GENOME-WIDE EXPRESSION ANALYSIS IMPLICATES WORKING MEMORY ASSOCIATED GENES IN THE GENERAL LEARNING ABILITIES OF OUTBRED MICE

by

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ABSTRACT OF THE DISSERTATION

Genome-wide expression analysis implicates working memory associated genes in the general learning abilities of outbred mice

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Previously, we have reported the existence of a general learning factor in genetically heterogeneous mice, and this factor is in many ways analogous to general intelligence in humans. This previous work established that the processes underlying general learning abilities in mice are, as in humans, related to working memory capacity and specifically selective attention. In the present set of experiments, using gene-expression microarray technology that allowed us to quantify the expression of ~25,000 genes simultaneously, we assessed the gene expression profiles of the best and worst learners from a sample of 60 mice (in two replications). For each group we compared four different brain regions (prefrontal cortex, the remaining cortex, cerebellum and hippocampus). The most

consistent pattern of differential expression was found in the prefrontal cortex, here a set of genes associated with the efficacy of dopamine functioning (i.e., Drd1a, Darpp-32, Rgs9) were upregulated in the fastest learners. As prefrontal dopamine functioning is associated with working memory, these results dove-tail with our previous behavioral results that demonstrated a relationship between working memory capacity and general learning abilities. This relationship was further verified through a quantitative PCR analysis where we demonstrated a significant correlation between the expression of these prefrontal dopamine genes and the general learning abilities of 48 mice. In total these results suggest that working memory and specifically dopamine signaling efficacy in the prefrontal cortex may be crucial for the establishment of general learning abilities.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

General Introduction

Individual differences in human cognitive abilities have been the subject of intense research for well over a century (Jensen, 1998). Perhaps the most influential and controversial finding in this area of research was the discovery of general intelligence. Spearman (1904) was the first to report the existence of a general intelligence factor when he noted that performances of individuals across a wide range of cognitive tasks tended to correlate in such a way that a single factor could account for a large portion of the underlying variance (Spearman, 1904). This finding has since been replicated many times and it has been estimated that the general intelligence factor can explain between 25-50% of the variance on any cognitive task. In other words, cognitive performance is influenced both by domain-specific abilities (e.g., spatial ability) as well as by a general, domain-independent ability (general intelligence). It has even been argued that general intelligence is one of the most heritable of cognitive traits (Sternberg, & Kaufman, 1998; Plomin, 2001; Plomin & Spinath, 2002; Macintosh, 1998; Jensen, 1998). However, attempts to address similar questions in animals have for the most part been infrequent and unsystematic, despite their potential to add to our understanding of cognition (Plomin, 2001). Instead, animal research has focused primarily on understanding single domains of learning (e.g., spatial abilities or Pavlovian conditioning). While this tactic has been successful in delineating the neuroanatomical substrates of certain forms of learning (e.g., Christian & Thompson; 2003; Eichenbaum, 2000; Phelps & LeDoux, 2005; White & McDonald, 2002), it has tended to leave unexplored those aspects of learning that are common across all domains.

Previously we, as well as others, have reported the existence of a general learning factor in outbred mice which appears in many ways to be analogous to general intelligence in humans (Matzel et al., 2003; Matzel et al., 2006; Kolata et al., 2005; Kolata et al., 2007; Galsworthy et al., 2005; Locurto et al., 2006). Specifically we found that when genetically heterogeneous mice are assessed on a battery of learning tasks (e.g., Lashley III maze, passive avoidance, spatial water maze, odor discrimination, fear conditioning) designed to tax different sensory/motor, information processing and motivational systems, approximately 30-40% of the variance in performance across the tasks could be explained by a single factor. This factor was demonstrated to be independent of stress reactivity and sensory / motor abilities as these modalities did not correlate with the general learning factor (Matzel et al, 2006). Directly modulating stress reactivity through pharmacological means (i.e., chlorodiazepoxide) also did not change the structure of the factor (Grossman et al., 2008). While exploratory measures were found to positively correlate with general learning abilities this relationship did not appear to be causative, as manipulating exploratory tendencies through behavioral means had no subsequent effect on general learning abilities (Light et al., 2008). Taken together these studies demonstrate that the robust primary factor that we have found to explain roughly 35% of the performance of mice across the learning battery is indeed a learning or cognitive-specific factor.

While our model extracts a general *learning* factor in mice, human tests of general intelligence investigate more general cognitive abilities (not necessarily learning abilities). Nevertheless there appears to be a relationship in humans between learning abilities and general intelligence. Human studies have shown that the rate of acquisition

of a skill is positively correlated with performance on intelligence batteries (Ackerman, 1987; Ackerman, 2005; Jensen, 1987). More specifically, Fitts and Posner (1967) described learning as a three stage process (cognitive, associative and autonomous). It is performance during the initial cognitive stage that most highly correlates with general intelligence test performance (Ackerman, 2005). During this stage, performance is slow and requires sustained effort and is detrimentally affected by extraneous attentional demands. Similarly, in our battery, learning was assessed during the initial acquisition phase; where the animals exhibited considerable individual differences. Therefore there appears to be a close conceptual correspondence between general learning abilities in mice and general intelligence in humans. This has led some to suggest they may be analogous constructs (Blinkhorn, 2003).

The discovery of a mouse analogue to general intelligence suggests the possibility that the basic structure of cognitive abilities is preserved across species and may be a fundamental construct in all vertebrae. The extent to which the murine cognitive architecture matches those of humans was further demonstrated by a combine analysis of the data from over 240 mice that had been assessed in the learning battery described above (Kolata et al., 2008). From this study we reported that, as in human cognition, there exists a hierarchical structure to the factors influencing learning in mice whereby a general factor influences domain-specific factors and performance on individual tasks. Specifically we showed that individual differences in spatial learning tasks were impacted by a domain-specific spatial learning factor which in turn was influenced by a general learning factor. These results suggest that the mouse is a good

model to investigate the factors underlying general intelligence absent domain-specific abilities.

Research with human subjects attempting to understand the underlying basis of general intelligence is extensive but interpretation is complicated by inherent differences in verbal ability and/or environmental background, complications that are not inherent to work with nonverbal laboratory animals. Furthermore, due to ethical considerations efforts to study the neural correlates of general intelligence are limited mainly to non-invasive imaging techniques such as functional magnetic resonance imaging (fMRI). These techniques, while useful, often have limitations which impact the depth to which they underlying mechanisms can be plumbed. Despite these limitations, great strides have been made in discovering both the behavioral and the neural correlates of general intelligence performance in humans.

Of all of the potential behavioral correlates of general intelligence that have been investigated with human subjects (e.g., reaction time, processing speed) perhaps none have been more consistently and robustly related than working memory capacity (e.g., Colom et al., 2008; Colom et al., 2004; Conway et al., 1996; Conway et al., 2003; Engle et al., 1999). Indeed this striking relationship led Kyllonen and Christal (1990) to boldly proclaim that, ‘‘reasoning ability is (little more than) working memory capacity.’’ In this context, working memory can be thought of as a limited capacity system that maintains goal relevant information across a delay and often does so in the face of attentional interference by salient distracters (Baddeley and Hitch, 1974). From this model it follows that working memory is composed of two independent processes. The first component is a short-term memory element that acts to maintain a memory trace across a

delay. The second component is a processing element that can increase the memory trace activation for goal relevant information while simultaneously inhibiting activation of salient distracting cues (Baddeley, 2003). This processing component is sometimes referred to as controlled or selective attention (Posner and Snyder, 1975). While the short-term memory component may be domain specific (i.e., there may be many short-term memory elements each sub-serving different domain-specific information), the selective attention component is more domain-general (Gazzaley et al., 2005) and therefore it may underlie the relationship between general intelligence and working memory.

Early studies that attempted to relate working memory to higher cognitive abilities often relied on simple span type memory task. In these types of tasks a subject is asked to recall of list of recently presented numbers, words or objects. Classically, Miller (1956) demonstrated that in such a task subjects are usually able to recall on average 7 items. Individual differences in the capacity limit in these short-term memory tasks often failed to correlate with higher cognitive abilities. For instance, Daneman and Carpenter (1980) found that simple span tasks did not correlate with reading comprehension. However, in the same study they reported that complex span tasks did predict reading comprehension. Complex span tasks (i.e., working memory tasks), as opposed to simple span, tax both short-term memory as well as controlled attention. In a complex span task a subject may be asked to read a series of sentences and then to recall the last word from each sentence. This contrast between short-term memory and working memory tasks led the authors to conclude that since working memory requires both short-term memory as well as controlled attention, and since simple span did not predict comprehension, then

controlled attention must underlie the relationship between working memory and higher cognitive abilities.

The dissociation between short-term memory and controlled attention has been repeatedly found in relation to general intelligence. Engle et al. (1999) attempted to model the relationship between working memory and general intelligence. Through a latent variable technique they found that while working memory and short-term memory were related constructs when the common variance between working memory and short-term memory was statistically removed, only the residual working memory component significantly predicted general intelligence. Since both the short-term memory as well as the working memory tasks contained a memory component but only the working memory tasks placed selective attentional demands on the subjects, the authors concluded that the relationship between working memory and intelligence was due to individual differences in selective attention. These results mirror the findings we have reported using our model of general learning abilities in mice.

We previously reported a positive correlation between the aggregate performances of individual outbred mice in the learning battery (e.g., general learning abilities) described above and their subsequent ability to accommodate competing demands on their spatial working memory capacity (Kolata et al. 2005). Specifically, we observed that when mice were required to perform in two radial arm mazes concurrently (a manipulation intended to place demands on working memory capacity), their performance in the target maze positively correlated with their general learning abilities. These results were suggestive of a relationship between working memory capacity and general learning abilities in mice and were consistent with the relationship between

working memory and general intelligence. However, this above experiment could not discern the relative contribution of the different aspects of the working memory system to the observed correlation with general cognitive abilities, and a second series of experiments (Kolata et al., 2007) was designed to assess these relative contributions. First, simple span abilities were assessed by requiring mice to maintain the memory of up to six visual symbols associated with food rewards. A moderate correlation was observed between this measure of simple span and individual animals' aggregate performance in a battery of six learning tasks. A second task was employed with which we could assess the efficacy of these animals' selective attention. In this task the animals had to use odor to guide their search for food while at the same time ignoring salient distracting odors which had previously been rewarded in a different context. Under these conditions of high interference, animals' accuracy was highly correlated with general learning abilities. Thus, as with work with human subjects, our results suggest that the critical variable in the relationship between general cognitive abilities and working memory is the reliance of working memory on selective attention.

While limited in their scope converging evidence has begun to emerge from neuroanatomical studies using human subjects, which suggests that selective attention and general intelligence may be highly related constructs (i.e., that there is a great deal of overlap between the brain regions engaged by selective attention, working memory and general intelligence). Before reviewing the brain regions putatively involved in performance on common measures of intelligence it will first be necessary to briefly review the working memory networks of the mammalian brain. Most of the relevant data has been derived recently from the application of imaging techniques (e.g., fMRI) in

human populations, although relevant neurophysiological data has also been obtained from non-human primates.

