better predictor of general intelligence in mice than working memory span (Kolata et al, 2007). Therefore the presence of overlapping cues in the previous experiment is a particularly strong candidate for the relevant component of radial arm maze training for it to increase general learning abilities in mice.

A modified control group was also used in this experiment. Control mice received equivalent food deprivation and identical food rewards, delivered in their home cages, in addition to equivalent handling. It was a concern that mice in the previous experiment could have benefitted from the food deprivation schedule or familiarity with novel food rewards rather than the actual working memory training. Should the present experiment fail to replicate a difference in general learning abilities between mice that receive overlapping cues and mice that do not receive working memory training, either food deprivation or receipt of food rewards would have to be accepted as the cause of the difference rather than working memory training.

Results:

Prior to radial arm maze training, there were no differences among groups in the elevated plus maze for percent of open entries (Figure 7A), $F(2, 24) = 1.14$, n.s. After training regimens were complete, the groups differed significantly for percent of entries

into open quadrants of an open field (Figure 7B), $F(2, 24) = 5.49$, $p < .05$. Post-hoc analysis revealed significant differences between the group receiving overlapping cues and both the group receiving no overlapping cues ($p < .05$) and mice which received no working memory training ($p < .01$). The group receiving no overlapping cues did not differ from mice which received no working memory training.

When performing in two radial arm mazes simultaneously, mice in both groups made significantly fewer errors across trials (Figure 8A). However, mice in the condition with overlapping cues performed significantly better than those without overlapping cues, $F(1, 17) = 5.31$, $p < .05$. There was also a strong trend towards an interaction between group and trial, $F(11, 187) = 1.82$, $p = .052$. Whether this effect is due to task difficulty or some effect of experiencing overlapping cues earlier in training cannot be determined here.

In the mouse-adapted Stroop test there was a strong trend towards a main effect of group (Figure 8B), $F(2, 24) = 3.31$, $p = .054$. A planned comparison revealed a significant difference between mice that received overlapping cues and mice that did not ($p < .05$). There was also a trend towards an interaction between group and trial, $F(4, 48) = 2.39$, $p = .06$.

In the water maze, mice learned the task, as evidenced by a significant reduction of latencies to find the hidden platform across trials (Figure 9A), $F(9, 216) = 4.13$, $p < .0001$. The main effect of group was not statistically significant, though there was a trend towards working memory trained mice outperforming mice which received no working memory training, $F(1, 25) = 2.24$, n.s. Planned comparisons revealed a strong trend towards mice that received overlapping cues outperforming mice which received no

working memory training ($p = .053$). There was no interaction between group and trial, $F(18, 216) = .90$, n.s. In the probe trial, a trend was seen towards a main effect of group for time spent in the target quadrant (Figure 9B), $F(2, 26) = 2.033$, $p = .15$. Planned comparisons revealed a strong trend towards mice trained with overlapping cues outperforming mice which received no working memory training ($p = .058$). No other groups were statistically different.

In the Lashley III maze, mice learned the task, as evidenced by a significant reduction of errors across trials (Figure 10A), $F(4, 96) = 19.12$,$p < .000001$. There was also a main effect of group, $F(1, 25) = 2.66$. Planned comparisons revealed significant differences between the group that received overlapping cues and the group that received no overlapping cues ($p < .05$) as well as mice which received no working memory training ($p < .05$). There was no statistical difference between the group that received no overlapping cues and mice which received no working memory training. There was also no interaction between group and trial, $F(8, 96) = .96$, n.s. In fear conditioning, there was no statistical difference among groups (Figure 10B), $F(2, 24) = .25$, n.s.

In odor discrimination, mice learned the task, as evidenced by a significant reduction of errors over trials (Figure 11A), $F(3, 72) = 75.96$, $p < .000001$. There was a main effect of group, $F(2, 24) = 4.04$, $p < .05$. Planned comparisons revealed significant differences between the group that received overlapping cues and mice which received no working memory training($p < .05$) and the group and received no overlapping cues and mice which received no working memory training ($p < .05$). The two groups that received radial arm maze training did not differ. There was a trend towards an interaction between group and trial, $F(6, 72) = 2.20$, $p = .052$.

In passive avoidance, there was a trend towards a main effect of group for step-down latency ratios (Figure 11B), $F(1, 25) = 3.25$, $p = .08$. Post-hoc tests revealed group differences between only the group that received overlapping cues and mice which received no working memory training ($p < .01$). The group that received no overlapping cues was intermediate to the other two groups, but did not differ statistically from either.

To obtain general learning scores for all mice, a Pearson's product-moment correlation matrix was created from the data obtained from the five tasks in the learning battery (Table 2A), which was then subjected to principal components analysis. A primary factor was extracted with an eiganvalue of 1.73, which accounted for 35 percent of the variance (Table 2B). From this primary factor, factor scores were extracted to represent animals' general learning scores. There was a main effect of group on factor scores (Figure 12A), $F(2, 24) = 10.57$, $p < .001$. Post-hoc comparisons revealed significant differences between the group that received overlapping cues during working memory training and both the group that received no overlapping cues during working memory training ($p < .05$) and mice that received no working memory training ($p < .0005$), as well as between the group that received no overlapping cues and mice that received no working memory training ($p < .05$).

We again asked if working memory training differentially affected the top or bottom half of the distribution of learning abilities (Figure 12B). In the top half of the distribution, there was a main effect of group, $F(2, 10) = 8.47$, $p < .01$. Planned comparisons revealed a significant difference between only the group receiving overlapping cues and mice which received no working memory training ($p < .005$). Trends towards differences between mice receiving overlapping cues and mice receiving

no overlapping cues ($p = .09$) as well as mice receiving no overlapping cues and mice which received no working memory training ($p = .059$) were found. In the bottom half of the distribution, the same pattern of results was observed. There was a significant main effect of group, $F(2, 10) = 13.97$, $p < .005$. Planned comparisons revealed significant differences between mice receiving overlapping cues and mice which received no working memory training ($p < .0005$), mice receiving no overlapping cues and mice which received no working memory training($p < .05$) and mice receiving overlapping cues and mice receiving no overlapping cues ($p < .05$).

Discussion:

This experiment provides a second instance of working memory training causing an improvement in selective attention abilities. This increase in selective attention abilities again coincided in an increase in general learning abilities in mice. Further, this was again true in mice drawn from the upper and lower halves of the distribution of general learning abilities, indicating again that the increase in general cognitive performance observed here occurs regardless of innate learning ability levels.

Importantly, when the selective attention load of the complex radial arm maze task was diminished, the effect of this training on general learning abilities was diminished as well. While the effect was not eliminated (animals in the group without overlapping cues during working memory training still exhibited better general learning abilities than mice which did not receive working memory training), it was significantly reduced compared to animals that received overlapping cues during working memory training.

Two possibilities can account for this diminished, but not eliminated, effect of

working memory training with a reduced selective attention component. First, it is possible that working memory training by itself, regardless of selective attention training, is sufficient to cause a small increase in general learning abilities. This is possible because working memory span does predict general learning abilities in mice as well as general intelligence in humans. It just does so to a lesser degree than tests of working memory which tax the selective attention system more heavily.

Another equally valid possibility, though, is that overlapping cues were simply *reduced* rather than eliminated. Because animals were physically moved from one maze to the other, the experimenter could have served as an overlapping cue between the two mazes. In addition, all cues were distinct to the human eye between mazes, but we cannot assume here that they were equally distinct to our mice. For instance, lights were displayed in different patterns between mazes, but if the mouse navigated using a more general distinction such as “lights” it could have been an overlapping cue. The present data cannot determine which of these two, if either, accounts for the presence of a reduced, but not eliminated, increase in general learning abilities in mice in the group without overlapping cues.

Experiment III

In the human literature, some previous researchers have studied the effects of working memory training in pre-pubescent children. Specifically, the children with ADHD studied by Klingberg (2002) and the Tools curriculum used in preschool children (Diamond et al, 2007) involved training regimens administered prior to puberty. The mice studied in the previous two experiments of this study were comparable to a point in the human lifespan which is substantially older, akin to young-adult humans. Because these manipulations in pre-pubescent children appeared quite successful in modulating executive function, and in the case of Klingberg et al (2002) intelligence test performance, the possibility is raised that working memory training could be more effective at a younger age.

It is well documented in mice that pre-pubescence is a time period where neural plasticity is at a very high level relative to later in life (Greenough et al, 1987). It follows, then, that this heightened plasticity could result in a maximization of the neural effects of working memory training, whatever they may be. However, research suggests that working memory, particularly the selective attention component (Desimone & Duncan, 1995; Duncan et al, 2000; Kane & Engel, 2002; Smith & Jonides, 1999; for review, see Conway, Kane & Engel, 2003), is dependent on the integrity of the prefrontal cortex. Therefore it is possible that a mature prefrontal cortex is necessary for working memory training to be effective.

