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Dopamine D1 sensitivity in the prefrontal cortex predicts general cognitive abilities and is modulated by working memory training

Christopher Wass, Alessandro Pizzo, Bruno Sauce, Yushi Kawasumi, Tudor Sturzoiu, Fred Ree, Tim Otto, and Louis D. Matzel

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A common source of variance (i.e., “general intelligence”) underlies an individual’s performance across diverse tests of cognitive ability, and evidence indicates that the processing efficacy of working memory may serve as one such source of common variance. One component of working memory, selective attention, has been reported to co-vary with general intelligence, and dopamine D1 signaling in prefrontal cortex can modulate attentional abilities. Based on their aggregate performance across five diverse tests of learning, here we characterized the general cognitive ability (GCA) of CD-1 outbred mice. In response to a D1 agonist (SKF82958, 1 mg/kg), we then assessed the relationship between GCA and activation of D1 receptor (D1R)-containing neurons in the prelimbic region of the medial prefrontal cortex, the agranular insular cortex, and the dorsomedial striatum. Increased activation of D1R-containing neurons in the prelimbic cortex (but not the agranular insular cortex or dorsomedial striatum) was observed in animals of high GCA relative to those of low GCA (quantified by c-Fos activation in response to the D1 agonist). However, a Western blot analysis revealed no differences in the density of D1Rs in the prelimbic cortex between animals of high and low GCA. Last, it was observed that working memory training promoted an increase in animals’ GCA and enhanced D1R-mediated neuronal activation in the prelimbic cortex. These results suggest that the sensitivity (but not density) of D1Rs in the prelimbic cortex may both regulate GCA and be a target for working memory training.

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Dopamine and general cognitive abilities

Results

Experiment I: D1 sensitivity and general cognitive abilities

Here we assessed 48 genetically heterogeneous CD-1 mice on a battery of five learning tasks designed to tax different sensory/motor and information-processing systems. Each animal’s aggregate performance (i.e., rate of acquisition) across these five tests of learning served as an indicator of that animal’s general cognitive ability (GCA). The performance of individual animals across all learning tasks was first analyzed with a principal component analysis. This is a variable reduction procedure that uses an orthogonal transformation to convert a set of independent observations (potentially correlated variables) into a set of uncorrelated variables (i.e., principal components). The principal component factor analysis of animals’ performance on the five learning tasks (see Table 1) indicated that performance on all tasks loaded in a consistent direction under a primary factor (GCA factor). That factor (eigenvalue 1.79) accounted for ~30% of the variance in performance of individual animals across all tasks. A secondary factor, with an eigenvalue of 1.34, was also extracted and accounted for ~22% of the variance. However, performance variables did not load in a consistent direction on this factor, meaning that there was not a common source of variance influencing the animals’ performance across all tasks. Since the focus of our study is understanding the mechanisms modulating GCAs, the secondary factor was not further considered. From the primary factor, GCA factor scores were calculated for each of the animals. A factor score is essentially an average z-score of an animal’s performance on the five learning tasks, with each score weighted according to the individual task’s loading on the primary (GCA) factor. Thus an animal’s factor score is a quantification of that animal’s relative position in the distribution of GCAs represented by this sample.

Once each animal’s factor score was obtained (where higher factor scores = higher GCAs), we then compared the factor scores of the animals that received a D1 agonist to their c-Fos immunoreactive nuclei in the prelimbic cortex (t(22) = 0.48, P < 0.02) (Fig. 1A), agranular insular cortex (t(22) = 0.29, n.s.) (Fig. 1B), and the dorsomedial striatum (t(22) = 0.06, n.s.) (Fig. 1C). These results indicate that animals which have higher GCAs expressed an increased level of D1R-mediated neuronal activation in the prelimbic cortex. There was no significant correlation between factor scores and c-Fos immunoreactive nuclei for any of the above regions in the animals that were treated with saline: prelimbic cortex (t(22) = 0.08, n.s.), agranular insular cortex (t(22) = 0.26, n.s.), and the dorsomedial striatum (t(22) = 0.1, n.s.).