It was once believed that all aspects of working memory, including short-term maintenance of information, were instantiated in the prefrontal cortex. However, a new picture of working memory has since emerged in which it is assumed that domain-specific sensory information is transiently stored in modality-specific brain structures and the processing and attentional components of working memory are instantiated in the prefrontal cortex (although this region may also serve some storage functions). Evidence for this framework comes mostly from imaging studies that demonstrate that spatial working memory tasks activate memory systems in both the parietal cortex as well as executive-attentional networks located in the prefrontal cortex (Cohen et al., 1997). Specifically, Cohen et al. observed that during a spatial working memory task the temporal activation dynamics in relevant brain regions could be divided into two categories. Parietal cortex, as well as some prefrontal regions, expressed sustained activation during a working memory retention period (indicative of short-term maintenance of information) while most prefrontal regions expressed only transient activation whose peak varied with the extent of the memory load (indicative of executive-attentional components of working memory). Similarly, Rowe et al. (2000) showed that in a spatial working memory task, the dorsal lateral prefrontal cortex was activated during the selection of items from memory but not during the maintenance of those items. This was contrasted with the intraparietal cortex which was only active during the maintenance phase and not during selection. Todd and Marois (2004) further established the role of the parietal cortex in short-term storage of visual items. They demonstrated

that the amount of information that can be maintained in visual short-term memory is correlated with activity in the parietal cortex as measured by fMRI. Furthermore they demonstrated that the parietal activity during visual short-term memory tasks occurred during the encoding and maintenance period of the task but not at the time of retrieval.

The above data suggests that there is a disassociation between those areas involved in the short-term maintenance of information and those regions involved in processing of that information. However, there is significant overlap between maintenance and processing as evidenced by studies showing that spatial working memory is impaired when, during a delay period, subjects are prevented from attending to the memorized locations of relevant objects (Awh and Jonides, 2001). Similarly, Gazzaley et al. (2005) demonstrated that the magnitude and the speed of neural processing in the visual association cortex are modulated by modality-independent top-down attentional networks in the prefrontal cortex. These frontal networks could enhance or suppress perceptual baseline visual association cortex activity depending on whether relevant stimuli were being attended to or ignored. Therefore, it appears that the entirety of "working memory" is not represented in any one region of the brain but that instead it involves a complex interplay between many networks located throughout the brain including the parietal lobe and the prefrontal cortex, the latter of which appears primarily relevant to the processing of information during working memory tasks.

Moving from understanding general intelligence on purely a behavioral level to a deeper neural anatomical level of analysis is a holy grail of intelligence research. Witness Jensen, who stated "The highest priority in g research…is to discover how certain anatomical structures in the brain…cause individual differences in g". (Jensen, 1998, p.

579). In search of this elusive goal, Jung and Haier (2007) conducted a comprehensive review of imaging studies that attempted to locate brain regions involved in general intelligence and concluded that the cortical networks in the prefrontal cortex, parietal cortex, and the occipital lobe are all equally involved in general intelligence tasks. The authors described these results as fitting a parietal–frontal integration theory of intelligence (P-FIT). However, perhaps a more parsimonious explanation of these results is to state that these are the very same regions most commonly associated with working memory. In fact, given the high degree to which general intelligence and working memory are related, it is not surprising that the networks involved in working memory are also engaged by general intelligence tasks.

This putative relationship between working memory and the brain networks that subserve “intelligence” is highlighted by the results of a study by Gray et al. (2003). In that study, the authors measured the general intelligence scores of 48 subjects using Raven's Advanced Progressive Matrix task (which is a good predictor of general intelligence). They then used fMRI to image the networks engaged by a working memory task with a high selective attention demand. Not surprisingly, they found that individuals with higher intelligence scores performed better on the working memory task. However, they also found that activity in the prefrontal and parietal cortex mediated the relationship between intelligence and working memory performance. These are the brain regions most commonly associated with both intelligence and working memory.

Imaging studies investigating general intelligence seem to suggest that both the regions associated with domain-specific short-term maintenance of working memory information, such as parietal and occipital cortex, as well as regions associated with the

processing component of working memory (i.e., prefrontal cortex) are all engaged by intelligence tasks. At a rudimentary level, this may seem paradoxical, since by definition, general intelligence is domain-independent. However, one may conclude from these studies that all of the sub-tasks in the intelligence batteries impinged on some of the same domain-specific abilities (e.g., visual information and processing), accounting for the common activation of parietal and occipital regions. Although it would be premature to make any definitive statement, this work as a whole suggests the possibility that the unifying brain region orchestrating all intelligence tasks, regardless of the information being processed, is the prefrontal cortex, as the prefrontal cortex appears to be the common mediator of both "intelligence" and selective attention.

While studies using human subjects have begun to delineate the potential mechanisms that underlie general intelligence, these types of studies are often limited to non-invasive techniques and are thus limited in their potential scope. To address this issue, in the present set of experiments we attempted to use our animal model of general learning abilities to move beyond what is feasible using human subjects, in an attempt to further the understanding of the processes and neural mechanisms that underlie general intelligence. Specifically, we hoped to identify sets of genes which show differential expression patterns in animals from the top and from the bottom regions of the distribution of general learning abilities. Given the close correspondence between selective attention / working memory and general intelligence on both the behavioral and neural levels, we hypothesized that working memory-related genes would be among those sets of genes which are predictive of general learning abilities.

Experiment 1

In order to begin the search for the potential cellular and molecular mechanisms that may underlie general learning abilities, we analyzed the gene expression levels for ~25,000 genes of mice from the top and bottom of the distribution of general learning abilities. In this way we attempted to find unique gene expression patterns in four different brain areas (prefrontal cortex, the remaining cortex, hippocampus, and cerebellum) that could separate the fastest learners from the slowest. It is worth noting that this work is not technically feasible with human subjects, indicative of another point of converging operations necessitating both human and animal work. This analysis was done using two biological replications of 30 mice from which in each replication a general learning score distribution was generated and the top 4 animals (fastest learners) and the bottom 4 animals (the slowest learners) were selected for whole-genome gene transcription analysis (Figure 1).

Materials and Methods

Subjects: CD-1 mice exhibit considerable behavioral variability, and thus are particularly well suited for studies of individual differences. These mice are an outbred strain that was derived in 1926 from an original colony of non-inbred Swiss mice consisting of 2 males and 6 females. Estimates of genetic variation in this line have indicated that despite over 50 years of breeding they are very similar to wild mouse populations (Rice & O'Brien, 1980). For this study, 60 male CD-1 mice (two replications of 30 mice each) were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mice arrived in our laboratory between 66–80 days of age, and ranged from 25–34 grams at the start of

testing. Testing began when the mice were 90–110 days of age, an age which corresponds with young adulthood. The mice were housed individually in clear shoebox cages in a temperature and humidity controlled colony room and were maintained on a 12 h light/dark cycle. In order to minimize any effect of individual differences in stress reactivity to handling, prior to the start of the experiment all of the animals were handled for 90 sec/day, five days/week over a period of two weeks prior to the start of behavioral testing.

Behavioral Methods:

The 60 CD-1 mice used in this replication were assessed (in 2 independent replications) on the 5 learning tasks which make up the core tasks used to evaluate general learning abilities (i.e., the Lashley III maze, passive avoidance, spatial water maze, associative fear conditioning and odor guided discrimination). These tasks were chosen so that they place unique sensory, motor, motivational, and information processing demands on the animals. In this way the only common underlying learning domain they share is the most general (i.e., a general learning ability factor). Briefly, passive avoidance is an operant conditioning paradigm in which the animals must learn to be passive in order to avoid aversive light and noise stimulation. The spatial water maze encourages the animals to integrate spatial information to efficiently escape from a pool of water. Odor discrimination is a task in which animals must discriminate and use a target odor to guide their search for food. Lastly, fear conditioning (assessed by freezing) is a conditioning test in which the animals learn to associate a tone with the presentation of a shock. In all of these tasks the animals were trained well past the point of asymptotic performance. In this way the total amount of learning was equated as much as possible between all the

animals. This was done so as to minimize any affect the extent of learning might have on gene expression.

Lashley III Maze (LM): This maze consisted of a start box, three interconnected alleys and a goal box. Previous studies have shown that over successive trials the latency to find the goal box decreased as well as the number of wrong turns. When extra-maze cues are minimized, the animals tend to use egocentric methods to locate the goal box (e.g., fixed motor patterns).

A Lashley III maze, scaled down for use with mice, was constructed from black Plexiglas and located in a dimly lit room (10 Lux at the floor of the maze). A 3 cm diameter white circle was located in the center of the goal box, and 45 mg Bio-serv food pellets (dustless rodent grain) was placed in the cup to motivate the animal's behavior.

Food-deprived animals were acclimated and trained on two successive days. Prior to acclimation they were exposed to three pellets of the reinforcer in their home cage. On the acclimation day, each mouse was confined in each of the first three alleys of the maze for four minutes and the final alley containing the goal box for six minutes – where three pellets were placed in goal box. At the end of each period, the animal was physically removed from the maze and placed in the next alley. This was done so as to acclimate the animals to the apparatus prior to testing. On the training days, the animals were placed in the start box and allowed to freely navigate the maze during which time their latency to locate the food and the number of wrong turns was recorded. Upon successfully consuming the pellet, the animals were returned to their home cage for an 18 min. inter-

trial interval during which time the maze was cleaned. The animals completed five trials during the first day of training and three trials on the second.

Passive Avoidance (PA): In this assay, animals learn to suppress their exploratory tendency in order to avoid aversive stimuli. The animals are placed on a platform and when they step down they are administered aversive stimuli, in this case a bright light, noise and loud tone.

A chamber with a white grid floor 16 x12 cm (l x w), illuminated by a dim light, was used for both acclimation and testing. An enclosed platform (70 x 45 x 45 cm, l x w x h) constructed of black Plexiglas and elevated 5 cm above the grid floor was located at the back of the chamber. There was only one opening from the platform facing the grid floor which allowed the animal to step down onto the floor. The exit from the platform could be blocked remotely by a clear Plexiglas guillotine-style door. When an animal left the platform and made contact with the grid floor it initiated the aversive stimuli. The aversive tone (180 dB, 2.4-3.7 kHz) was generated by a piezoelectric buzzer (RadioShack, 273-057). The aversive light was generated by a 75W halogen flood light.

During training, the animals were placed on the platform with the door closed, confining them in the enclosure. After 5 min., the door was opened and the latency of the animal to leave the platform and make contact with the floor was recorded. After they made contact, the aversive stimuli were initiated and the door was lowered, exposing them to the stimuli for 4 sec. after which they were allowed access to the enclosure again. This procedure was repeated for two additional trials. For purpose of ranking the animals

the ratio of the step down latency on the second trial to step down latency on the first trial (prior to any learning) served as the index of learning.