Because research in gerbils shows that the medial prefrontal cortex continues to mature, in terms of dopaminergic innervation, until PND 90, the possibility exists that working memory training would be ineffective in pre-pubescent mice, although

maturation begins to slow down after PND 30 (Dawirs et al, 1993). However, further study of gerbils complicates the issue because social environmental enrichment promotes better maturation of the medial prefrontal cortex as well as working memory as assessed in a delayed alternation task (Winterfeld et al, 1998). Therefore working memory training could itself create the mature prefrontal cortex necessary for its effect on intelligence.

This experiment was intended to examine the possibility that working memory training could differentially affect mice in different stages of development. Therefore it is a replication of Experiment I in all respects except the age of one group of animals during working memory training. Here all mice were assessed for native exploratory tendencies at 32 days of age and one group began working memory training at 34 days, an age considered to correspond to the cusp of pre-pubescence and puberty (Hedrich, 2004) and completed training at 77 days of age. A second group began training at 60 days, an age comparable to that observed in Experiment 1 and Experiment 2. A third group received all task-nonspecific elements of working memory training (handling, food deprivation, and food rewards) as in Experiment 2.

Subjects:

Thirty CD-1 outbred mice obtained at 21 days of age were used in this experiment. They remained group housed until PND 28, at which time there were housed individually and maintained on ad libitum food and water unless noted otherwise in a temperature-controlled vivarium on a 12-hour light/dark cycle. They were allowed to acclimate to the vivarium for one week while group housed and were subsequently handled (removed from the home cage and held by the experimenter for 60s/day) for one

week prior to behavioral testing, after being individually housed.

Apparatus and Procedure:

All apparati and procedures for Experiment III were identical to those used in Experiment II with a few exceptions concerning the age of the mice when they experienced some of the tasks. These mice entered the elevated plus maze at 32 days of age and working memory training began at 34 days of age, pre-pubescence, for one group. A second group received working memory training at an age comparable to the comparable treatment, i.e. in Experiment I. All mice entered the open field as at 120 days and the learning battery started at 122 days. Control mice were split into two sub-groups, one of which was treated as in Experiment 2 but at PND 34, the other of which was treated as in Experiment 2 at PND 75.

Results:

Prior to radial arm maze training, there were no differences among groups in the elevated plus maze for percent of open entries (Figure 13A), $F(2, 27) = .36$, n.s. After training regimens were complete, in a second assessment of exploration in the open field, unlike in the first two experiments, there was still no main effect of group (Figure 13B), $F(2, 27) = .57$, n.s.

When performing in two radial arm mazes simultaneously, both groups made significantly fewer errors to find all sixteen food rewards over trials (Figure 14A), $F(11, 220) = 1.97$, $p < .05$. Mice of different ages did not differ in their performances on the working memory task, $F(1, 20) = .76$, n.s., nor was there an interaction between group and trial, $F(11, 220) = 1.24$, n.s. Subsequently, when tested for selective attention abilities in the mouse-adapted Stroop test, there was a main effect of group (Figure 14B),

$F(2, 26) = 18.02$, $p < .0001$. Planned comparisons revealed differences between both groups trained on the radial arm maze and mice which did not receive working memory training ($p < .001$ for both comparisons), however there was no difference between the two experimental groups which suggests that, although mice trained on a working memory task still out-perform mice which are not trained on a working memory task, the age at which working memory training was administered had no impact on selective attention when assessed in young-adulthood.

In the water maze, mice learned the task, as evidenced by significant reductions of latencies to find the hidden platform over trials (Figure 15A), $F(9, 234) = 7.39$, $p < .0001$. There were no group differences on this task, $F(2, 26) = .13$, n.s. There was no interaction between group and trial, $F(2, 234) = 1.06$, n.s. Group differences were not observed in the probe trial (Figure 15B), $F(2, 27) = 1.21$, n.s.

In the Lashley III maze, mice learned the task, as evidenced by a significant reduction in errors to find the food reward across trials (Figure 16A), $F(4, 108) = 23.34$, $p<.0000001$. There was a trend towards a main effect of group, $F(2, 27) = 2.04$, $p = .15$. Planned comparisons revealed a strong trend towards a difference between the group which began working memory training in pre-pubescence and mice which received no working memory training ($p = .054$). There was no interaction between group and trial, $F(2, 108) = .28$, n.s.

In fear conditioning, animals learned to associate the tone and shock as demonstrated by increased freezing to the tone across trials, $F(2, 50) = 58.80$, $p < .0001$. However, there was no statistical difference among groups (Figure 16B), $F(2, 25) = 1.26$, n.s. and no interaction between group and trial, $F(4, 50) = 2.03$, n.s.

In odor discrimination, mice learned the task, as evidenced by a significant reduction in errors to find the food reward across trials (Figure 17A), $F(3, 81) = 252.06$, $p < .000001$. There was no main effect of group, $F(2, 27) = .98$, n.s. There was a trend towards an interaction between group and trial, $F(6, 81) = 1.98$, $p = .07$. In passive avoidance, there was no main effect of group for step-down latency ratios (Figure 17B), $F(2, 27) = .80$, n.s.

To obtain general learning scores for all mice, a Pearson's product-moment correlation matrix was created from the data from all five tasks in the learning battery (Table 3A), which was then subjected to principal components analysis. A primary factor was extracted with an eiganvalue of 1.89, which accounted for 31.5 percent of the variance (Table 3B). From this primary factor, factor scores were extracted to represent animals' general learning scores. There was a strong tendency toward a main effect of group on factor scores (Figure 18A), $F(2, 27) = 3.34$,$p = .051$. Post-hoc comparisons revealed differences between mice which began training in pre-pubescence and mice which received no working memory training ($p < .05$) and a strong trend towards mice trained in young-adulthood and mice which received no working memory training ($p = .055$). There was no difference between mice that received the working memory training regimen at different ages.

Again it was necessary here to see if working memory training differentially affected the top or bottom half of the distribution of learning abilities (Figure 18B). In the top half of the distribution, there was a main effect of group, $F(2, 12) = 4.46$, $p < .05$. Planned comparisons revealed a significant difference between mice trained in pre-pubescence and mice not trained on a working memory task ($p < .05$) and a trend towards

mice trained in young-adulthood outperforming mice not trained on a working memory task ($p = .07$). In the bottom half of the distribution, the same pattern of results was observed. There was a strong trend towards a main effect of group, $F(2, 12) = 3.79$, $p = .053$. Planned comparisons revealed differences between mice trained on the working memory task in pre-pubescence and mice not trained on a working memory task ($p < .05$) and mice trained on a working memory task in young-adulthood and mice not trained on a working memory task ($p < .05$), but not between experimental groups.

Discussion:

From this experiment, there is little evidence to support the notion that training animals on a working memory task in pre-pubescence has a larger effect on general cognitive abilities than training them on the same working memory task post-puberty. This experiment, of course, has severe limitations. Most importantly, the duration of training is so long that it necessary extends into post-pubescence for mice. This is unavoidable in mice given the life cycle of a mouse and the length of the training. However, it remains possible that training in human infants would have a greater effect in pre-pubescence than later in life.

It is useful here to examine the timeline used with the group of mice which received working memory training beginning in pre-pubescence. This group experienced a rather lengthy period of rest between when working memory training ended and when testing in the open field and the subsequent learning battery began. The effect of working memory training on general learning abilities persisted in this group despite the relative deprivation of living in their home cages. This suggests several important points. First, it suggests that the effects of working memory training do not decline with time, at least on

the scale of weeks, without maintenance. That is, mice (and presumably humans) who undergo working memory training would reap the benefits of that training over long periods of time without the need for re-training and maintenance programs.

Second, it suggests that the effects of working memory training on the brain persist over this same time period. This is very important because it limits the list of possible mechanisms through which working memory training exerts its effects on the brain to those which would persist over a long period of time such as epigenetic effects or structural changes rather than something more transient. That is, something must become fundamentally different about the brain of a mouse that has been trained using one of these regimens and that difference has to be such that it does not tend to decrease with time, even in the face of relatively deprived living conditions.

Finally, the finding that working memory training is effective at improving both executive function and general cognitive abilities across these two very different developmental time points opens the door to the possibility that it can be effective across the lifespan. Further research is necessary to verify this, but it stands to reason that working memory training would effectively increase general cognitive abilities for the entire adult portion of life up until the onset of cognitive decline in old age. It is therefore plausible, if one considers our mice which have not undergone working memory training as reared in an impoverished environment (Winterfield et al, 1998), that an individual who grows up in an environment less conducive to intellectual development can overcome that deficit, even in mid-life. Most importantly, it opens the door to research on the effects of working memory training late in life, when cognition has already begun to decline.