Groups of animals representing different GCAs were then compared based on their aggregate performance across all learning tasks. That is, the factor scores (of each individual) were ranked, and the top, middle, and bottom thirds of these ranked scores were used to construct groups of animals representing high, intermediate, and low GCAs. Marked differences in the average number of D1R-mediated c-Fos immunoreactive nuclei in the prelimbic cortex were observed in animals of high, intermediate, and low GCAs (representative slices provided in Fig. 2). The average number of c-Fos immunoreactive nuclei in the prelimbic cortex (see Fig. 3A) was then compared between groups using an ANOVA which revealed a main effect of group, F(2,21) = 3.59, P < 0.05. An LSD post hoc analysis revealed a significant difference between animals of high GCA and low GCA in the prelimbic cortex, F(2,21) = 2.25, n.s.) or the dorsomedial striatum (F(2,21) = 1.12, n.s.) (Fig. 3B,C).

<table>
<thead>
<tr>
<th>Table 1. Experiment 1’s factor loadings from the principal components analysis (n = 48) for performance on the five learning tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning task</td>
</tr>
<tr>
<td>Lashley III maze</td>
</tr>
<tr>
<td>Water maze</td>
</tr>
<tr>
<td>Passive avoidance</td>
</tr>
<tr>
<td>Fear conditioning</td>
</tr>
<tr>
<td>Odor discrimination</td>
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<tr>
<td>Eigenvalue</td>
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<tr>
<td>Variance explained</td>
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</table>
A sample of 32 CD-1 mice were assessed for their learning performance in the five learning tasks, which were once again subjected to a principal components analysis in order to derive each individual animal’s factor score (indicative of GCA). A primary factor was extracted with an eigenvalue of 1.94, which accounted for 32% of the variance in performance across the five tasks (Table 2). From the primary factor, factor scores were extracted to represent animals’ GCAs. A secondary factor was also extracted with an eigenvalue of 1.39 which accounted for 21% of the variance. Performance measures did not load in a consistent direction on this factor, indicating that this factor did not capture a common source of variance across all tasks. This factor will not be further considered. Since this experiment was intended to determine whether there was a differential level in the density of D1Rs in the prelimbic cortex between animals of high GCA compared to those of low GCA, eight animals with the highest GCAs and eight animals with the lowest GCAs were assessed for their levels of D1R protein by Western blotting procedures (Fig. 4A,B).

Once the density of D1 protein in the prelimbic cortex was quantified for each subject, we then assessed whether there were differences in the density of D1Rs between high GCA animals and low GCA animals. In order to accomplish this we averaged the density of the eight animals with the highest GCA (13.3 ± 1.49SEM) and compared that to the average density of the eight animals with the lowest GCA (13.68 ± 2.09SEM) using an independent samples t-test. Results indicate that there were no significant differences in the average density of each group, t(14) = −0.15, n.s. (Fig. 4C). In addition to between-group comparisons (i.e., animals of high and low GCA), we also performed a simple correlation comparing D1R density level to factor scores, which again found no relationship between these two variables, r(14) = 0.004, n.s. These results indicate that the number of D1Rs does not differ between animals of high GCA and low GCA. Thus the increases in D1R-mediated neuronal activation observed in Experiment 1 were not likely the result of differential densities of D1 receptors.

**Experiment 3: Effect of working memory training on cognitive performance and D1 sensitivity**

It has been reported that the imposition of a working memory training regimen with a high demand on selective attention can promote an increase in the general cognitive performance of mice (Light et al. 2010; Matzel et al. 2011). Here we ascertained if working memory training targeted the same dopaminergic signaling mechanisms (D1R-containing neurons) that underlie innate cognitive abilities. One might expect such a modulation since receptor use is a critical determinant of rate of turnover, and turnover regulates sensitivity (Gygi et al. 1999; Ferguson 2001; Olesen et al. 2004). Since D1Rs in prefrontal networks are preferentially active during the execution of working memory tasks, intense working memory training might reasonably be expected to promote an increased rate of turnover and hence sensitivity.