Spatial Water Maze (WM): This task requires the animals to locate a submerged platform in a pool of opaque water. Absent distinct intra-maze cues, animals' performance in this maze is highly dependent on the integration of extra-maze spatial cues. The animals are motivated by their aversion to water. The latency and the path length to locate the platform decrease over successive trials, despite entering the pool from different locations.

A round pool (140 cm diameter, 56 cm deep) was filled to within 20 cm of the top with water that is clouded with a nontoxic, water soluble black paint. A hidden 14 cm diameter black platform was located in a fixed position 1 cm below the surface of the water. The pool was enclosed by a ceiling high black curtain on which five different light patterns (which served as spatial cues) were fixed at various positions.

On the day prior to training, each animal was confined to the platform for 360 sec. by a clear Plexiglas cylinder that fits around the platform. On the next two training days, the animals were started from one of three positions for each trial such that no two subsequent trials start from the same position. The animal is said to have successfully located the platform when it places all four paws on the platform and remains for 5 sec. After locating the platform or swimming for 90 sec., the animals were left or placed on the platform for 10 sec. They were then removed for 10 min. and placed in a holding box before the start of the next trial. Each animal completed 14 total trials (6 on the first training day, and 4 on each of the following two days). The latency to find the platform

was recorded for each trial. During the first replication the path length distances to locate the platform were also recorded using custom Matlab software (Mathworks, Natick, MA).

Associative Fear Conditioning (FC): In this task the animals received a tone (CS) paired with a mild foot shock (US). Two distinct experimental chambers were used (a training context and a novel context). Each box was contained within a sound and light-attenuating chamber. The training box (16.5 x 26.5 x 20 cm) was brightly lit with a clear Plexiglas front/back, and one stainless steel and one clear Plexiglas side wall. The floor was composed of a steel grid (5 mm spacing) from which a 0.6 mA constant current footshock could be delivered from a shock scrambler (Lafayette Instruments, Lafayette, IN). The novel chamber (23 x 21.5 x 19 cm) was dimly lit and all of the walls and the floor were composed of clear Plexiglas. In both boxes the tone (60 dB, 2.9 kHz) was delivered by a piezoelectric buzzer (Med Associates, EV-203a).

The animals were acclimated to the training and novel contexts by placing each animal in both boxes for 20 min. on the day before training. Training on the subsequent day occurred in a single 18 min. session during which the animals received three noise-shock pairings after 6 min., after 10 min and after 16 min. The CS presentation consisted of a pulsed (0.7 sec on, 0.3 sec off) 20 sec. tone. Immediately following the tone offset the shock (US) was presented for 500 msec. The following day the animals were placed in the novel chamber where they received the same presentation of tones but without the shock.

To quantify the conditioned fear responses, freezing responses were videotaped and both the time spent freezing 20 sec. prior to the initiation of the tone as well as freezing during the tone were scored by an independent observer. The conditioned response to the CS was said to be freezing during the tone presentation minus freezing prior to the tone. For purpose of ranking the animals, CS freezing during the second training trial was used.

Odor Discrimination (OD): Rodents are adept at using odor to guide their reinforce behavior. This task is modified from one used by Sara, et al. (1999) but scaled down for use with mice. In this task, mice navigated through a field using unique odors to guide them. The animals learned to choose the food cup that contained the target smell when given three choices. The plastic food cups used contained a cotton swab at the bottom holding 25 µl of odor (anise, banana or coconut flavored extract) which was covered with a wire mesh. The food cup locations were rearranged on each trial but the accessible food was always marked by the target odor (in this case mint).

The odor discrimination chamber consisted of a black Plexiglas 60 cm square field with 30 cm high walls located in a dimly lit room with good ventilation. One of three plastic food cups was placed in three corners. Only the target cup had the food (30 mg portion of chocolate flavored puffed rice) accessible on top of the wire mesh. The other two cups had food located under the wire mesh, allowing the mice to smell the food but not access it.

Each animal had one day of acclimation and one day of testing. The night prior to the acclimation day, food was removed from each animal's home cage. The next day each mouse was placed in the box for 20 min. with no food cups present. At the end of

the day each animal received three pieces of the reinforcer in their home cage. On the training day each animal received six trials in which they were placed in the corner of the training chamber which did not contain a food cup. On the first trial an additional reinforcer was placed on the edge of the target cup (mint). At the end of each trial the food cups and the starting location were rearranged but mint always remained as the target odor. For each trial both latency to locate the food and number of errors were recorded (where an error is making contact with or sniffing within 2 cm of an incorrect food cup). For purpose of this analysis, the average errors across trials 2 and 3 served to index learning.

Brain Dissection: Two weeks following the completion of the learning battery the animals were sacrificed and their brains extracted. Specifically, the animals' were live decapitated according to standard animal ethical protocols and their brains quickly dissected to remove the relevant brain regions (the prefrontal cortex, the remaining cortex, hippocampus, and cerebellum). The tissue was immediately placed in a solution of RNAlater (Ambion) to preserve RNA integrity.

RNA Isolation: Total RNA isolation followed the recommend protocol described in the RiboPure RNA Isolation Kit (Ambion). Tissue samples were first homogenized in a TRI reagent solution and combined with 1-bromo-3-chloroprapane. The resulting mixture was centrifuged and the aqueous solution removed. The total RNA containing solution then underwent purification using glass fiber cartridges. For each brain region the resulting RNA from the best learners were pooled and the same was done for the worst learners (resulting in a total of 8 pools per replication). The total RNA was maintained at -70°C for long term storage.

cDNA Synthesis and Microarray Hybridization: cDNA synthesis and microarray hybridization were carried out at the Keck Microarray Facility at Yale University (New Haven, CT). The gene expression analysis utilized the Illumina Sentrix MouseRef-8 BeadChip containing target probes for ~25,000 annotated mouse genes.

As per the Keck Microarray Facilities procedures, the preparation of labeled cRNA for hybridization onto Illumina BeadChips followed the recommended Illumina protocol using a TotalPrep RNA Amplification kit (Applied Biosystems). Double stranded cDNA and biotin-labeled cRNA were synthesized and purified from 500 ng of total RNA. Purification of the cRNA followed, and integrity of the cRNA was assessed by running aliquots on the Bioanalyzer prior to hybridization.

Hybridization buffer from the BeadChip kit (Illumina) was mixed with 1500 ng of biotin-labeled cRNA, heated to 65°C for 5 minutes, and then loaded onto the BeadChip. The BeadChips were sealed in a hybridization chamber and placed in an oven at 58°C with a rocker for 16-20 hours. After the hybridization, the BeadChips were washed and stained in a series of washes and stains as outlined in the Illumina protocol. The BeadChips were then scanned on the Illumina Iscan. Scanned files were loaded into BeadStudio software for analysis and arrays were background normalized.

Results and Discussion:

General Learning Ability: In all of the learning tasks, animals' performance was measured during acquisition, where there are considerable individual differences in performance. However, training was extended beyond this point to asymptotic performance levels thus insuring that differences in performance levels or total learning

were not reflected in differences in gene expression. In both of the replications the correlations between each of the learning tasks revealed a positive manifold, i.e., all correlations were consistently positive, suggesting a common source of variance. A subsequent unrotated principal component factor analysis revealed that in both replications about 41%, eigenvalue = 2.03 and 2.12 respectively, (Table 1A and 1B) of the variations in the animals' performances between the tasks could be explained by a single factor ("general learning" factor). In the combined analysis in order to minimize the impact of any variations across the multiple replications that contributed to this study, for purposes of factor analysis, animals' performance on each task was converted to a z score, thus mitigating slight variations in training parameters that might impact acquisition rates. In this analysis an unrotated principal component analysis revealed 41%, eigenvalue = 2.04, of the variation in performances could be explained by the general learning factor (Table 1C). No secondary factor with an eigenvalue > 1 was found so a rotated factor analysis was not possible. The lack of a secondary factor is consistent with previous large analyses using our learning battery (Kolata et al., 2008). In order to quantify individual animals' general learning abilities, we calculated factor scores for each animal. A factor score is analogous to the average z-score for each animal in each task, weighted by the degree to which each task contributes to the primary factor (the general learning factor) in the factor analysis. Thus, a factor score of zero would indicate an "average" learner, while a low score would be indicative of a fast learner; conversely, a high score would designate a poor learner.

From each replication, using factor scores, we identified the top 4 fastest learners (fast) and bottom 4 slowest learners (slow). The difference in between the slowest

learners and the fastest learners were not only reflected in their general learning abilities but also in the individual learning tasks. In all of the tasks the slowest learners performed significantly worse than the fastest learners (Figure 2). In four of the five tasks an analysis of variance (ANOVA) comparing the performance of the fast learners to that of the slow learners across the learning trial revealed a significant effect of group (water maze: $F(1, 14) = 6.97$, $p<.05$; fear conditioning: $F(1, 14) = 7.84$, $p<.05$; Lashley maze: $F(1, 14) = 27.79$, $p<.01$; passive avoidance: $F(1, 14) = 6.78$, $p <.05$). While there was no significant overall effect of group in odor discrimination, $F(1, 14) = 2.8$, $p = .11$, perhaps due to the rapid learning in both groups, a post hoc planned comparison revealed that the groups significantly differed on trials 3-5, $F(1, 14) = 19.54$, $p <.001$.

Gene Expression: In each of the two replications the gene expression levels for ~25,000 genes of the fast learners were compared to those of the slow learners in each of four different brain regions. Since the samples were pooled no estimate of variance was possible and therefore a cut off was chosen for the ratio of fast/slow expression levels for each gene to identify differential expression (cutoff = 1.34 as this is the limit of detection for the Illumina chip, see Figure 3). Table 3 and 4 lists the differently expressed genes in the cerebellum / hippocampus and prefrontal cortex / cortex respectively. To formalize whether the list of differently expressed genes belonged to a particular gene ontology (GO) we used the GOrilla tool (Eden et al., 2009). Of all the brain regions examined only the prefrontal cortex revealed a significant biological function represented by the differentially expressed genes. In the prefrontal cortex the GOrilla assigned the function ‘dopamine D1 receptor activity’ a significant p-value ($p = 5.13E-4$).

Discussion: The prefrontal cortex represented the most consistent region in terms of gene expression as it was the only region where the differently expressed genes appeared to represent an underlying biological function, namely dopamine signaling. Specifically, protein phosphatase 1, regulatory (inhibitor) subunit 1B (Ppp1r1b), regulator of G-protein signaling 9 (Rgs9) and dopamine receptor subtype 1a (Drd1a) seem to show differential expression in the best and the worst learners in the prefrontal cortex. Ppp1r1b encodes the protein, dopamine- and cyclic AMP-regulated phosphoprotein (Darpp-32) and as this is the more common nomenclature in the literature it will be referred to as Darpp-32 in the present text. Darpp-32 is located in forebrain neurons containing D1 receptors (Walaas and Greengard, 1984). As Feinberg et al. (1998) demonstrated, in response to dopamine stimulation of D1 receptors Darpp-32 is phosphorylated and becomes a potent protein phosphatase 1 inhibitor (PP1) by acting through adenylate cyclase. PP1 in turn has been shown to act as a suppressor of learning and memory by negatively regulating downstream proteins and kinases important for synaptic plasticity (Genoux et al., 2002; Ceulemans and Bollen, 2004). Conversely, D2 receptor stimulation has been shown to reduce Darpp-32 phosphorylation (Nishi et al., 1997). Interestingly, Rgs9 has been shown to suppress the response to dopamine acting through D2 but not D1 receptor stimulation (Rahman et al., 2003). Therefore these three genes (Darpp-32, Rgs9 and Drd1a) interact to increase the response to D1 receptor stimulation and enhance the suppression of PP1.