General Discussion

We have here demonstrated through a series of three experiments that training mice on a complex working memory task improves both executive function of those mice and their general learning abilities. The overall goal of increasing intelligence through training on a complex working memory task here was three-fold. First, we wanted to verify our mouse model of intelligence by demonstrating that it behaves similarly to human intelligence when manipulated. Second, we wanted to provide further support and guidance to the developing human literature on working memory training so that it may be optimally applied to healthy populations of humans. Third, we wanted to provide a purely behavioral manipulation of learning abilities in mice so that further study of learning and general intelligence in the mouse brain, in a between-groups manner, is possible.

Toward the first goal, further verification of our mouse model of intelligence, the present study provides further evidence that the cognitive trait studied here is analogous to general intelligence in humans. We have, up to this point, verified our model of general learning abilities by eliminating alternate hypotheses of what our primary factor might represent. For example, variations in stress reactivity (Matzel et all, 2006; Grossman et al, 2008) and exploration (Light et al, 2008) have been evaluated and subsequently eliminated as potential mediators of this general factor. The present set of experiments extend this line of reasoning by examining the impact on general learning abilities of a manipulation reported in the human literature to impact performance on common tests of intelligence. Because general learning abilities in mice here respond to a working memory training regimen similarly to the manner in which human intelligence

responds to an analogous training regimen, we have provided evidence that, not only is our primary factor a general learning factor, but that our general learning factor is, in this respect, analogous to general intelligence in humans.

Toward the second goal, further support for the efficacy of working memory training regimens in increasing intelligence, the present set of experiments provides strong evidence for an increase in general learning abilities through training regimens that tax the working memory system. This is consistent with recent work in the human literature (Diamond et al, 2007, Jaeggei et al; Klingberg et al, 2002; Tang & Posner, 2009). One might also make a case for the possibility that what is being observed in this study is an effect of environmental deprivation in the mice which did not receive working memory training. In other words, one might think that *any* manipulation done outside the home cage would have a corresponding increase in general learning abilities. This would provide yet another account of why our mice which experienced no overlapping cues showed an increase in general learning abilities. However, when simply exposed to novel environments for twenty minutes a day over the same period of time as the training observed here, no increase in general learning abilities was observed (Light et al, 2008).

The second part of our second goal is that we wanted to provide guidance to the developing human literature on the topic of working memory training regimens. The human literature has thus far been limited to varying the duration or difficulty of the working memory training rather than discerning components of that training which maximize its effect on subsequent tests of cognitive performance. The work presented here takes an important step toward achieving that goal by comparing a training regimen which acutely taxes the selective attention system with one which places lesser demands

on that process. From this manipulation we have here been able to determine that the selective attention load of the working memory task has a strong impact on the ability of a working memory training regimen to improve general cognitive abilities. While we cannot here claim that taxation of the selective attention system is solely responsible for the increase in general learning abilities, we can confidently state that it augments the effect of the training regimen.

It is important to note here that all research on working memory training, our study included, tends to differ from real-world applications of a working memory training regimen in a way that likely reduces the impact the training makes on the subject's intelligence. These manipulations are both too standardized and too short. It is nearly certain that some of the mice studied here were "left behind" during preliminary training and therefore had a harder time with the most difficult version of the task, performing in both radial arm mazes simultaneously. In the laboratory it is necessary to move forward when the group as a whole appears to be ready to do so, but in a real-world application one could easily wait for individuals who take more time. Because these individuals likely have more to gain from the training in the first place, this could greatly enhance the overall success of the training for increasing both executive function and intelligence.

Due to limited time and resources, these training regimens are far shorter than is possible in a real-world application. One could envision training that persists over the course of years, decades, and perhaps a lifetime. Individuals undergoing such training could conceivably achieve mastery of difficult training sets impossible to examine in a laboratory setting. Jaeggi et al (2008) provided possible evidence that a longer training schedule has a larger effect than a smaller one and, using their placebo control group,

Klingberg et al (2002) provided evidence (at least in children with ADHD) that training regimens which increase in difficulty over time are more effective than simple regimens which never become more difficult. From the present research, one can envision a training regimen where the selective attention demands of the training increase steadily over the lifespan of the participant, maximizing the regimen's effects on intelligence. All three of these lines of research point to the fact that the improvements we see in the laboratory are just a small taste of what could be possible in a real-world application of a personalized, long-term working memory training regimen.

The third goal of our study was to provide a manipulation whereby general learning abilities can be manipulated in order to allow future research to examine related changes in the brain between manipulated and unmanipulated groups. Toward that end, it is useful here to begin to narrow possible mechanisms through which working memory training can enhance general learning abilities using evidence from previous studies, as well as more recent evidence from our laboratory. Importantly, we will attempt to narrow down both the region of the brain where the largest change is likely and the specific system within that region that is most likely to have changed due to working memory training.

While current brain imaging techniques used in humans lack both the temporal and anatomical precision required to truly understand the structural properties associated with cognitive traits such as intelligence, they can provide evidence for a gross anatomic region in which to begin to study. Toward that end, Duncan et al (2000) used positron emission topography (PET) to study the brains of patients as they completed two versions of a set of three cognitive tasks. In one version, the tasks were highly correlated with

general intelligence, in the second version, they were not. The group then subtracted PET activity generated by the set of tasks orthogonal to g from activity generated by the set of correlated tasks. They found that the lateral prefrontal cortex had the most consistent subtracted activation across all sets of tasks examined.

We previously noted that Klingberg et al (2002) successfully used a working memory training regimen to improve both intelligence and executive function in children with ADHD and healthy adult subjects. The research group subsequently used brain imaging (here functional magnetic resonance imaging, fMRI) to examine whether their working memory training regimen caused brains of patients trained on the regimen to activate differently from brains of patients which did not receive the training regimen in response to the set of post-training tests. In that study, Olesen et al (2004) conducted two experiments, in which adult male subjects were trained on the same working memory regimen as the previous experiment for five weeks. In experiment 1, only 3 subjects were trained on the working memory regimen and compared to 11 control subjects, which were not trained on the working memory regimen, on pre and post-training measures. Measures included cognitive performances on tests previously studied as well as fMRI activation while the subject completed both a visuo-spatial working memory task and a control task. Cognitively, the group replicated their previous results, including increases on scores for Raven's progressive matrices and reduced latencies to complete a Stroop task in the group which received working memory training. fMRI results revealed an increase in prefrontal cortex activity in the group trained on the working memory regimen compared to participants not trained on the working memory regimen for the post-test session. Experiment 2 expanded on experiment 1 by training eight subjects who

were scanned 5 times over the 5-week training period. This experiment replicated the previous results.

These imaging studies provide us with strong evidence that the frontal cortex would be a good place to look for effects of working memory training on the brain. However, one might ask whether there is any evidence that enhancement in the prefrontal cortex could affect brain regions responsible for more domain specific learning effects. To this end, research on the hippocampus provides a direct relationship between attention and learning and memory in brain structures other than the prefrontal cortex. Kentros et al (2004) studied the activity of complex-spike pyramidal neurons in the CA1 region of the hippocampus that tend to fire when the animal is in a specific location in a given context, termed "place cells" by O'Keefe (1971). Place cell firing was observed across four tasks conducted in the same environment but with various attentional demands. In order from no attentional component to maximum, the tasks were as follows: no task (simple exploration of the apparatus), foraging for food (used by O'Keefe to observe place cells in his original paper), switching between a novel environment and the original environment, and a spatial learning task. In the spatial learning task, the environment was lit with bright light and a noise (both of which are aversive to rodents) and the mouse had to navigate to an unmarked location in the field that would turn off the aversive stimulation.

Stability of place cells (the consistency with which they fire in a given location) was shown to increase as a function of attentional demand. Additionally, using a subset of mice that did not learn the spatial task, they were able to compare the stability of place cells between "performers"(those which learned the task) and "non-performers"(those

which did not and were therefore eliminated from other analyses) and found that the place fields of performers were far more stable than those of non-performers. This provides evidence that "attention," or executive function, presumably generated in the prefrontal cortex, modulates learning in tasks associated with other brain regions, here specifically hippocampus-dependent spatial learning.

However, the frontal cortex is still too broad a target to begin research. It is still necessary to begin to narrow down what changes *within* the prefrontal cortex could result from working memory training. Towards this end, unpublished data from our laboratory provides preliminary support for a dopaminergic underpinning to general intelligence. Gene chip analysis of approximately 25,000 genes conducted between good learning and poor learning subsets of a typical set of genetically heterogeneous CD-1 mice found consistent up-regulation of three dopamine-related genes in the prefrontal cortex across three replications of the study. It is important to note here that fewer than 20 of 25,000 genes met this criterion. Confirmatory real-time PCR found that the expression of these three genes were also highly correlated to general learning abilities in a sample of nearly 60 mice. Specifically, the genes coded for the dopamine transporter darpp32, a component of the dopamine 1a receptor, and a g-protein related suppressor of dopamine's response to D2 signaling.