Here, animals received either working memory training (WMT, n = 30), simple exposure to the training apparatus (EXP, n = 28), or remained in their home cage (HOME, n = 26). In order to ensure that animals of high GCA were not spuriously assigned into a single experimental group, the groups were created so that each group had a sample of animals with similar innate exploratory tendencies assessed by levels of exploration in an open field. As innate exploratory tendencies have been shown to be co-regulated with an animal’s GCA, this served as a viable way to ensure that animals of similar GCAs were equally represented in each of our treatment groups (Matzel et al. 2006; Light et al. 2011). A subgroup of each of these groups (WMT, n = 14; EXP, n = 14; HOME, n = 12) was subsequently assessed for performance across the battery of learning tasks (described below). As in the prior experiment, the acquisition performance of these animals across all learning tasks was first analyzed with a principal component analysis. This analysis extracted a primary factor (GCA factor) with an eigenvalue of 1.27, which accounted for 30% of the variance in performance of individual animals across all tasks (see Table 3). A secondary factor was also extracted with an eigenvalue of 1.1 which accounted for 22% variance. However,
performance variables did not load in a consistent direction on this factor (indicating no common source of underlying variance), and so this factor will not be further considered. Factor scores were derived from the primary factor that represented the GCAs of individual animals. Factor scores were then segregated according to the treatment that the animals had previously received (i.e., WMT, HOME). When factor scores from the three treatment conditions were compared (see Fig. 5) a main effect of treatment was observed ($F_{2,19} = 6.23, P < 0.01$). Post hoc comparisons of factor scores revealed significant differences between the group that received working memory training (WMT) and the group that received simple exposure to the maze (EXP), $P < 0.05$, and between group WMT and the HOME cage control condition, $P < 0.01$. No significant difference was observed between groups EXP and HOME. These results indicate that 12 d of composite working memory training promoted an increase in the GCAs of treated animals.

Some of the animals received working memory training, simple exposure to the maze, or remained in their home cages, but underwent no behavioral testing in the learning battery (WMT, $n = 16$; EXP, $n = 14$; HOME, $n = 14$). Half of the animals in each of these groups received an intraperitoneal injection of a D1 agonist (SKF82958) and the remaining half received a saline injection (resulting in a total of six groups). One-hour post-injection, levels of c-Fos immunoreactive nuclei were assessed in the prelimbic cortex, agranular insular cortex, and the dorsomedial striatum. Marked differences in the average number of c-Fos immunoreactive nuclei (in response to the D1 agonist) were observed in the prelimbic cortex, suggesting that training-induced differences in GCA may reflect a mechanism analogous to that which is associated with innate differences in GCA. It should be noted that we did not observe any differences in the number of Fos immunoreactive nuclei between the groups that received only a saline injection. That is, all saline-treated animals, regardless of whether they underwent working memory training or remained in their home cages, had similar basal levels of Fos immunoreactivity. One possible explanation for these results could be that since no cognitive demand was placed on the animals there was not a need for an increase in neuronal activation, an effect that is consistent with that reported in the human literature (Cohen et al. 1997). An

comparisons are illustrated in Figure 6B. No significant differences were observed between any groups in the agranular insular cortex $F_{2,19} = 0.911$, n.s. (Fig. 6C), but there was a trend toward significance for a main effect of group in c-Fos immunoreactive nuclei in the dorsomedial striatum, $F_{2,19} = 3.5$, $P = 0.051$ (Fig. 6D). Further post hoc analysis revealed a trend toward a significant difference in the dorsomedial striatum between working memory trained animals and animals that were exposed to the apparatus for an equivalent amount of time ($P = 0.075$).

**Discussion**

The present experiments provide evidence that there is a differential level of D1R-mediated neuronal activation in the prelimbic cortex (the area in rodents that is thought to be homologous to the dlPFC in humans; Uylings et al. 2003) of animals that express high GCAs relative to animals of low GCAs. We had speculated that this difference in activation would arise as a consequence of a higher density of D1Rs, a result that would be consistent with our observation of elevated DRD1 mRNA levels in animals characterized as having high GCA (Kolata et al. 2010). However, no evidence for an increased density of D1R-containing neurons associated with higher cognitive abilities was observed here (Experiment 2). Nevertheless, it was observed (Experiment 3) that the imposition of a working memory training regimen promoted an increase in the neuronal activation of D1R-containing neurons in the prelimbic cortex, suggesting that training-induced differences in GCA may reflect a mechanism analogous to that which is associated with innate differences in GCA. It should be noted that we did not observe any differences in the number of Fos immunoreactive nuclei between the groups that received only a saline injection. That is, all saline-treated animals, regardless of whether they underwent working memory training or remained in their home cages, had similar basal levels of Fos immunoreactivity. One possible explanation for these results could be that since no cognitive demand was placed on the animals there was not a need for an increase in neuronal activation, an effect that is consistent with that reported in the human literature (Cohen et al. 1997). An
increase in basal activity (as would be reflected in elevated Fos immunoreactivity) may be disadvantageous owing to the necessary energy consumption relative to reserving that activity for times of use (e.g., a cognitive demand upon stimulation of the D1 receptor). Thus, the administration of the D1 agonist (which simulates a cognitive demand) might result in an increase in neuronal excitability in the group that underwent working memory training compared to the groups that did not receive that training. These results extend the results obtained with humans showing that working memory training designed to heavily tax selective attention produces a functional change in dopaminergic binding in the dorsolateral prefrontal cortex (McNab et al. 2009), and facilitates the execution of behaviors that in aggregate are indicative of fluid intelligence (for review, see Buschkuehl and Jaeggi 2010).