Among the other genes which showed differential expression in the prefrontal cortex, Scn1a (Sodium channel protein type I subunit alpha) stands out. This gene encodes the pore forming unit of a sodium channel subtype that is expressed

predominantly in interneurons (Ogiwara et al., 2007). The functional significance of this differential expression is not necessarily immediately obvious, however Posthuma et al. (2005) found that this gene was contained in a segment of the human chromosome in which genetic differences were associated with variations in general intelligence in humans.

While it does not necessarily follow that the gene expression results from other brain regions did not represent a 'real' difference between fast and slow learners, it does suggest that gene expression differences in the prefrontal cortex are the most likely to reflect a general learning-ability associated underlying process (i.e., dopamine related processes). This is intriguing in light of the fact that prefrontal dopamine function is thought to be involved in working memory and working memory is known to be related to intelligence in humans. For instance, **Sawaguchi** et al. (1991) demonstrated that local prefrontal injections of a D1 antagonist selectively impaired in a working memory task but had no effect on control tasks that required the same motor and sensory abilities but lacked a working memory load. Similarly, in regards to learning specifically, Kentros et al. (2004) demonstrated that in mice attention is required for stabilization of place fields in the hippocampus and that this process is blocked by a selective dopamine antagonist and enhanced by a dopamine agonist.

Two of the genes that showed differential expression in the prefrontal cortex, Rgs9 and Darpp-32, are known to regulate dopamine processing. However, these genes are mostly studied for their role in the striatum. Despite this, these genes have also been shown to be expressed in the cortex (Figure 4). Darpp-32 shows widespread cortical expression (Svenningsson et al., 2002). Rgs9 is thought to be specific to the striatum

however in mice it has been demonstrated to be expressed at low to moderate levels in the cortex (Gàzon et al., 2001; Bouhamdan et al. 2004). Similarly, the consortium at GENSAT (gene expression nervous system atlas) has revealed cortical expression of both Rgs9 and Darpp-32 in mice (Gong et al., 2003). Perhaps also surprising is that these genes showed different patterns of expression in the two replications (faster > slower in replication 2 and slower > faster in replication 1). This could be due to an inverted U type response whereby high or low dopamine function could impair working memory performance (e.g., Vijayraghavan et al., 2007). It is also possible that one or more of the samples that went into the sample pool were aberrant. If this was correct it would most likely be in the first replication as the fold change values in that replication for these genes seemed to be outliers. Due this aberration we hypothesized that the second replication more accurately represented the true effect.

Of the genes that showed differential expression outside the prefrontal cortex, perhaps one of the most intriguing is the transcription factor Neurod1 (Neurogenic differentiation 1) which was found to be differently expressed in the cerebellum. In adult mammals, Neurod1 has been shown to be preferentially expressed in the cerebellum and to have a critical role in the proliferation, and differentiation of granule cells in both the cerebellum and dentate gyrus (Lee, 1999; Figure 5). During development NeuroD1 is necessary for normal granule cell formation as the absence of NeuroD1 results in the death of the granule cells in posterior part of the cerebellum and in the dentate gyrus shortly after birth (Miyata et al., 1999). The functional significance of an increased expression of NeuroD1 in the fast learners in regards to general learning abilities, however, is not immediately obvious.

The increased hippocampal expression of the cell adhesion molecule, Leucine-rich repeat transmembrane neuronal protein 1 (Lrrtm1), in fast learners is also interesting as this gene is known to be important for synaptogenesis. Lrrtm1 is selectively located in glutamatergic excitory synapses and is highly expressed in hippocampal neurons. *In vitro* neurons lacking Lrrtm1 also exhibit a pre-synaptic deficit in synaptic vesicle clustering (Brose, 2009). The findings by Linhoff et al. (2009) which demonstrated that in culture Lrrtm1 is sufficient for excitory synapse formation have obvious implications for general learning abilities.

Experiment 2

The results from the genome-wide expression analysis performed in experiment 1 implicated working memory related genes in the prefrontal cortex as being involved in general learning abilities. While other regions also showed differential expression in learning-related genes the prefrontal cortex was the only region in which several genes related to one function (i.e., working memory) predicted general learning abilities. Therefore, the prefrontal cortex seems to be the region most likely to be engaged by general learning abilities, which is consistent with human literature (e.g., Jung and Haier, 2007; Gray et al., 2003). As our previous study pooled subjects, however, there was no means to estimate variance and as such there was no way to verify the robustness of these findings. In order to further explore this potential relationship, therefore, we used a quantitative polymerase chain reaction (QPCR) assay to quantify the expression levels of all of the genes (10 total) in prefrontal cortex that showed a fold change above the cutoff threshold. This was done not just for the 16 animals (8 animals from 2 replications) that contributed to the microarray analysis in the first experiment but for a total of 50 animals

(out of 60 animals) whose general learning abilities had been assessed in the first experiment. The total number of animals was reduced to 50 (the 5 animals removed from each replication came from the middle of the distribution of general learning abilities) so that more genes could assessed in QPCR. In doing so we hoped to ascertain both the robustness of the potential relationship between the working memory related genes and general learning abilities as well as the nature of that potential relationship (e.g., linear, inverted-U, etc).

Materials and Methods

Subjects: The 50 CD-1 mice used in this experiment were the same animals used in experiment 1.

Brain Dissection: Two weeks following the completion of the learning battery the animals were sacrificed and their brains extracted. Specifically, the animals' were live decapitated according to standard animal ethical protocols and their brains quickly dissected to remove the prefrontal cortex / prelimbic region. The tissue was immediately placed in a solution of RNAlater (Ambion) to preserve RNA integrity.

RNA Isolation: Total RNA isolation followed the recommend protocol described in the RiboPure RNA Isolation Kit (Ambion). Tissue samples were first homogenized in a TRI reagent solution and combined with 1-bromo-3-chloroprapane. The resulting mixture was centrifuged and the aqueous solution removed. The total RNA-containing solution then underwent purification using glass fiber filter cartridges. For each brain region the resulting RNA from the best learners were pooled and the same was done for the worst

learners (resulting in a total of 8 pools per replication). The total RNA was maintained at -70°C for long-term storage.

Gene Expression Quantification by QPCR:

QPCR was carried out at the Burnham Institute (La Jolla, CA). Taqman probes were chosen for each of the 10 genes plus one house-keeping gene (GAPDH) that were to be assayed. The probes chosen crossed at least one exon-intron junction so as not to be specific to any alternative splice forms. The total RNA was converted to cDNA and then ~1µg of sample was used for each reaction. All reactions were done in duplicate and relative concentrations values were calculated using a standard curve for known quantities of GAPDH.

Results and Discussion:

In addition to the 10 genes for which we quantified expression we also quantified one control / housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). This was done so as to both verify the efficacy of the QPCR as well as to control for differences in starting RNA concentrations by normalizing the expression values against this gene. However, it was found that GAPDH values were not equal between the fast learners and the slow learners. While there was not a significant relationship between GAPDH gene expression and general learning abilities there was a negative correlation such that faster learners tended to have more GAPDH mRNA transcripts. Therefore using this gene to normalize the results would necessarily skew the results away from finding any relationship with general learning abilities. Due to this correlation we used the expression values for Psmc3ip to normalize the data. This was done because there

was no relationship between the raw / unnormalized expression values for this gene and general learning abilities nor was there a relationship when this gene was normalized against GAPDH. Using Pscmc3ip to control for differences in starting RNA concentration, therefore, would most accurately represent the data.

The total expression values for each gene whose transcript copy numbers were quantified using QPCR are shown in Figure 6. As expected, Rgs9 showed a low to moderate expression in the prefrontal cortex. Rgs9 is mostly expressed in the striatum with more moderate expression found in the cortex (Figure 4). Similarly, nudix (nucleoside diphosphate linked moiety X)-type motif 6 (Nudt6) was expressed at very low levels. This gene is not expressed at high levels on basal conditions (Gomez-Pinilla et al., 1992). Conversely, ATPase, aminophospholipid transporter, class I, type 8A, member 1 (ATP8a1) showed the highest expression values and this is consistent with its general role in, among other things, vesicle exocytosis (Lenoir et al., 2007).

In experiment 1 three dopamine associated genes (Drd1a, Darpp-32 and Rgs9) showed different patterns of expression in the two replications (faster > slower in replication 2 and slower > faster in replication 1). We hypothesized that this could be due to an inverted U type response whereby high or low dopamine function could impair working memory performance (e.g., Vijayraghavan et al., 2007). Alternatively it was possible that one or more of the samples that went into the sample pool were aberrant. If this was correct we speculated that the aberration would most likely be in the first replication as the fold change values in that replication for these genes seemed to be outliers. The QPCR data verified this latter hypothesis as two of the 50 samples (1 of which contributed to the sample pool in the first replication) showed aberrantly high

values for the dopamine related genes (i.e., 50x higher values). These samples were thus removed from the data and were not subject to further analyses.

All of the dopamine associated genes showed consistent and significant negative correlations with general learning abilities (Darpp-32: $r(46) = -0.38$, $p < .05$; Rgs9: $r(46) = -0.44$, $p < .05$; Drd1a: $r(46) = -0.37$, $p < .05$) such that animals learned faster tended to show higher normalized gene expression values (Figure 7). Consistent with their common function (e.g., dopamine signaling) these genes were also highly correlated with each other ($r = 0.85 – 0.89$). Of the remaining genes only Nudt6 was significantly correlated with general learning abilities, $r(46) = -0.29$, $p < .05$. However, all of the genes tended to correlate in such a way that higher gene expression was associated with faster general learning abilities.

A principle component factor analysis including the performance on the five learning tasks that make up the learning battery as well as the normalized gene expression values from the 9 genes assessed by QPCR revealed a primary factor in which all of the expression values loaded in the opposite direction as the learning tasks (Table 4A). This suggests that the high expression is associated with fast learning. A secondary factor was also extracted in which only the dopamine associated genes load consistently in the opposite direction as the learning tasks. This suggests that the dopamine genes may uniquely predict general learning abilities. This can clearly be seen by a verimax rotated factor analysis, which attempts to find the most number of uncorrelated latent factors. This analysis revealed a secondary factor in which just the dopamine associated genes loaded consistently and strongly in the opposite direction as the learning tasks (Table

4B), suggesting that the dopamine associated genes may share a common variance that uniquely predicts general learning abilities.