From the human imaging literature, then, we have evidence that the prefrontal cortex is both implicated in general intelligence and changed in some manner by a working memory training regimen. From our own laboratory we have evidence that prefrontal cortex dopamine is implicated as a critical difference between good and bad learners. In combination, these results suggest the possibility that dopamine transmission

is somehow modified in the prefrontal cortex as a result of working memory training. Further examination of the animal working memory literature provides further support for this hypothesis.

In mice, Kellendonk et al (2006) genetically modified mice to over-express the dopamine D2 receptor in the striatum. While mice over-expressing the D2 receptor were found to be normal in terms of locomotor activity, general anxiety, and performance on reference memory tasks, they were impaired on tasks of working memory as measured by a win-shift task in a radial arm maze and a delayed reinforced alternation task. Interestingly, reversal of the D2 over-expression did not reverse these working memory deficits, indicating that the presence of the receptors themselves are not the cause of the deficit, but rather cause some chronic change that results in the deficit. The downstream effect was localized to the prefrontal cortex. Dopamine innervation of prefrontal cortex was unchanged as measured by tyrosine hydroxylase-positive vericosities, but a decrease in dopamine turnover was indicated in the mice which previously overexpressed striatal D2 receptors. Specifically, transgenic mice demonstrated an increase in dopamine levels and a reduced ratio of metabolites to dopamine, specifically the DOPAC:DA ratio and HVA: DA ratio.

Additionally, Kellendonk et al (2006) examined D1 receptor activation in the medial prefrontal cortex in these transgenic mice. Using an injection of the D1 receptor agonist chloro-APB- hydrobromide, activation of D1 receptors was measured by immunohistochemical analysis of expression of the immediate early gene *c-fos*. Transgenic mice were found to have an increased activation of D1 receptors in the medial prefrontal cortex. Further, real-time PCR analysis revealed no differences in D1 or D5

receptor mRNA levels. Over-expressed D2 receptors in the striatum seemingly somehow sensitized D1 receptors in the prefrontal cortex and this sensitization presumably had a negative impact on working memory function but did not produce a more general cognitive impairment.

Further evidence for prefrontal cortex dopamine's role in working memory comes from research on gerbils. Winterfield et al (1998) altered dopamine innervation of the prefrontal cortex by providing one group of gerbils with a socially and environmentally enriched environment. The group demonstrated that the animals provided with socially and environmentally enriched environments showed greater dopaminergic innervation of the prefrontal cortex on PND 90. Consequently, these gerbils also displayed better working memory abilities, as assessed in a delayed reinforced alternation task in a Y-maze. This is evidence that enrichment paradigms could exhibit their cognitive effects through the working memory system. Further, it is another line of research which points to prefrontal cortex dopamine as necessary for working memory and perhaps working memory training's enhancement of general cognitive abilities.

Somewhat disparate sets of experiments, then, have provided converging evidence that prefrontal cortex dopamine is responsible, in some manner, for working memory and perhaps general cognitive abilities. It is therefore now possible, using the working memory training regimen presented in this study, to examine this possibility in a causal manner. Specifically, mice trained on the working memory task should differentially utilize dopamine D1 receptors in the prefrontal cortex. It would be interesting to additionally examine D2 receptors in the striatum due to their demonstrated relation to D1 receptors in prefrontal cortex (Kellendonk et al, 2006). Further, the

reduced selective attention version of the training used in Experiment 2 could be used here to determine if reducing the selective attention load reduces the neurological effect of training in the same manner that it reduces its effect on general learning abilities.

At the outset of this study, our measure of executive control, the Stroop test, was thought to be a confirmatory test. That is, if we had found no enhancement of general learning abilities by working memory training, we could then assure ourselves that working memory, or executive function, was enhanced by the manipulation as predicted, thereby confirming a successful training regimen even in the absence of general learning effects. Research on executive function, however, provides evidence that its increase as a result of working memory training is yet another important finding of the present study.

Executive function has been implicated in many mental disorders including attention deficit hyperactivity disorder (ADHD) and schizophrenia. If these mental disorders are, in fact, based at least in part on a deficit in executive control, then working memory training regimens might not only be useful in improving the mental capacities of "normal" people, but can also help to improve at least those two clinical populations as well.

The best evidence to date for working memory's training potential to alleviate symptoms of attention deficit hyperactivity disorder have been previously discussed in this report. Briefly, improvements in overall behavior was demonstrated by Diamond et al (2007) when their Tools curriculum was administered to preschool children and Klingberg et al (2002) demonstrated a large reduction in head movements of subjects with ADHD after working memory training. However, it is important to additionally note that many of the proposed underlying mechanisms for ADHD tend to include

prefrontal cortex dopamine (review in Castellanos and Tannock, 2002) and that drugs used to alleviate the symptoms of ADHD show both improvements in tests of attention and corresponding alterations of both dopamine and norepinephrine in the prefrontal cortex (Berridge, 2006).

In schizophrenia, Hutton et al (2002) studied the disorder in relation to executive function among first-episode patients. Like many prior studies, they found that patients with schizophrenia were impaired in many learning tasks including those that taxed spatial working memory and those that required the participant to plan a response. Interestingly, no deficit was found for a task of set-shifting ability, which is commonly used to test executive function. This result stands in contrast to studies of patients who have progressed beyond their first schizophrenic episode and suggests a progressive degeneration of executive function as trademark of the disorder (Hutton et al, 1998). Perhaps more importantly for the purposes of this report, schizophrenic patients have characteristics alteration in dopamine. For example, post-mortem examination of brains of schizophrenic patients have been shown to have reduced dopamine projections in layer 6 of the prefrontal cortex (Akil et al, 1999). Further, Lindstrom et al (1999) demonstrated elevated dopamine levels in both the striatum and prefrontal cortex. This could presumably result in a sensitization of D1 receptors in prefrontal cortex in a manner similar to the previously described transgenic study by Kellendonk et al (2006).

Finally, then, application of the mouse model described here may be uniquely appropriate to studies relevant to these clinical populations, because we can now make the problem of a neural basis for executive function tractable. Should the neurological basis for executive function be mapped out, it opens the door for targeted

pharmacological manipulation which could work, in conjunction with a working memory training regimen, to greatly improve the prognosis of these clinical disorders.

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Figure Captions

Figure 1A. In the elevated plus maze (pre-treatment), percent of crossings into open arms plotted as a function of group. Brackets indicate standard errors.
Figure 1B. In the open field (post-treatment), percent of entries into open quadrants plotted as a function of group. Brackets indicate standard errors.
Figure 2A. In the radial arm maze, total errors during the final (simultaneous performance) phase plotted as a function of trial. Brackets indicate standard errors.
Figure 2B. In the mouse-adapted stroop test, total errors plotted as a function of trial for each group. Note that each trial depicted consists of a summation of one trial in each context. Brackets indicate standard errors.
Figure 3A. In the water maze, latency to find the hidden plaform plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 3B. In water maze, total time spent in the target quadrant during the final 30 seconds of the probe trial plotted as a function of group. Brackets indicate standard errors. Dashed line indicates random performance.
Figure 4A. In the Lashley III maze, number of errors made before finding food plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 4B. In fear conditioning, ratio of the latency to complete 25 licks during the white noise to the latency to complete 25 licks prior to the white noise plotted as a function of group. Brackets indicate standard errors.
Figure 5A. In odor discrimination, total errors plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 5B. In passive avoidance, ratio of latency to step down after aversive stimulus to latency to step down prior to aversive stimulus plotted as a function of group. Brackets indicate standard errors.
Figure 6A. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group. Brackets indicate standard errors.
Figure 6B. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group for both the top and bottom half of the each group's learning distribution. Brackets indicate standard errors.
Figure 7A. In the elevated plus maze (pre-treatment), percent of crossings into open arms plotted as a function of group. Brackets indicate standard errors.
Figure 7B. In the open field (post-treatment), percent of entries into open quadrants plotted as a function of group. Brackets indicate standard errors.
Figure 8A. In the radial arm maze, total errors during the final (simultaneous performance) phase plotted as a function of trial for both groups. Brackets indicate standard errors.
Figure 8B. In the mouse-adapted stroop test, total errors plotted as a function of trial for each group. Note that each trial depicted consists of a summation of one trial in each context. Brackets indicate standard errors.
Figure 9A. In the water maze, latency to find the hidden plaform plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 9B. In water maze, total time spent in the target quadrant during the final 30 seconds of the probe trial plotted as a function of group. Brackets indicate standard errors. Dashed line indicates random performance.