The question of whether or not increases in mRNA levels should correlate with protein expression has been a central concern in the field of biology. In some instances, mRNA levels correlate highly with levels of protein expression (Futcher 1999), whereas in other cases, no correlation exists between the two (Gygi et al. 1999). The central supposition that DNA is transcribed into RNA and directly determines protein levels presumes that this transcription occurs independently of other rate-limiting factors. Although the results of mRNA analyses aid in the elucidation of how specific phenotypes may manifest, a myriad of factors mediating these processes need to be taken into account since an increase in mRNA simply increases the likelihood that the protein target will be differentially expressed. Factors such as protein half-life (rate of protein turnover), mutations in the mRNA causing them to be silenced (possibly through RNA interference or DNA methylation), or transporter variations can all determine protein levels (Greenbaum et al. 2003).

Our present experiments do not allow us to determine what factors impacted the level of D1R expression in high GCA animals, although an increase in the rate of receptor turnover may underlie the increase in DRD1 mRNA levels in high GCA animals that we have previously reported (Kolata et al. 2010). Importantly, an increase in receptor turnover rates would also correspond to an increase in the level of sensitivity of the D1R (Ferguson 2001). When a D1 agonist binds to the receptor it facilitates specific signaling cascades and, once that signaling cascade is initiated, the receptor is then removed from the membrane through sequestration. It has been long thought that sequestration’s primary role was for receptor desensitization, but more recent evidence has shown that this process effectively promotes receptor re-sensitization which positively regulates receptor signaling (Ferguson 2001; Pierce et al. 2002). Therefore an enhanced rate of receptor turnover would enhance neuronal signaling. That increase in a neuron’s signaling potential may underlie the increase in neuronal activity observed in animals of high GCA compared to those of low GCA.

Simulations of prefrontal cortex firing patterns have led to the hypothesis that D1 modulation of the prefrontal cortex implements a gating function that serves to regulate the maintenance of information in active memory in order to protect the memory from interference (i.e., focusing attention on task-relevant information). This attentional gating feature is thought to be regulated by top-down processing mechanisms. According to this model, D1Rs in the prefrontal cortex underlie the maintenance of relevant information by increasing the tonic activity (via increasing the

Table 2. Experiment 2’s factor loading from the principal component analysis (n = 32) for performance on the five learning tasks

<table>
<thead>
<tr>
<th>Learning task</th>
<th>General cognitive ability factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lashley III maze</td>
<td>0.75</td>
</tr>
<tr>
<td>Water maze</td>
<td>0.42</td>
</tr>
<tr>
<td>Passive avoidance</td>
<td>0.74</td>
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<tr>
<td>Fear conditioning</td>
<td>0.64</td>
</tr>
<tr>
<td>Odor discrimination</td>
<td>0.10</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>1.94</td>
</tr>
<tr>
<td>Variance explained</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Figure 3. Exogenous application of SKF82958-induced c-Fos immunoreactive nuclei. Three groups of animals were formed based on the top, middle, and bottom third of the distribution of factor scores (reflective of GCAs) obtained from the principal component analysis of learning test performance (high factor scores = better general cognitive performance). Values are expressed as the mean ± SEM. (A) Comparison of the mean number of c-Fos immunoreactive nuclei in the prelimbic cortex of animals that had been characterized as having high, intermediate, or low GCA revealed a significant difference between animals of high GCA and low GCA (P < 0.05). (B) Comparison of the mean number of c-Fos immunoreactive nuclei in the agranular insular cortex of animals characterized for their GCA; no significant differences were observed. (C) No significant difference between groups was observed when the mean number of Fos immunoreactive nuclei in the dorsomedial striatum was compared.
gain) of dopaminergic neurons, thus protecting the memory from interference (Cohen et al. 2002; Costa 2007; Costa et al. 2007). This increase in gain promotes persistent neuronal firing in order to stabilize the actively stored memory. In order to then incorporate/update the contents of working memory to ensure that a behavior is guided toward a goal, dopamine D2 receptors are activated in the dorsomedial striatum which “opens the gates” by increasing the phasic activity of dopaminergic neurons and allowing the memory to be updated (Cohen et al. 2002; Wickens et al. 2007). This model has been supported by previous research showing that pharmacological blockade of D2 receptors or DA denervation in the dorsomedial striatum is crucial to the efficient shifting of behaviors (i.e., “cognitive flexibility”) and important in facilitating corticostriatal plasticity (Centonze et al. 2001; O’Neill and Brown 2007).