While all of the gene expression values tend to correlate with each other and thus load together on a principal component analysis (Table 4A), the dopamine genes seem to share a unique underlying variance which may predict general learning abilities. To further test this hypothesis a rotated factor analysis including all of the genes' expression values was performed. This analysis revealed a primary factor (dopamine factor) in which the three dopamine genes loaded strongly and the remaining genes not as much (Table 5A). A secondary factor was extracted which explained the remaining variance minus the unique variance shared by the dopamine genes. From this analysis a factor score was extracted from the dopamine factor and from the factor which explained the remaining variance. This new dopamine factor quantified the unique variance shared by the dopamine genes minus any shared variance they had with the rest of the genes. When this dopamine factor and the factor explaining the rest of the gene expression variance were included in a factor analysis with the learning tasks, only the dopamine factor loaded strongly with the learning tasks (Table 5B). Furthermore the dopamine factor correlated significantly with the general learning factor ($r(46) = -0.44$, $p < .05$).

Discussion:

The results from the second experiment confirmed and extended many of the findings from the microarray gene expression analysis. Specifically, the QPCR data demonstrated that a relationship exists between individual differences in the speed of learning and expression of three dopamine related genes (Darpp-32, Rgs9 and Drd1a). The QPCR

data also clarified a puzzling finding from the microarray data whereby we found opposite directions of effect for these genes in the two replications. Further analysis in experiment 2 confirmed that this was due to one aberrant sample. The functional significance of the up-regulation of these three dopamine related genes in faster learners is most likely directly tied to the suppression of PP1. These three genes could act in concert to enhance synaptic plasticity by suppressing PP1 following dopamine stimulation. In turn this could act to enhance the activity of prefrontal networks that are involved in working memory. Interestingly, in humans there is evidence that certain haplotypes in the Darpp-32 gene are associated with increased performance on executive functioning / working memory type tasks (Meyer-Lindenberg et al., 2007).

Outside of the three dopamine related genes only one other gene, Nudt6 which is also known as basic fibroblast growth factor (bFGF), showed a significant correlation with general learning abilities. While this gene is only expressed in a select subgroup of neurons it is expressed by astrocytes where it acts as a potent trophic factor for neurons (Gómez-Pinilla et al., 1992). In culture bFGF has been shown to promote the survival of prefrontal cortical neurons (Morrison et al., 1986). Outside the brain bFGF has been shown to promote angiogenesis (Cross and Claesson-Welsh, 2001). In addition, bFGF is up-regulated in mice that underwent voluntary wheel running for 4 days as compared to sedentary controls – implicating this gene in the positive cognitive effects of exercise (Gómez-Pinilla et al., 1997). The potential implications of the upregulation of this gene for general learning abilities are two-fold. It is possible given the relatively low levels of bFGF found in our samples that the differences were indicative of differential levels of prefrontal vascularization in fast and slow learners. Poor blood flow would have obvious

detrimental effects on cognitive performance. This is demonstrated by the correlation between age-related cognitive decline and cerebral blood-flow (Marchal et al., 1992). The second possibility is that the direct trophic effect bFGF exerts on neurons enhances neuronal survival in fast learners. This may be directly related to general learning abilities (e.g., enhanced survival increases the efficacy of synaptic plasticity). Conversely, it may be side-effect of potentially increased neuronal activity that may accompany fast learning abilities. For instance, enhanced activity of PP1 through Darpp-32 phosphorylation could exert stress on neurons, as when activated PP1 works to conserve energy through a recycling of protein factors, and the reversal of the cell to an energy-conserving state (Ceulemans and Bollen, 2003). In turn trophic factors, such as bFGF, may be needed to maintain cell survival in face of this increased stress.

General Discussion

The present set of experiments was designed to begin to search for the potential molecular and cellular correlates of general learning abilities. In doing so we hoped to be able to confirm and extend the already extensive literature into the neural correlates of general intelligence in humans. In those studies, activity in the prefrontal cortex as well as other regions engaged by executive functions have been shown to be predicative of general intelligence performance (e.g., Gray et al., 2003; Jung and Haier et al., 2007). These previous results support the behavioral studies which have repeatedly shown a correlation between working memory performance and general intelligence (e.g, Colom et al., 2008; Colom et al., 2004; Conway et al., 2002; Conway et al., 1996; Engle et al.,

1999). Thus together they offer converging evidence for a central role for working memory, and specifically selective attention, in general intelligence. However, an understanding of the nature of this relationship on a more molecular level has for the most part eluded researchers using human subjects due to obvious ethical considerations. Due to these considerations our animal model of general learning abilities offers an opportunity otherwise not available.

The main findings from our current study demonstrate a relationship between general learning abilities and dopamine functioning in the prefrontal cortex. Specifically, we showed that three dopamine related genes (Darpp-32, Rgs9, and Drd1a) are significantly correlated with general learning abilities. While it is perhaps premature to speculate about the functional consequence of an up-regulation in these three genes in faster learners, it seems likely that they interact to boost the suppression of protein phosphatase 1 (PP1) and thereby enhance synaptic plasticity and the efficacy of D1 mediated signaling. Activation of D1 dopamine receptors causes a cascade of events which phosphorylates Darpp-32, which in turn inhibits PP1 (Fienberg et al., 1998). An increase in the main functional unit of D1 receptors (Drd1a) as well as Darpp-32 could therefore act in concert to enhance this suppression. Conversely, D2 dopamine receptor activation reduces phosphorylation of Darpp-32 which in turn releases inhibition of PP1 (Nishi et al., 1997). However, Rgs9 dampens the downstream effects of D2 activation (Rahman et al., 2003). Therefore, an increase in Rgs9 also may act to enhance the suppression of PP1. An increase in synaptic plasticity and D1 mediated dopamine signaling efficacy in the prefrontal cortex potentially could act to enhance working memory function and therefore increase general intelligence. For instance, it is known

that that during a working memory tasks activity of dopaminegic midbrain neurons are enhanced and dopamine levels in the prefrontal cortex increase (Shultz et al., 1993; Watanabe et al., 1997). Similarly, studies have shown that differences in Darpp-32 in humans are associated with increased neostriatial volume, enhanced connectivity between the striatum and the prefrontal cortex and better performance on working memory tasks (Meyer-Lindenberg et al., 2007).

While the exact role that dopamine plays in the prefrontal cortex during a working memory tasks are not fully worked out, one intriguing model is worth noting here. Durstewitz et al. (1999) modeled the functioning of prefrontal cortex neurons during a working memory task with and without dopaminergic input. Their simple neural network was designed to mimic the persistent activity that actual prefrontal cortex neurons maintain during a delay period. That is the neurons maintain a memory trace of goal relevant information even in the absence of the original cues and in the face of interference. Their model demonstrated that dopamine inputs into this network served to stabilize these persistent memory traces and protect them from interference. This finding fits nicely with the hypothesized role of the prefrontal cortex in working memory and in general intelligence. That is, the prefrontal cortex acts to maintain selective attention towards goal relevant information and to ignore salient but irrelevant distracters. In light of this model our finding implicating dopaminergic pathways in general learning abilities integrate into the existing general intelligence literature using human subjects.

While we found significant correlations between general learning abilities and a number of genes, most of which were related to dopamine, and while we found that these genes loaded on the same general learning abilities factor as the learning tasks in a

principal component factor analysis, the amount of variance explained by any individual gene was low. While each gene alone explains a small amount of variance, it is likely that combined they may offer more explanatory value. However, the gene expression values for each gene were highly correlated, suggesting that the variance explained by any one gene is highly related to the variance explained by all of the genes together. Indeed, when we extracted the common variance shared by all of the dopamine related genes it still did not explain a very large portion of the underlying variance in general learning abilities (about 20%). This indicates that there is much more to general learning abilities then can be explained by variations in dopaminergic function. Indeed this should be predicted by the normal distribution of general learning scores. If there were only a handful of genes that underpinned general learning abilities, then the distribution would be expected to be multi-modal. The extreme example of this is if there was only one gene explaining general learning abilities then the distribution should necessarily be bi-modal. For instance, there is only one gene variant that explains sickle-cell anemia and therefore there are basically just two possible states (normal or having sickle-cell anemia). Given the current results it may be hypothesized that working memory and prefrontal dopamine signaling are specifically related to general learning abilities but there may be many genes and many pathways involved. It may be that the nature of genome-wide expression assays somewhat limited the scope of what we could possibly find as the threshold for detection of differential expression is fairly high. In addition the nature of the experimental design limited us to finding mostly linear relationships. It is most likely that if the present results are found to be robust, we have captured just a small fraction of the pathways involved.

We chose to look for differences in gene expression between fast and slow learners during basal conditions. That is to say that we looked for differences that are not directly related to specific learning events. It is highly likely that additional genes could be identified that show differential expression when expression is assessed immediately following a learning event. However, animals with high cognitive abilities appear to be qualitatively different those animals with low cognitive abilities prior to any specific learning event. This difference is highlighted by the immediate divergence in performance that is seen between these two groups at the very earliest stages of acquisition of a learning task. In addition, these animals differ on tasks that have no learning component such as working memory and selective attention tasks. Furthermore, in such an analysis it would be difficult to separate gene expression differences that were related specifically to general learning abilities to those that were related to the amount of learning that has occurred as animals of higher cognitive abilities, by their very nature, learn faster. Therefore, if the animals are assessed at a point during acquisition, then how much the animals will have learned will differ. Thus it would be difficult if to disassociate differences that cause the animals to learn at different rates from differences that are the result of how much the animals have learned.

Given the present results, an intriguing next step would be to investigate how the environment interacts with these pathways. It has been demonstrated that general intelligence is not entirely predetermined. For instance, we have recently shown that working memory training is sufficient to enhance general learning abilities based on animals' aggregate performance across a battery of five learning tasks (Light et al., 2009). Therefore, one could hypothesize that working memory training could act to up-

regulate the expression of the critical dopamine related genes in the prefrontal cortex. Along these lines it would also be intriguing to investigate the means by which this hypothesized enhancement occurs. For instance, there may be variations in the methylation patterns in the promoter regions for the critical dopamine related genes. These differences could act to suppress or possibly enhance the transcription of the genes. Working memory training may cause epigenetic changes that result in enhancing general learning abilities. It is worth noting that a number of genes that were found to be differently expressed in the good and bad learners were related to transcriptional regulation and epigenetic mechanisms. For instance, several histone proteins (e.g., Hist1h2bf, and Histh2bf), which are a key component of epigenetic gene expression regulation, were found to differ between the groups. Similarly, Smad2 is involved in transcriptional regulation and Paip2 inhibits mRNA translation.

Overall the present set of experiments contribute to the already existing literature positing a role for working memory in general learning abilities that has been demonstrated both behaviorally (in mice and humans) and by using functional neural imaging (in humans). However, these results also add to this literature by suggesting specific mechanisms (prefrontal dopamine signaling mediated by Darpp-32) that may underlie general learning abilities. If these results are found to be robust then it could lead to an understanding of the basic structure of cognitive abilities that has been evolutionarily conserved across perhaps all mammals.