Figure 10A. In the Lashley III maze, number of errors made before finding food plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 10B. In fear conditioning, ratio of the latency to complete 25 licks during the white noise to the latency to complete 25 licks prior to the white noise plotted as a function of group. Brackets indicate standard errors.
Figure 11A. In odor discrimination, total errors plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 11B. In passive avoidance, ratio of latency to step down after aversive stimulus to latency to step down prior to aversive stimulus plotted as a function of group. Brackets indicate standard errors.
Figure 12A. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group. Brackets indicate standard errors.
Figure 12B. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group for both the top and bottom half of the each group's learning distribution. Brackets indicate standard errors.
Figure 13A. In the elevated plus maze (pre-treatment), percent of crossings into open arms plotted as a function of group. Brackets indicate standard errors.
Figure 13B. In the open field (post-treatment), percent of entries into open quadrants plotted as a function of group. Brackets indicate standard errors.
Figure 14A. In the radial arm maze, total errors during the final (simultaneous performance) phase plotted as a function of trial for both groups. Brackets indicate standard errors.
Figure 14B. In the mouse-adapted stroop test, total errors plotted as a function of trial for each group. Note that each trial depicted consists of a summation of one trial in each context. Brackets indicate standard errors.
Figure 15A. In the water maze, latency to find the hidden plaform plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 15B. In water maze, total time spent in the target quadrant during the final 30 seconds of the probe trial plotted as a function of group. Brackets indicate standard errors. Dashed line indicates random performance.
Figure 16A. In the Lashley III maze, number of errors made before finding food plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 16B. In fear conditioning, ratio of the latency to complete 25 licks during the white noise to the latency to complete 25 licks prior to the white noise plotted as a function of group. Brackets indicate standard errors.
Figure 17A. In odor discrimination, total errors plotted as a function of trial for each group. Brackets indicate standard errors.
Figure 17B. In passive avoidance, ratio of latency to step down after aversive stimulus to latency to step down prior to aversive stimulus plotted as a function of group. Brackets indicate standard errors.
Figure 18A. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group. Brackets indicate standard errors.
Figure 18B. From principal components analysis of all learning tasks, general learning ability (primary factor score) is plotted as a function of group for both the top and bottom

half of the each group's learning distribution. Brackets indicate standard errors.

Table 1A: Correlation matrix for 27 animals from Experiment I

	FC	PA	WM	LM	OD
Fear Conditioning (FC)	1.00	-.01	-.09	.18	-.21
Passive Avoidance (PA)	-.01	1.00	.26	.08	.24
Water Maze (WM)	-.09	.26	1.00	.35	.04
Lashley III Maze (LM)	.18	.08	.35	1.00	-.02
Odor Discrimination (OD)	-.21	.24	.04	-.02	1.00

Table 1B: Unrotated Principal components analysis for 27 animals from Experiment I

	Factor 1	Factor 2
Fear Conditioning	-.104177	.706792
Passive Avoidance	.659630	-.211752
Water Maze	.771271	.170275
Lashley III Maze	.572879	.572411
Odor Discrimination	.381543	-.644596
eiganvalue	1.514589	1.316546
Variance Explained	.302918	.263309

Table 2A: Correlation matrix for 27 animals from Experiment II

	WM	FC	OD	PA	LM
Water Maze (WM)	1.00	-.26	.21	.10	.10
Fear Conditioning (FC)	-.26	1.00	.15	. 40	-.23
Odor Discrimination (OD)	.21	.15	1.00	.41	.16
Passive Avoidance (PA)	.10	.40	.41	.33	1.00

Table 2B: Unrotated Principal components analysis for 27 animals from Experiment II

	Factor 1	Factor 2
Water Maze	.260475	.647170
Fear Conditioning	.384864	-.832506
Odor Discrimination	.736276	.072646
Passive Avoidance	.868167	-.167069
Lashley III Maze	.467911	.520156
eiganvalue	1.730723	1.415647
Variance Explained	.346145	.283129

Table 3A: Correlation matrix for 30 animals from Experiment III

	EP	WM	FCN	OD	PA	LM
Elevated Plus Maze (EP)	1.00	-.21	-.02	.30	.06	.06
Water Maze (WM)	-.21	1.00	.20	.17	-.04	.37
Fear Conditioning (FC)	-.02	.20	1.00	.01	.07	.23
Odor Discrimination (OD)	.30	.17	.01	1.00	.34	.42
Passive Avoidance (PA)	.06	-.04	.07	.34	1.00	.24
Lashley III Maze (LM)	.06	.37	.23	.42	.24	1.00

Table 3B: Unrotated Principal components analysis for 30 animals from Experiment III

	Factor 1	Factor 2
Elevated Plus Maze	.212605	-.711360
Water Maze	.489182	.648657
Fear Conditioning	.359705	.420995
Odor Discrimination	.748555	-.371299
Passive Avoidance	.523596	-.331691
Lashley III Maze	.802513	.167104
eiganvalue	1.892403	1.379832
Variance Explained	.315401	.229972

Figure 1

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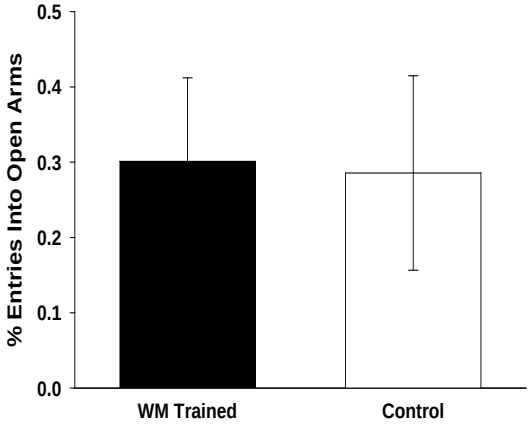

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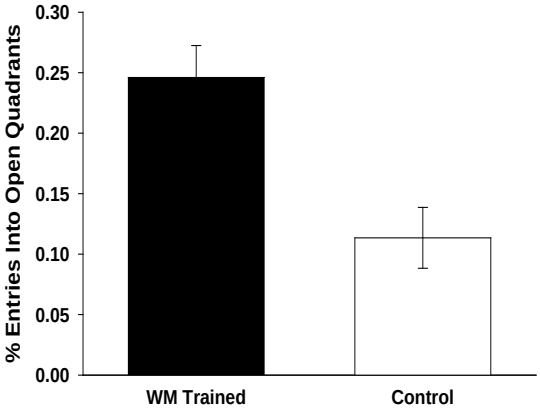

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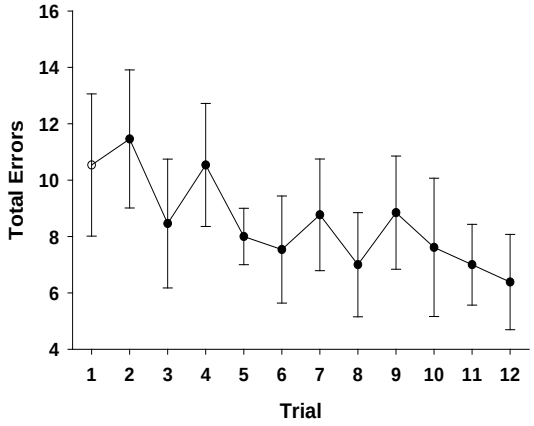

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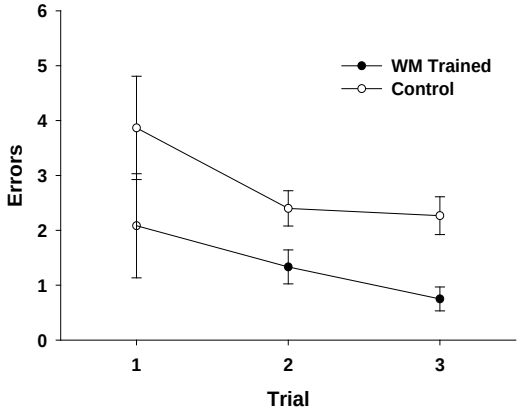

Figure 3

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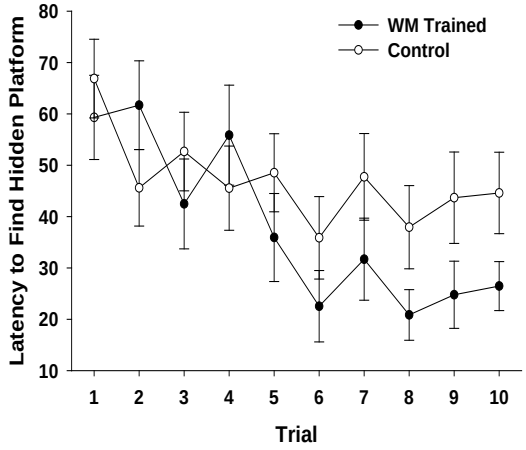