This model of attentional regulation of information fits well with the present results in that animals with a higher level of GCAs exhibited more robust D1R-mediated neuronal activation. Such a characteristic would not only improve performance on a working memory task, but owing to the role of working memory in the execution of more basic learning tasks, would promote improvements in more general cognitive abilities, as working memory training did here.

The above hypothesis is consistent with the observation in Experiment 3 that animals which underwent extensive working memory training exhibited a consequent increase in D1R-mediated neuronal activation. Since working memory training required animals to actively maintain a memory of locations in the face of interfering external stimuli, that taxon (and its use of the D1R) may have increased the sensitivity of D1R-containing neurons. It is notable in this regard that implementation of a similar working memory training regimen also resulted in improved performance on specific tests of selective attention (Light et al. 2010; Matzel and Kolata 2010; Matzel et al. 2011).

One pathway that could incorporate the current findings with the model presented above may arise from the D1R’s ability to inhibit protein phosphatase 1’s (PP1) negative regulation of downstream proteins and kinases. PP1’s inactivation results from the stimulation of D1Rs, which activate adenylate cyclase. Adenylate cyclase then converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which then phosphorylates protein kinase A (PKA) which in turn phosphorylates Darpp-32. When Darpp-32 is phosphorylated by PKA, it becomes a potent inhibitor of PP1 (Neve et al. 2004; Williams and Castner 2006). The suppression of PP1 leads to an increase in neuronal excitability which results in an increase in downstream proteins and kinases important for synaptic plasticity and the facilitation of learning and memory (Calabresi et al. 2000; Centonze et al. 2001; Genoux et al. 2002; Neve et al. 2004; Allen et al. 2006).

Table 3. Experiment 3’s factor loading from the principal component analysis (n = 40) for performance on the five learning tasks

<table>
<thead>
<tr>
<th>General cognitive ability factor</th>
<th>Lashley III maze</th>
<th>Water maze</th>
<th>Passive avoidance</th>
<th>Fear conditioning</th>
<th>Odor discrimination</th>
<th>Eigenvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.48</td>
<td>0.75</td>
<td>0.68</td>
<td>0.70</td>
<td>0.20</td>
<td>1.79</td>
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<td></td>
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<td>0.36</td>
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The discussion above is congruent with the hypothesized role that D1Rs in the prefrontal cortex may play in the modulation of animals’ GCAs through their regulation of the efficacy of selective attention (a component of working memory). Taking the current results together with the model presented above, it seems likely that the increase in neuronal activation could be due to a differential sensitivity of the D1R-containing neurons in animals of high GCA compared to animals of low GCA. An increased level of sensitivity would allow for an actively stored memory to be less prone to interference through a process of gain modulation. Although the current experiment cannot ascertain what the direct cause is of the increase in receptor sensitivity, it does suggest that behavioral training regimes and/or pharmacological manipulations could potentially serve to increase an individual’s general intelligence.

Materials and Methods

Experiment I: D1 sensitivity and general cognitive abilities

Subjects

Forty-eight male CD-1 outbred mice were obtained from Harlan Laboratories (Indianapolis, IN) at 45–50 d of age, and weighed ~25–30 g. The subjects were singly housed in clear standard shoe box cages in a humidity- and temperature-controlled vivarium maintained on a 12-h light–dark cycle. To minimize differential stress responses exhibited by the animals due to experimenter handling, the animals were handled by an experimenter for 90 sec a day, 5 d per wk, for a period of 2 wk prior to the start of behavioral testing.