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FIGURE LEGENDS

FIGURE 1: Flow diagram showing the overall design of experiment 1. One sample of 60 mice was divided into 2 replications. These replications were independently assessed on general learning abilities. The top and bottom of each distribution were then used to assess gene expression of ~250,000 genes

FIGURE 2: Graphs showing the performance of the animals with the best general learning abilities (fast) compared to the animals with the worst (slow) from the original sample of 60 mice (8 per group). The tasks shown are the 5 tasks that make up the learning battery.

FIGURE 3: Representative log-intensity graph showing differential gene expression in the prefrontal cortex. X-axis is the log of the average gene expression values for each gene. Y-axis is the log2 of the ratio of gene expression (fast learners / slow learners). Dashed lines show cutoff threshold. Highlighted genes are examples of three genes (Ppp1r1b (Darpp-32), Rgs9 and Scn1a) that replicated in 2 replications.

FIGURE 4: Images showing prefrontal cortex expression of A) Darpp-32 B) Rgs9 in adult mice. The images are taken from GENSAT and labeling uses green fluorescent protein.

FIGURE 5: Images showing expression of NeuroD1 in granule cells of A) cerebellum in adult mice B) dentate gyrus in D7 mice. The images are taken from GENSAT and labeling uses green fluorescent protein.

FIGURE 6: Overall gene expression of each gene used in the experiment 2 from the prefrontal cortex. QPCR was used to assess the expression

FIGURE 7: Correlations between normalized gene expression (y-axis) and general learning abilities (x-axis – lower scores = faster learning).

A) Significant negative correlation with Darpp-32
B) Significant negative correlation with Drd1a
C) Significant negative correlation with Rgs9

TABLE LEGENDS

TABLE 1: Factor analyses of the performance of the two independent biological replications reveal stable general learning scores.

A) Replication 1
B) Replication 2
C) Combined factor analysis with all 60 animals from both replications

TABLE 2: Differently expressed cerebellum and hippocampus genes as assessed in the genome-wide microarray. Direction of effect refers to how that gene's expression differed in the good learners relative to the bad learners.

TABLE 3: Differently expressed prefrontal cortex and cortex genes as assessed in the genome-wide microarray. Direction of effect refers to how that gene's expression differed in the good learners relative to the bad learners.

TABLE 4: Factor analyses including the prefrontal gene expression and the learning scores

A) Unrotated factor analysis reveals a first factor mostly accounting for the gene expression and a second factor on which the learning tasks load
B) Rotated factor analysis reveals a secondary factor on which the dopamine-associated genes load with the learning tasks

TABLE 5: Factor analyses showing the unique relationship between the dopamine-associated genes and general learning ability

A) A maximal-likelihood rotated factor analysis including all of the prefrontal genes reveals a primary factor which accounts for mostly the variance in the dopamine-associated genes and secondary factor which explains the remaining variance
B) A maximal-likelihood rotated factor analysis including the learning tasks and the factor scores extracted from Table 5A reveal that the dopamine-associated genes share a unique variance with the learning tasks.

TABLE 1

A

	General Learning Factor
Lashley Maze	0.79
Water Maze	0.62
Fear Conditioning	0.55
Passive Avoidance	0.57
Odor Discrimination	0.63
Eigenvalue	2.03
% Variance Explained	41%

B

	General Learning Factor
Lashley Maze	0.73
Water Maze	0.67
Fear Conditioning	0.40
Passive Avoidance	0.90
Odor Discrimination	0.40
Eigenvalue	2.12
% Variance Explained	42%

C

	General Learning Factor
Lashley Maze	0.76
Water Maze	0.64
Fear Conditioning	0.47
Passive Avoidance	0.77
Odor Discrimination	0.51
Eigenvalue	2.05
% Variance Explained	41%

TABLE 2

Cerebellum			
Gene	**Description**	**Direction of Regulation in Fast Learners**	**Function**
Neurod1	neurogenic differentiation 1	UP	regulation of transcription
Paip2	polyadenylate-binding protein-interacting protein 2	UP	translation repressor activity, nucleic acid binding
Rala	v-ral simian leukemia viral oncogene homolog A (ras related)	UP	small GTPase mediated signal transduction
Adat2	adenosine deaminase, tRNA-specific 2	DOWN	tRNA processing
Git2	G protein-coupled receptor kinase-interactor 2	DOWN	ARF GTPase activator activity
Histh2bf	histone cluster 1, H2bf	DOWN	nucleosome assembly
SMad2	MAD homolog 2	DOWN	chromatin binding
Tmem14C	transmembrane protein 14C	DOWN	integral to membrane
Hippocampus			
Gene	**Description**	**Direction of Regulation in Fast Learners**	**Function**
Cart	CART prepropeptide	UP	neuropeptide signaling pathway
Dncl2b	dynein light chain roadblock-type 2	UP	microtubule
Hist1h2bf	histone cluster 1, H2bm	UP	nucleosome assembly
Lrrtm1	leucine rich repeat transmembrane neuronal 1	UP	synaptogenesis
Acot13	acyl-CoA thioesterase 13	UP	regulate intracellular levels of acyl-CoA
Gabbr1	gamma-aminobutyric acid (GABA) B receptor, 1	DOWN	GABA-B receptor activity
Kifap3	kinesin-associated protein 3	DOWN	calcium-dependent cell-cell adhesion
Mbp	myelin basic protein	DOWN	myelination
Ppp3r1	protein phosphatase 3, regulatory subunit B, alpha isoform	DOWN	calmodulin-regulated protein phosphatase
SMad2	MAD homolog 2	DOWN	chromatin binding

TABLE 3

Prefrontal Cortex			
Gene	**Description**	**Direction of Regulation in Fast Learners**	**Function**
Atp8a1	Atpase	UP	ATP binding
Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	UP	required for microRNA-induced gene silencing
Kcnh1	potassium voltage-gated channel, subfamily H (eag-related), member 1	UP	Delayed-rectifier potassium channel
Nudt6	nudix (nucleoside diphosphate linked moiety X)-type motif 6	UP	Trophic factor
Slc25a18	solute carrier family 25 member 18	UP	transport of glutamate across the inner mitochondrial membrane
Scn1a	sodium channel, voltage-gated, type I, alpha	UP	Pore forming unit voltage-gated sodium channel
Darpp-32	dopamine, cAMP-regulated phosphoprotein of 32,000 kDa	DOWN/UP	phosphoprotein phosphatase inhibitor activity
Rgs9	regulator of G-protein signaling 9	DOWN/UP	negative regulation of signal transduction
Drd1a	dopamine receptor D1A	DOWN/UP	dopamine D1 receptor activity
Psmc3ip	proteasome (prosome, macropain) 26S subunit, ATPase 3, interacting protein	DOWN	DNA binding
Cortex			
Gene	**Description**	**Direction of Regulation in Fast Learners**	**Function**
Kcnh1	potassium voltage-gated channel, subfamily H (eag-related), member 1	UP	Delayed-rectifier potassium channel
SMad2	MAD homolog 2	DOWN	chromatin binding
Psmc3ip	proteasome (prosome, macropain) 26S subunit, ATPase 3, interacting protein	DOWN	DNA binding
Cldn12	claudin 12	DOWN	tight junction

TABLE 4

A

	Factor 1	Factor 2
Lashley Maze	0.17	0.69
Water Maze	0.24	0.49
Fear Conditioning	0.01	0.60
Passive Avoidance	0.44	0.72
Odor Discrimination	0.47	0.36
Atp8a1	-0.95	0.19
Ddx6	-0.91	0.21
Drd1a	-0.89	-0.12
Kcnh1	-0.93	0.18
Nudt6	-0.79	0.13
Darpp32	-0.92	-0.09
Rgs9	-0.69	-0.35
Scn1a	-0.87	0.31
Slc25a18	-0.92	0.26
Eigenvalue	7.47	2.22
% Variance Explained	53%	16%

B

	Factor 1	Factor 2
Lashley Maze	0.02	0.71
Water Maze	-0.09	0.55
Fear Conditioning	0.15	0.58
Passive Avoidance	-0.22	0.81
Odor Discrimination	-0.34	0.48
Atp8a1	0.96	-0.07
Ddx6	0.93	-0.05
Drd1a	0.82	-0.37
Kcnh1	0.94	-0.07
Nudt6	0.79	-0.08
Darpp32	0.85	-0.34
Rgs9	0.56	-0.53
Scn1a	0.92	0.05
Slc25a18	0.96	0.00
Eigenvalue	7.06	2.63
% Variance Explained	50%	18%

TABLE 5

A

	Dopamine Factor	Remaining Variance
Atp8a1	0.34	0.92
Ddx6	0.39	0.85
Drd1a	**0.78**	0.54
Kcnh1	0.38	0.88
Nudt6	0.24	0.75
Darpp32	**0.78**	0.56
Rgs9	**0.98**	0.15
Scn1a	0.24	0.89
Slc25a18	0.31	0.90
Eigenvalue	2.81	5.18
% Variance Explained	31%	57%

B

	Factor 1
Lashley Maze	0.49
Water Maze	0.35
Fear Conditioning	0.30
Passive Avoidance	0.97
Odor Discrimination	0.32
Dopamine Factor	-0.68
Remaining Variance	-0.01
Eigenvalue	1.99
% Variance Explained	28%