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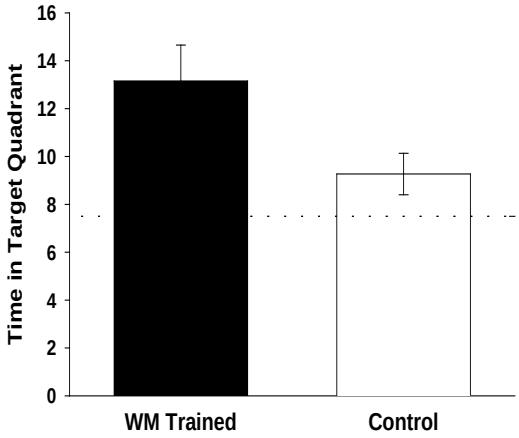

Figure 4

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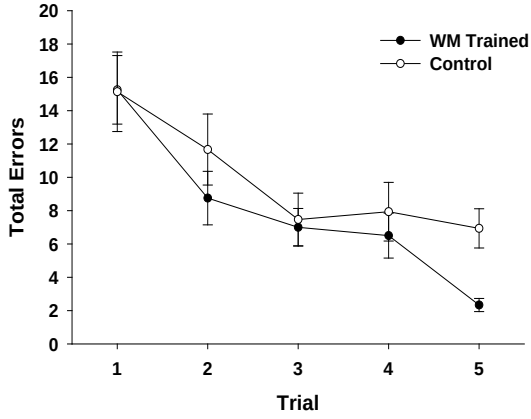

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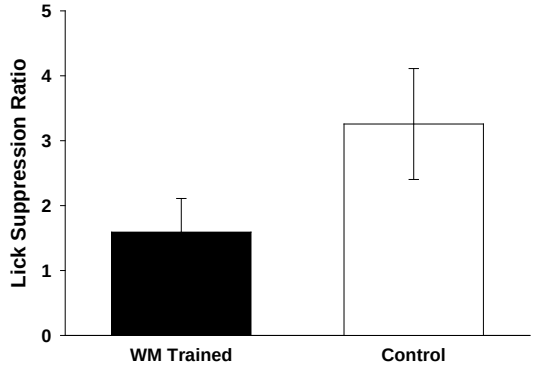

Figure 5

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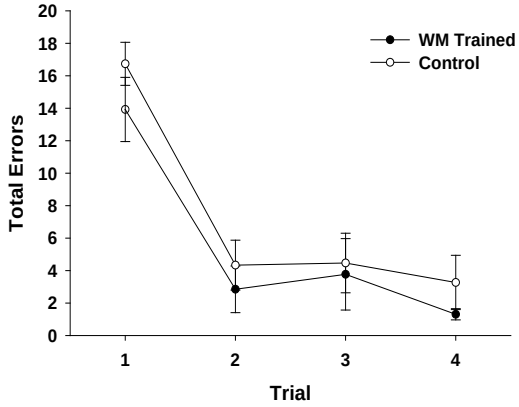

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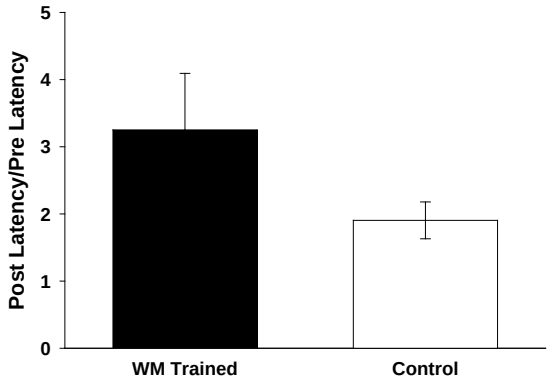

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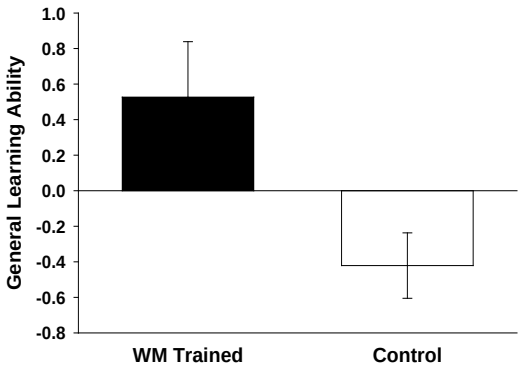

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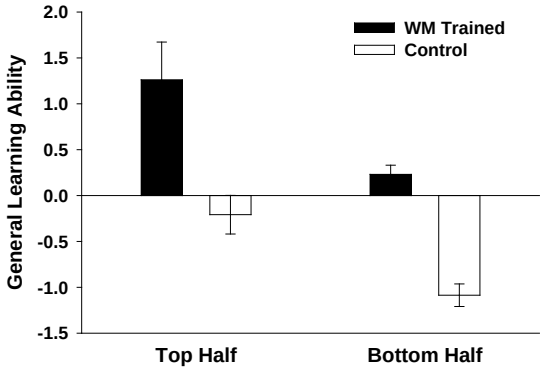

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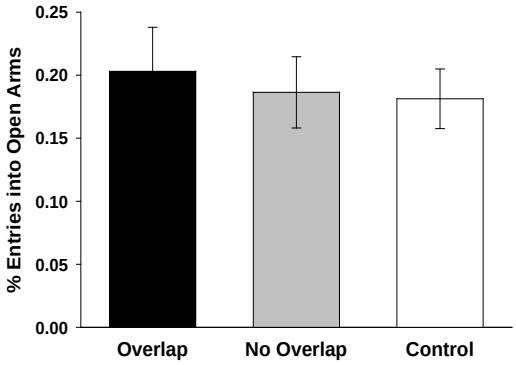

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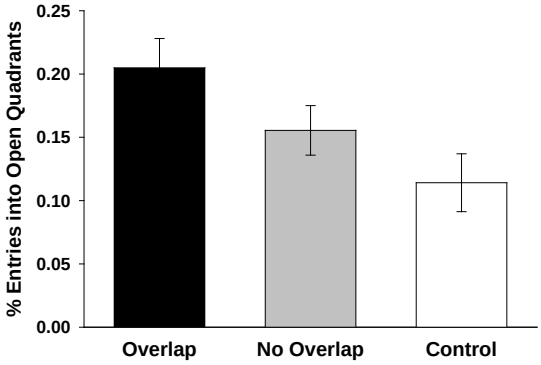

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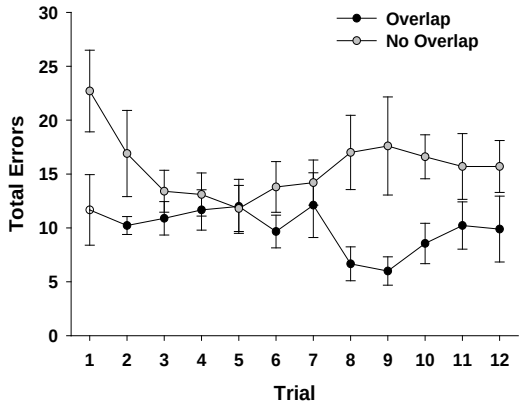

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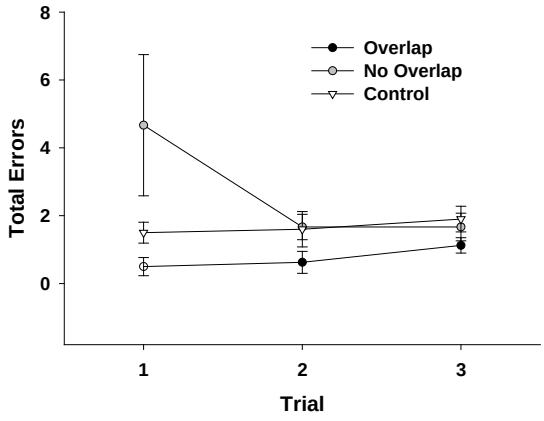

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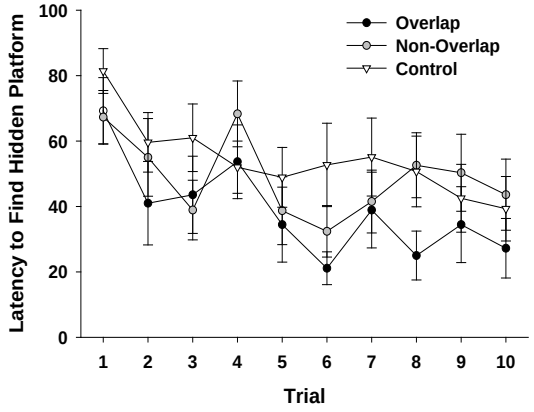