Learning battery

Learning, attentional, and reasoning abilities are co-regulated in CD-1 mice (Matzel et al. 2003; Kolata et al. 2007; Wass et al. 2012). Here GCA was assessed as aggregate performance of animals across a battery of five learning tasks (as first described in Matzel et al. 2003). These tasks were chosen for inclusion specifically because they represent different domains of learning, and are dependent on different sensory/motor and motivational systems. Likewise, these forms of learning are (varyingly) dependent on different neuroanatomical systems. Thus variance shared across all tasks is likely to represent those cognitive abilities that are shared across tasks, i.e., general cognitive ability. All of the animals were tested on the five tasks in the following order: Lashley III Maze, spatial water maze, passive avoidance, associative fear conditioning, and odor-guided discrimination. Three days of rest intervened between each successive task. For tasks utilizing food reinforcers, animals were food-deprived 48 h prior to training by allowing only 90 min of access to food within 2 h of the end of the light cycle. All of the procedures for the five tasks have been recently described in Wass et al. (2012); therefore, only brief descriptions of the tasks, as well as some of the neuroanatomical areas implicated in the processing of each task, will be discussed here.

Lashley III maze. In this task, animals were required to navigate from a starting location through a series of four interconnected alleys.
in order to receive a positive reinforcer located in a goal box connected to the fourth alley. In order to successfully traverse this maze without making errors (wrong turns which would result in a dead end or backtracking to a previously visited alley), the animal must utilize egocentric navigation (remembering a sequence of right or left turns). Some of the neuroanatomical areas thought to be involved in mediating learning in this task are the anterior cingulate cortex (Kesner et al. 1989; Kolb et al. 1994), prelimbic cortex (Kesner et al. 1989), and the dorsolateral striatum (De et al. 2005; Palencia and Ragozzino 2005).

Passive avoidance. Passive avoidance is an operant conditioning task where an animal learns to suppress movement in order to avoid an aversive stimulus. This “passive” response is exemplified in Passive avoidance. Passive avoidance is an operant conditioning task (De et al. 2005).

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Spatial water maze. In this task, an animal was immersed in a pool of opaque water in which it must locate a submerged platform. The performance of animals improved across trials (indicated by a reduction in path lengths and latencies to locate the platform) despite starting from multiple locations. Such a procedure mitigates the use of egocentric navigation and promotes the use of allocentric navigation (i.e., using stable extramaze cues in order to discern the location of the submerged platform). Neuroanatomical areas thought to be involved in modulating performance in this task include the hippocampus (Save and Neuroanatomical areas thought to be involved in modulating learning during this task are the prelimbic cortex (Maren 2001), prelimbic cortex (Kesner et al. 1989) and the dorsolateral striatum (De et al. 2005).

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sections were cut. Once the brain sections were cut, tissue punch es corresponding to the prelimbic cortex were taken. Each tissue punch yielded ~3 mg of tissue.

Once the intended tissue samples were collected, the samples were suspended in ice-cold lysis buffer with a protease inhibitor cocktail (#78430, Thermo Scientific). Protein extracts were then purified by centrifugation and centrifuged at 12,000 rpm for 10 min at 4°C. Supernatants were then extracted, flash frozen in liquid nitrogen, and stored at −70°C until use. Protein concentrations were then determined in duplicates using a Bradford Assay Kit (Bio-Rad). Samples of 25 μg of protein were separated by gel electrophoresis using 10% TGX precast gels (#456-1033, Bio-Rad) and blotted onto 0.4-μm pore size PVDF membrane using the mini-Protene tetra cell electrophoresis system (Bio-Rad). The membranes were then incubated with mouse anti-DRD1 mono-
clonal antibody (1:250, Chemicon International) and rabbit anti-
actin polyclonal antibody (1:5000, Abcam) in 20mM Tris HCL pH 7.5 containing 0.9% w/v NaCl, 0.1% v/v Tween-20, and 5% w/v nonfat dry milk (Carnation) for 2 h at room temperature. The membrane was then washed three times for 10 min before being incubated in anti-mouse-IgG and anti-rabbit-IgG conjugated to horseradish peroxidase (1:500, Millipore). Protein bands were then detected using a chemiluminescent substrate ECL kit (#2650, Millipore) and visualized with a Bio-Rad Fluor-S multi-imager and normalized to actin. Band densities were then ana-
yzed using Image J software provided by NIH.