FIGURE 1

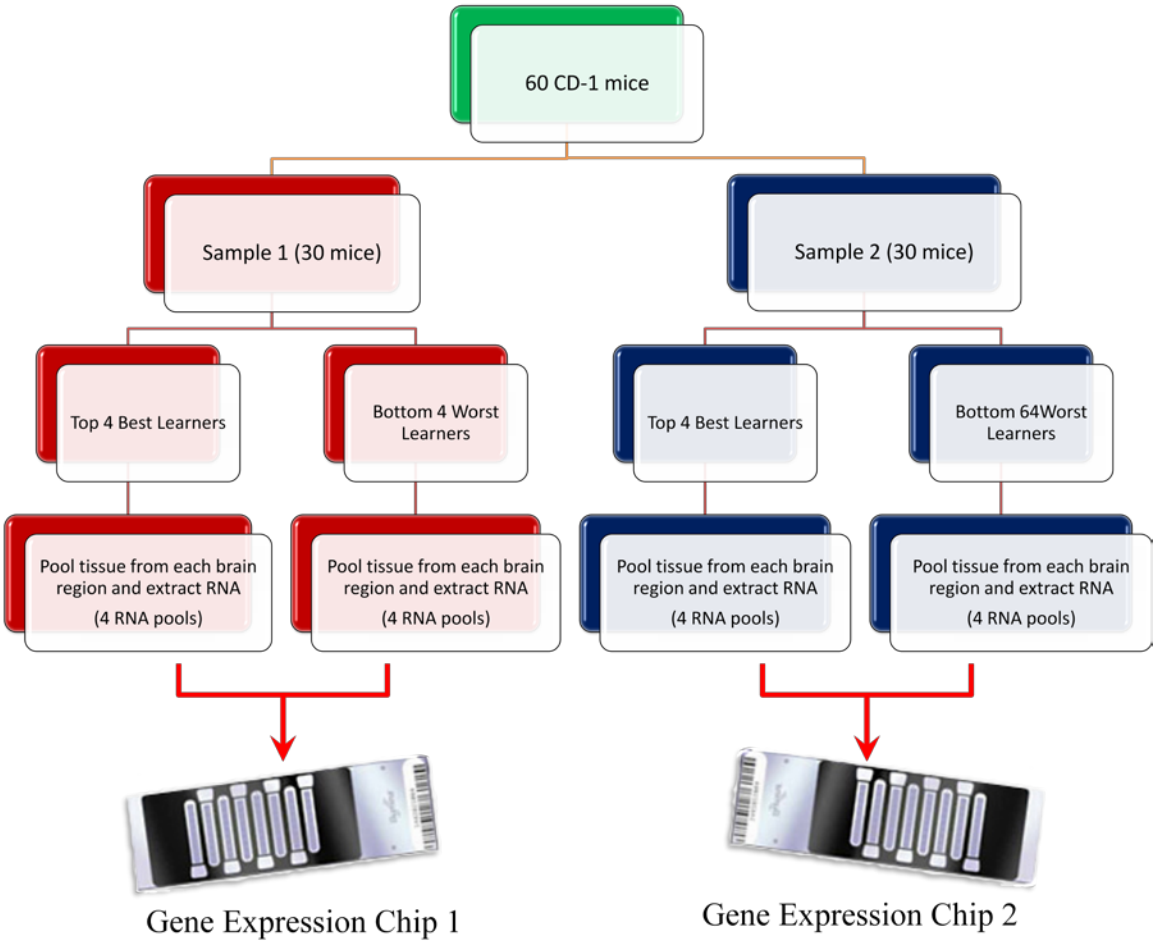

60 CD-1 mice
Sample 1 (30 mice)
Sample 2 (30 mice)
Top 4 Best Learners
Bottom 4 Worst Learners
Top 4 Best Learners
Bottom 64Worst Learners
Pool tissue from each brain region and extract RNA (4 RNA pools)
Pool tissue from each brain region and extract RNA (4 RNA pools)
Pool tissue from each brain region and extract RNA (4 RNA pools)
Pool tissue from each brain region and extract RNA (4 RNA pools)
Gene Expression Chip 1
Gene Expression Chip 2

FIGURE 2

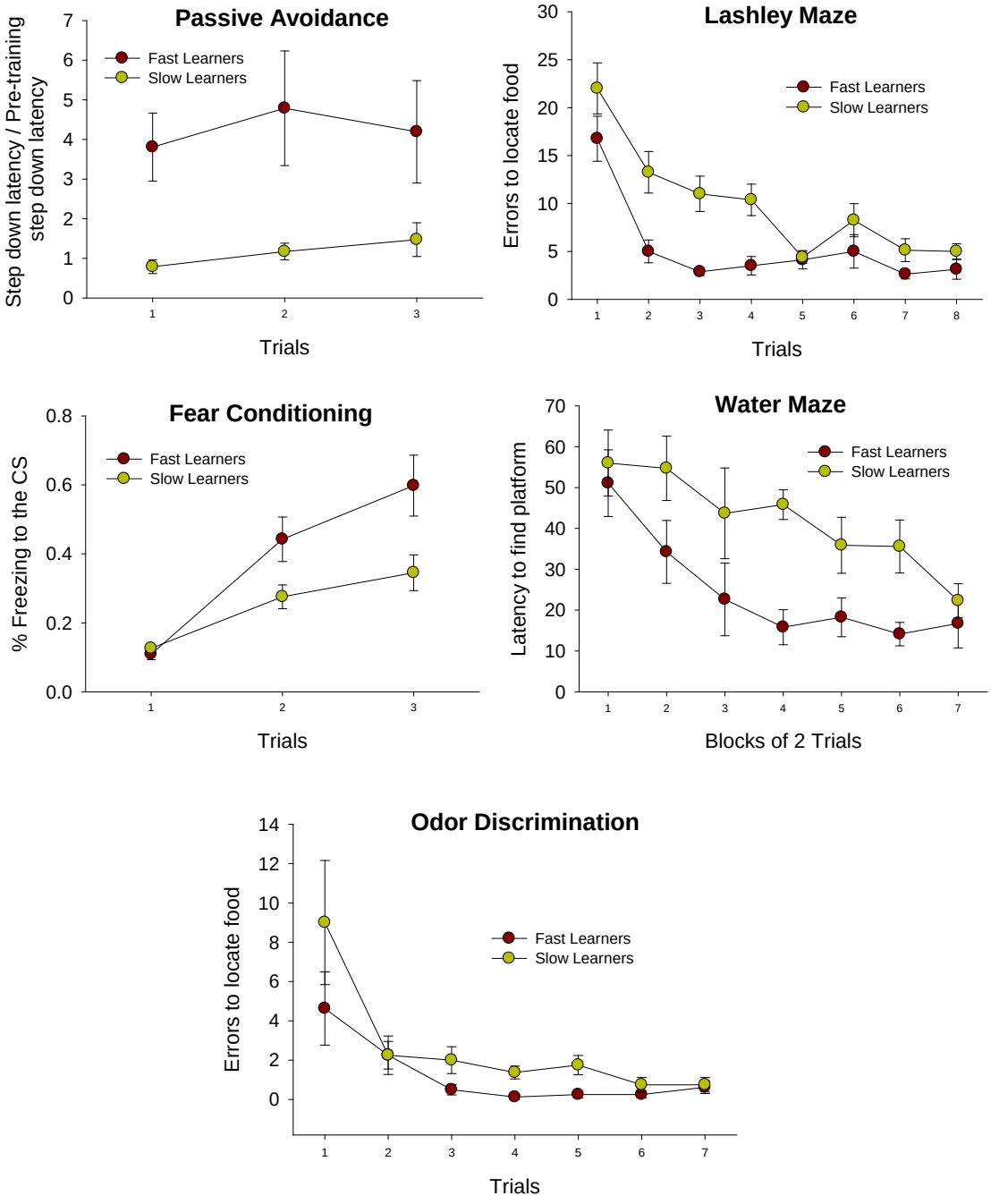

Passive Avoidance
Step down latency / Pre-training step down latency
Fast Learners
Slow Learners
Trials
Lashley Maze
Errors to locate food
Fast Learners
Slow Learners
Trials
Fear Conditioning
% Freezing to the CS
Fast Learners
Slow Learners
Trials
Water Maze
Latency to find platform
Fast Learners
Slow Learners
Blocks of 2 Trials
Odor Discrimination
Errors to locate food
Fast Learners
Slow Learners
Trials

FIGURE 3

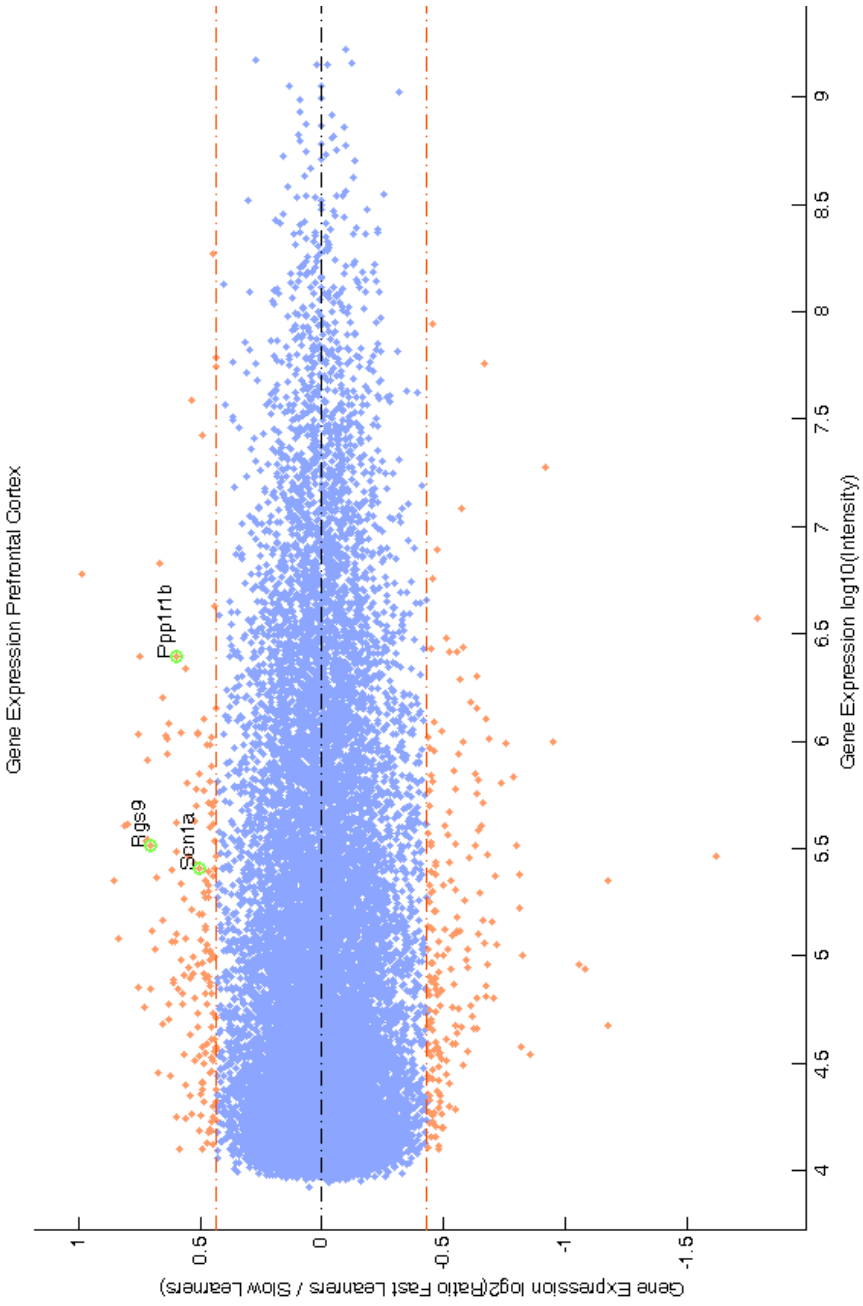

Gene Expression Prefrontal Cortex
Ppp1r1b
Rgs9
Scn1a
Gene Expression log2(Ratio Fast Learners / Slow Learners)
1
0.5
0
-0.5
-1
-1.5
Gene Expression log10(Intensity)
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9