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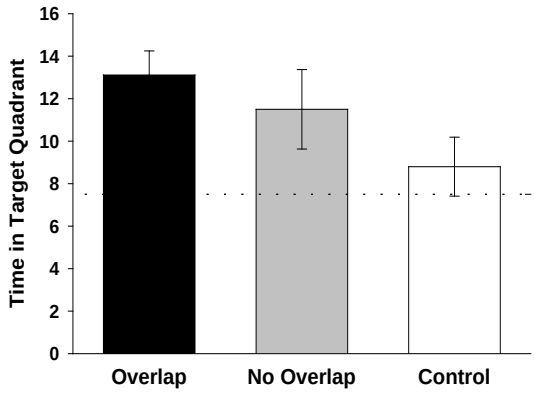

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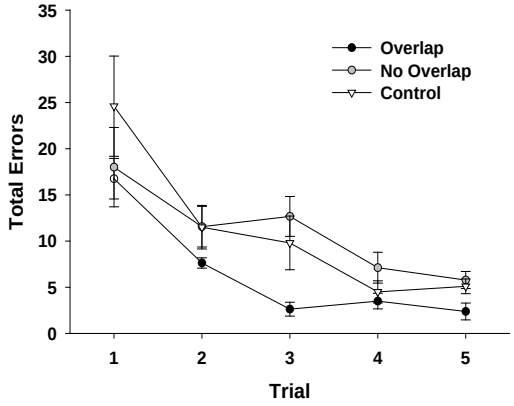

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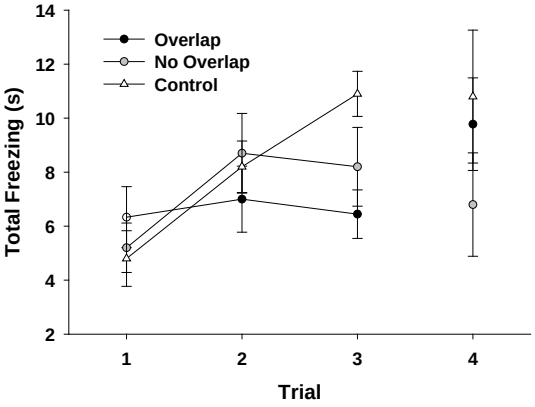

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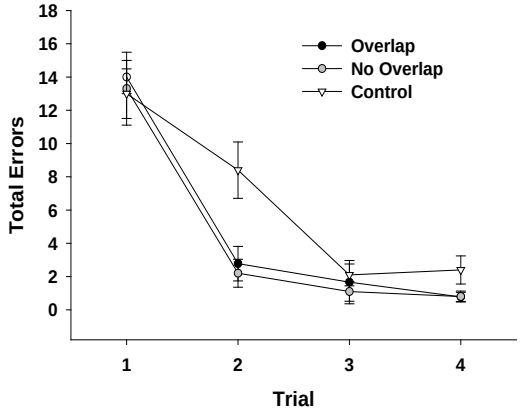

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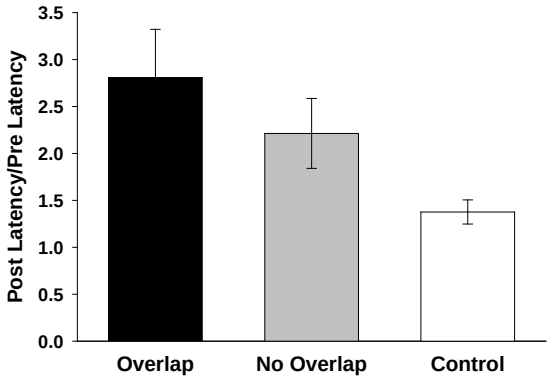

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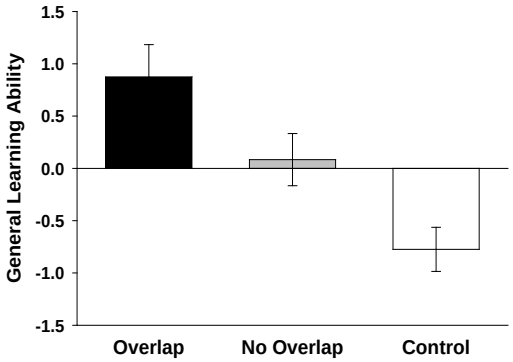

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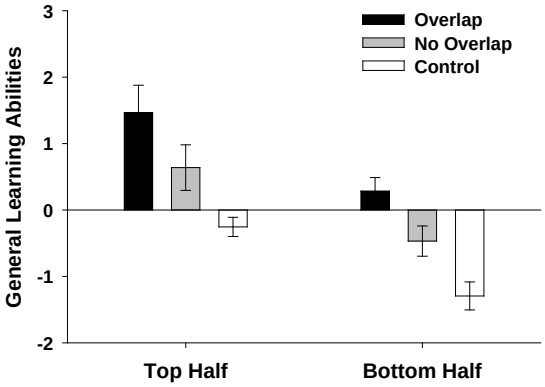

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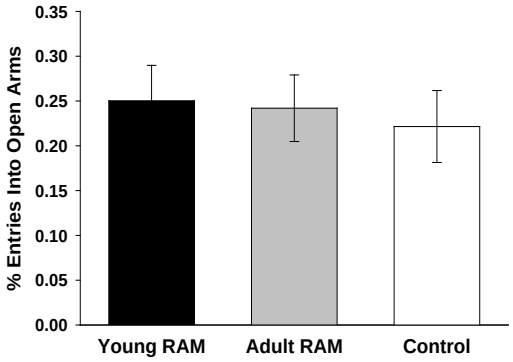

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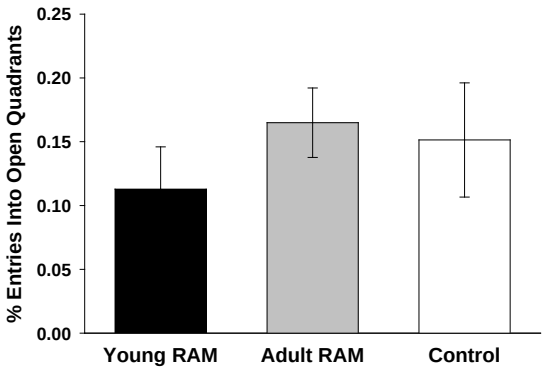

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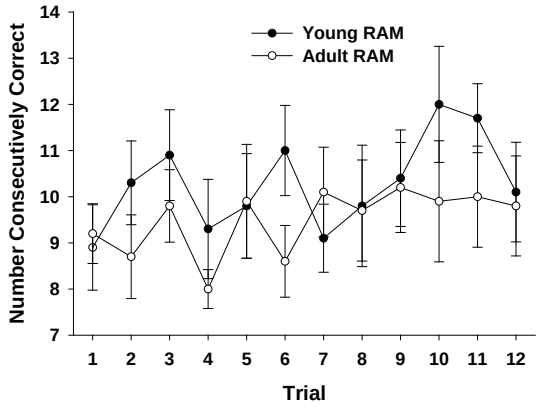

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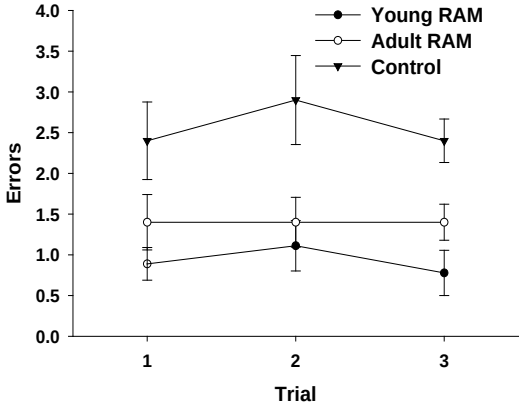

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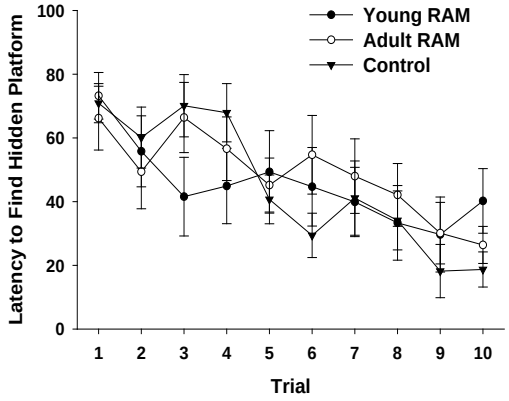

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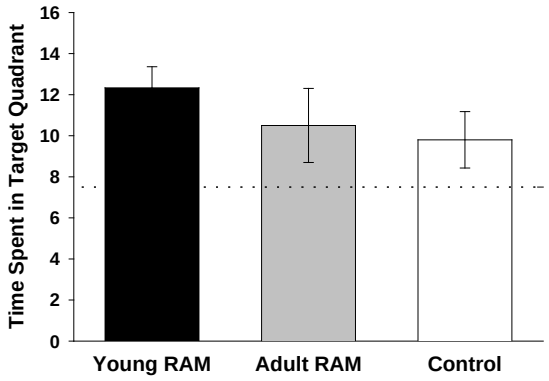