**Experiment 3: Effect of working memory training on cognitive performance and D1 sensitivity**

**Subjects**

Eighty-five CD-1 outbred mice were obtained from Harlan Laboratories (Indianapolis, IN) at 45–50 d of age and weighed be-
tween 25 and 30 g. Housing and maintenance conditions were identical to those described in Experiment 1.

**Working memory training**

This experiment utilized a three-group design. One group received working memory training (WMT, n = 30), one received an equivalent amount of time exposed to the training apparatus without being trained (EXP, n = 28), and one remained in their home cages and received only an equivalent amount of handling and rein-
forcers (HOME, n = 26). To ensure that a random selection of animals was assigned to each of the groups, we first assessed the animals' innate propensity for exploration in an open field (a behavior that tends to predict GCA). Once the animals' propensi-
sity for exploration was quantified, they were divided into three groups with similar exploratory tendencies constituting group WMT, EXP, and HOME.

A complete description of these procedures appears in Light et al. (2010). Briefly, mice in the WMT group were trained to as-
ympote on two distinct radial arm mazes (one black with walls around the center hub, one gray with no walls), where the animals collected food at the end of each of the eight arms. The mazes were located in the same room such that they shared common extra-
maze visual stimuli (geometric patterns comprised of small LED "Christmas" bulbs, pictures, and architectural details that can be used by the animal as search cues).

Each trial in each maze began with one piece of food at the end of each arm. Animals were initially trained on each maze indi-
vividually. Trials (i.e., completion of one maze) were administered once per day (alternating the black and gray mazes across days), and this training was complete in 32 d (i.e., all animals received 16 trials in each maze). At the end of this training, performance of all animals had stabilized and all animals made at least six con-
secutive correct choices on the last two trials in each maze. At this point, animals continued training, but now received two trials a day, one in each maze (with a 4-h inter-trial interval). This train-
ing proceeded for 4 d (during which the order of testing in the two mazes alternated across days). Subsequent to this initial training (during which the animals had received a total of 20 trials in
each maze), the animals then performed concurrently on both mazes once a day for 12 d (constituting working memory train-
ing). During this training, mice alternated choices in blocks of three in each maze (i.e., were allowed to find three pellets in the black maze, three pellets in the gray maze, three pellets in the black, three pellets in the gray, two pellets in the black, two pellets in the gray). This training required the animals to concurrently maintain a memory of the choices in each maze. This task has been asserted to have a high attentional demand, since the memory of previously visited arms is dependent on extramaze vi-

cual cues that are common to the two mazes (i.e., the mazes are located in a single room). Thus, this training taxed both the main-
tenance of information as well as working memory capacity and selective attention (see Matzel and Kolata [2010] for an expanded rationale).

During each of the training periods described above, subjects in the EXP group were placed in the apparatus for an amount of time that equaled matched animals in the WMT group. The EXP group also received reinforcers in the apparatus, but the pellets were not located at the end of each arm; rather, they were located at the beginning of each arm nearest the central hub so that work-
ing memory was not taxed (since the animal could see the food and would not have to actively maintain the locations that it had previously visited). This group ensured that the effects of working memory training were not solely due to the animals being exposed to a novel environment or the level of activity associated with the working memory training procedure.

**Learning battery**

A subgroup of animals that received working memory training (n = 14), exposure to the training apparatus (n = 14), or remained in their home cages (n = 12) was assessed for the animals' cogni-
tive performance across the battery of five learning tasks in order to ensure that the training procedure effectively enhanced the GCA of animals that received training. The order the tasks were implemented was identical to that in Experiment 1 (see above).

**cFos immunohistochemistry**

Two weeks following the completion of the working memory training regimen, the three groups of animals that received no behavioral testing (WMT, n = 16; EXP, n = 14; HOME, n = 14) were further subdivided in half (resulting in a total of six groups). Three groups received (via i.p. injection, 1 mg/kg) the D1 agonist SKF82958 (Chloro-APB-Hydrobromide), and three received an in-
jection of 0.09% saline. Further histological procedures/analyses for the expression of c-Fos were performed identically to those of Experiment 1.

**Acknowledgments**

This work was supported by the National Institute on Aging (AG022698), the Office of Naval Research (N000141210873), the Busch Foundation (to L.D.M.) and the National Science Foundation (IOS 0919159) to T.O.

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Received June 4, 2013; accepted in revised form August 1, 2013.