FIGURE 4

A

B

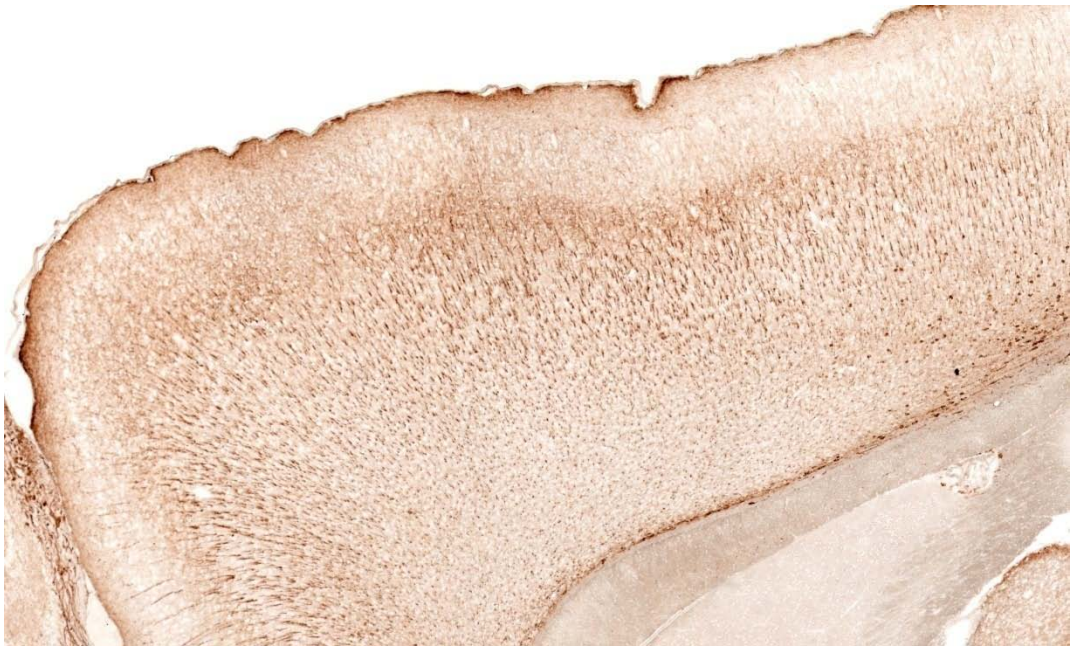
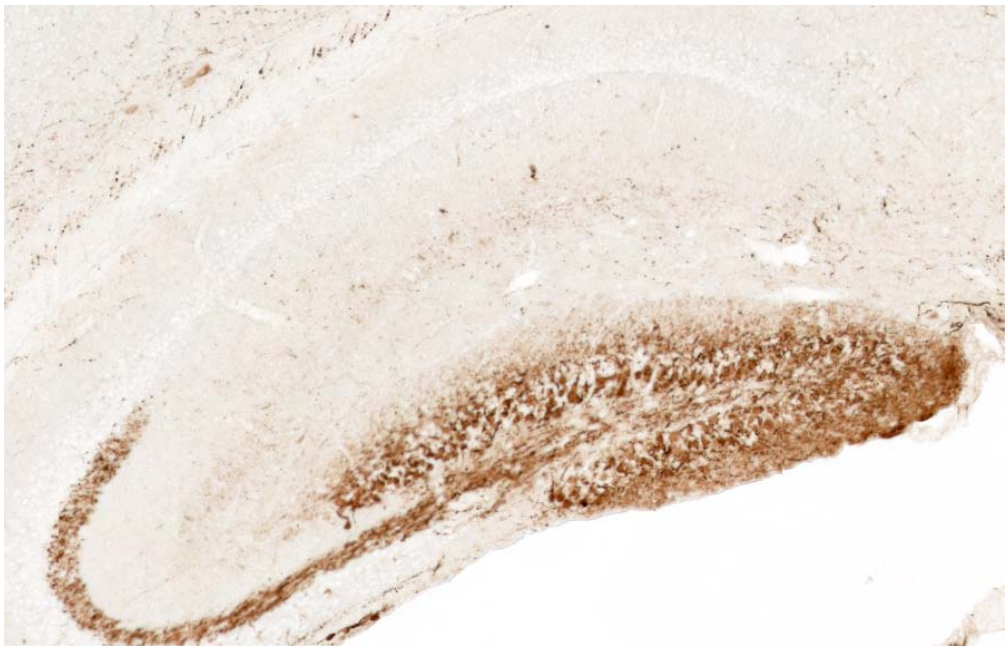


FIGURE 5

A



B

FIGURE 6

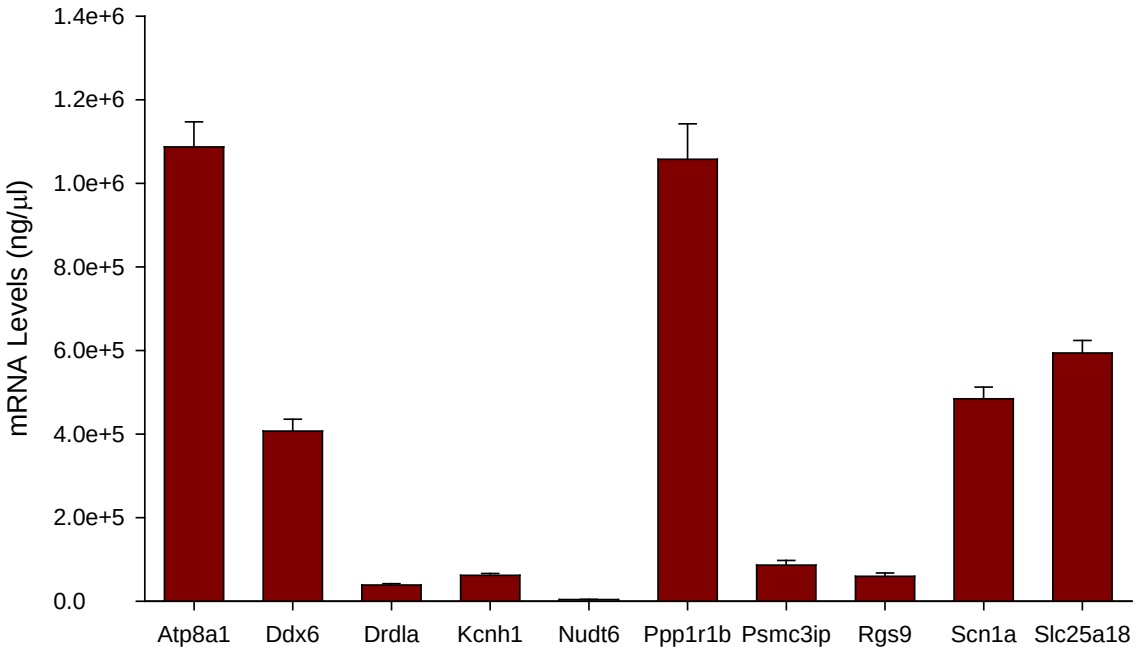
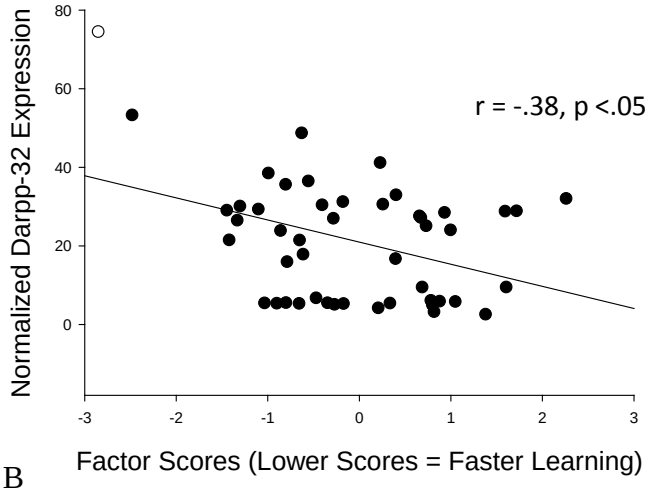
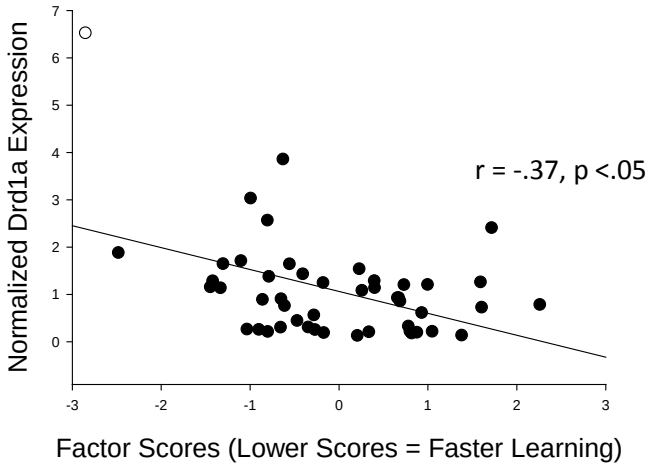

1.4e+6
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0
mRNA Levels (ng/μl)
Atp8a1
Ddx6
Drdla
Kcnh1
Nudt6
Ppp1r1b
Psmc3ip
Rgs9
Scn1a
Slc25a18

FIGURE 7

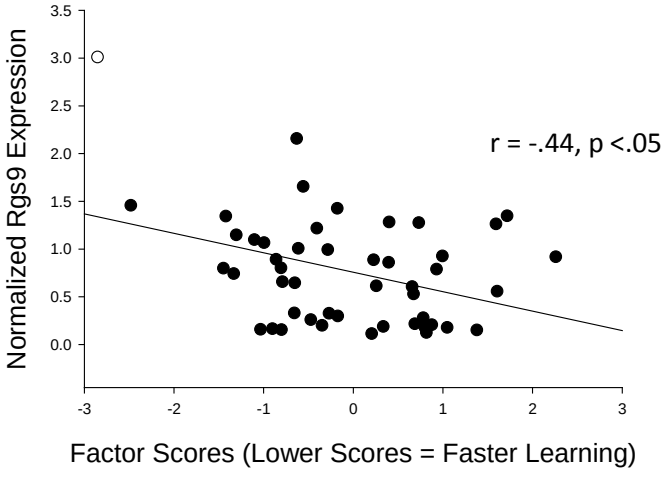
A


Normalized Darpp-32 Expression
r = -.38, p <.05
Factor Scores (Lower Scores = Faster Learning)

B


Normalized Drd1a Expression
r = -.37, p <.05
Factor Scores (Lower Scores = Faster Learning)

C


Normalized Rgs9 Expression
r = -.44, p <.05
Factor Scores (Lower Scores = Faster Learning)

Curriculum Vitae

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1999-2003 University of Pennsylvania, Philadelphia, Pa
B.A. in cognitive science (behavioral neuroscience concentration), minor in computer science

2003-2005 Rutgers University, New Brunswick, NJ
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Teaching

2003-2009 Graduate Teaching Assistant, Rutgers University, 2003-present
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Peer Reviewed Journal Articles

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

Matzel, L.D., Townsend, D.A., Grossman, H., Han, Y.R., Hale, G., Zapulla, M., Light, K., & 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*, 86, 228-240.

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