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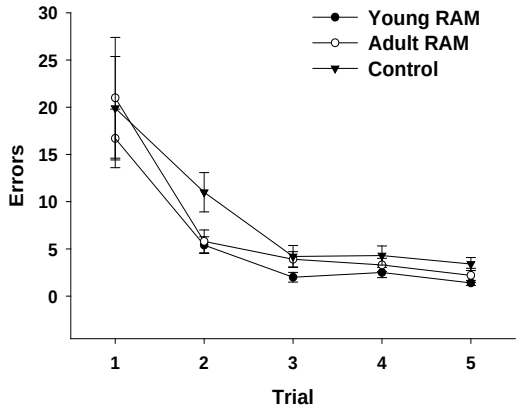

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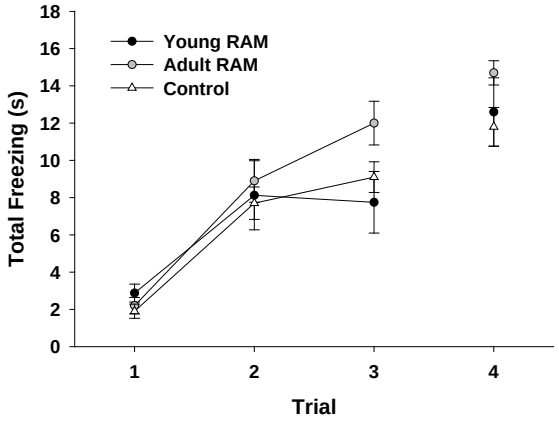
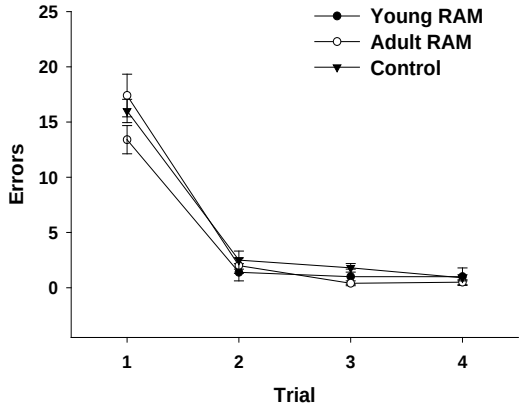


Figure 17

A



B

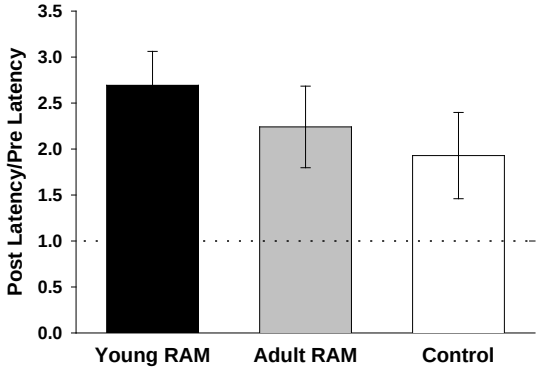
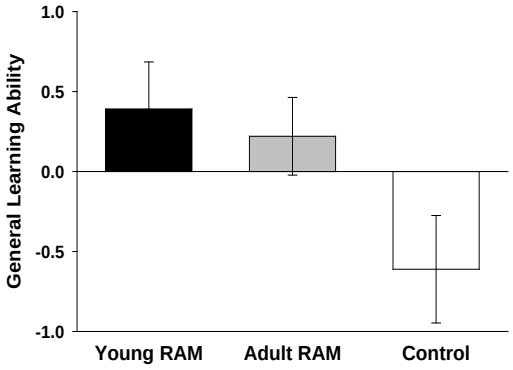
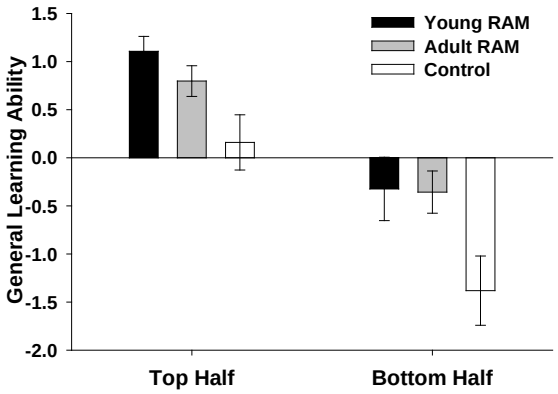


Figure 18

A



B



KENNETH R. LIGHT

Psychology Department – Behavioral Neuroscience Area

EDUCATION:

Degree	Institution	Date	Department
B.A.	Ramapo College	2003	Psychology
M.S.	Rutgers University	2006	Psychology
PhD	Rutgers University	Expected 2010	Psychology

SCHOLARLY INTERESTS:

1. The neurological basis of learning and memory.
2. The neurological basis of consciousness.
3. The neurological basis of perception.

PROFESSIONAL EXPERIENCE:

9/03 - present	Teaching Assistant, Rutgers University
9/06 – 12/06, 9/07 - 12/07	Part Time Lecturer, Rutgers University

RESEARCH EXPERIENCE:

Behavioral:

Mouse: Morris water maze, Lashley III maze, Associative fear conditioning, odor discrimination, passive avoidance, radial arm maze, Hebb-Williams maze, elevated plus maze, open field, and various sensory and motor assays.

Human: Administration of visual stimuli for perception experiments.

Neurochemical: EIA, IHC and HPLC.

Surgical: Live decapitation, trunk blood collection, brain dissection, and perfusion.

INSTRUCTIONAL EXPERIENCE:

Quantitative Methods in Psychology (Statistics)
Conditioning and Learning

HONORS:

2003 Ramapo: B.A. Summa Cum Laude
2003 Ramapo: Outstanding Student in Psychology
2007 Rutgers: Excellence in Teaching By a Graduate Student (1 of 2 issued per year)

PUBLISHED REPORTS:

Light, K., Grossman, H., Kolata, S. & Matzel, L.D. (2009). Rates of habituation underlie the relationship between general learning abilities and exploration in CD-1 outbred mice. *In preparation.*

Light, K., Kolata, S., Wass, C., Denman-Brice, A., Zagalsky, R. & Matzel, L.D. (2009). Working memory training promotes general cognitive abilities in genetically heterogenous mice. *Proceedings of the National Academy of Sciences, in submission.*

Matzel, L.D., Wass, C., Kolata, S., Light, K. & Colas, D. (2009). Age-related impairments of new memories reflect failures of learning, not retention. *Learning and Memory, in press.*

Matzel, L.D., Grossman, H., Light, K., Townsend, D., & Kolata, S. (2008). Age-related declines in general cognitive abilities of Balb/C mice are associated with disparities in working memory, body weight, and general activity. *Learning and Memory,* 15, 733-746.

Light, K., Kolata, S., Hale, G., Matzel, L.D. (2008) Up-Regulation of exploratory tendencies does not enhance general learning abilities in juvenile or young-adult outbred mice. *Neurobiology of Learning and Memory,* 90, 317-329.

Kolata, S., Light, K., Matzel, L.D. (2007) Factor analysis reveals both domain specific as well as domain general learning abilities in outbred CD-1 mice. *In preparation.*

Kolata, S., Wu, J., Light, K., Schachner, M., & Matzel, L.D. (2008) Impaired working memory duration but normal learning abilities in mice conditionally deficient in the adhesion molecule close homologue of L1.

Grossman, H., Hale, G., Light, K., Kolata, S., Townsend, D. A., Goldfarb, Y., Kusnecov, A., Matzel, L. D. (2007). Pharmacological modulation of stress reactivity dissociates general learning ability from the propensity for exploration. *Behavioral Neuroscience*, 121(5), 949-964.

Kolata, S., Light, K., Grossman, H., Hale, G., Matzel, L.D. (2007) Selective attention is a primary determinant of the relationship between working memory and general learning ability in outbred mice. *Learning and Memory*, 22, 22-28.

Matzel, L. D., Townsend, D. A., Han, Y. R., Grossman, H., Hale, G., Zappulla, M., Light, K. and Kolata, S.(2006) Exploration in outbred mice covaries with general learning abilities irrespective of stress reactivity, emotionality, and physical attributes. *Neurobiology of Learning and Memory*, *84(3)*, 228-240.

Kolata, S., Light, K., Townsend, D. A., Hale, G., Grossman, H. C. and Matzel, L.D.(2005). Variations in working memory capacity predict individual differences in general learning abilities among genetically diverse mice. *Neurobiology of Learning and Memory*, *84(3)*, 241-246.