Dopaminergic Sensitivity in the Prefrontal Cortex is Associated with Variations in General Cognitive Abilities and is a Target for Working Memory Training

By

Christopher David Wass

A thesis submitted to the

Graduate School-New Brunswick

Rutgers, the State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Psychology

Written under the direction of

Louis D. Matzel Ph.D.

And approved by

_____

_____

_____

New Brunswick, New Jersey

October 2012

ABSTRACT OF THE THESIS

Dopamine Signaling and General Cognitive Abilities

By CHRISTOPHER DAVID WASS

Thesis Director:

Professor Louis D. Matzel Ph.D.

An individual's performance across diverse tests of cognitive ability tends to co-vary, indicative of a common source of underlying variance (i.e., "general intelligence"). Recent evidence indicates that the processing efficacy of working memory predicts the level of general intelligence in humans and general cognitive abilities (GCA) in non-human animals (e.g. rodents and monkeys). One component of working memory, namely selective attention, has been reported to highly co-vary with general intelligence, and evidence suggests that dopamine D1 signaling in the medial prefrontal cortex (mPFC) critically modulates attentional abilities. Here, we characterized the GCA of 48 CD-1 outbred mice based on their aggregate performance across five diverse tests of learning. Using immunohistochemical techniques following administration of a D1 agonist (SKF82958, 1 mg/kg), we examined the relationship between GCA and endogenous sensitivity of D1 receptors in the mPFC, the dlPFC, and the striatum. Results indicate a differential sensitivity of D1 receptors in the mPFC (but not the dlPFC or striatum) between animals of high GCA and low GCA (quantified by cFos activation in response to

the D1 agonist). In Experiment 2, we assessed whether the enhancement in D1 receptor sensitivity levels were the result of an increased amount of D1 receptors in the mPFC. Results indicated that animals of high GCA and low GCA express no differences in the density of D1 receptors in the mPFC. Subsequently we examined whether the imposition of a working memory training regimen (with a high demand on selective attention) modulates the same dopaminergic signaling mechanisms that were associated with innate GCA. Working memory training promoted an increase in animals' GCA and enhanced the sensitivity of D1 receptors in the mPFC. These findings suggest that the sensitivity (but not number) of D1 receptors in the mPFC may both regulate GCA and may be a target for working memory training that promotes GCA.

Acknowledgments

I would like to thank Dr. Louis Matzel for his guidance while conducting this research as well as his assistance and mentorship during all aspects of my graduate training. I would also like to thank the members of my thesis committee, Dr. Alex Kusnecov and Dr. Timothy Otto for their constructive comments on the thesis. Finally I would like to thank my peers, both graduate and undergraduate, as well as Thomas Grace for their excellent assistance.

TABLEOF CONTENTS

LIST OF TABLES

LIST OF FIGURES

General Introduction:

Evidence suggests that working memory and general intelligence are highly co-regulated (1, 2,3). However studies of humans are constrained in their ability to assess the mechanisms that underlie general intelligence and the elucidation of these neural and molecular mechanisms is considered to be a principal goal of contemporary intelligence research (4). Much like humans, the efficacy of an animal's working memory processing components, namely working memory capacity and selective attention, is co-regulated with their general cognitive abilities (GCA; 5,6) and also as in humans, may be a causal determinant of general cognitive performance (7). We have previously shown that the imposition of a working memory training regimen facilitates an increase in animals' GCA (6). Since working memory and general intelligence have been shown to be highly co-regulated, here we assessed whether innate GCA and the beneficial impact of working memory training on GCA shared common substrates.

Recent fMRI imaging studies of humans have shown that the dorsolateral prefrontal (dlPFC) cortex, as well as the parietal cortex, are engaged during working memory-based tasks with a high selective attention load (8,9,10). It has also been asserted that D1 activity levels in the dlPFC and parietal cortex may play a role in modulating the relationship between working memory performance and intelligence (11). Furthermore, it has been suggested that working memory training enhances an individual's fluid intelligence (7) as well as producing functional changes in D1 dopaminergic binding in the prefrontal cortex (11,12,13).

Research on non-human animals has implicated the medial prefrontal cortex (mPFC; an area thought to be analogous to that of the dlPFC in humans) in the regulation

of working memory/attentional abilities (14,15). Lesions of this sub-region have been shown to impair an animal's performance in working memory tasks such as the radial arm maze (16) as well as tasks designed to assess an animal's attentional abilities such as the five-choice serial reaction time task (14,17,18,19). Pharmacological manipulations of D1 signaling in the mPFC have determined that an increase in the activation of D1 receptors within the mPFC enhances the performance of animals that have been categorized as having "poor" attentional abilities (15). Conversely, injections of D1 antagonists impair the attentional performance of animals that have been classified as having "good" attentional abilities (20,21).

Previously we have used a microRNA analysis to examine 25,000 genes in the frontal cortex of genetically heterogeneous CD-1 mice that have been characterized for their GCA. It was revealed that roughly 10 genes were up-regulated in animals with high GCA relative to animals with low GCA. Of those 10 relevant genes, three in particular form a functional dopaminergic cluster (Darpp-32, Rgs9, and Drd1a) which have the potential to modulate the sensitivity of dopaminergic binding to the dopamine D1 receptor (22).

In conjunction with the results above, our microarray analysis suggests that the efficacy of D1 signaling in mPFC may modulate individuals' attentional abilities and in turn the general cognitive performance of both human and non-human animals. To test this hypothesis we first examined the endogenous sensitivity levels of D1 receptors in animals that have been quantified for their GCA. Experiment 2 then aimed to assess whether or not any enhancements in D1 receptor sensitivity levels were directly related to the density of D1 receptors in the mPFC. Furthermore, Experiment 3 aimed to assess

whether the implementation of working memory training (with a high selective attention load) affected the same dopaminergic signaling mechanisms that may innately regulate GCA.

Experiment 1

Materials and Methods

Subjects: A sample of 48 male CD-1 outbred mice were obtained from Harlan Laboratories (Indianapolis, IN) at 45-50 days of age, and weighed 25-30 grams. The subjects were singly housed in clear standard shoe box cages in a humidity and temperature controlled vivarium which was maintained on a 12 hour light/dark cycle. In order to minimize any differential stress responses exhibited by the animals due to experimenter handling, the animals were handled by an experimenter for 90 seconds a day, five days per week, for a period of two weeks prior to the start of behavioral testing.

Learning Battery

It was previously determined that learning, attentional, and reasoning abilities are co-regulated in CD-1 mice (5,40, 41). Here GCA was assessed as aggregate performance of animals across a battery of learning tasks (as first described in 40). To this end, we evaluated animals' performance on five diverse tasks that impinged on different domains of learning, sensory/motor, and motivational systems. All of the animals were tested on these 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 in the learning battery. For tasks utilizing food reinforcers, animals were food deprived 48 hours prior to training by allowing only

90 min of access to food within two hours of the end of the light cycle. All of the procedures for the five tasks have been recently described in (41), therefore they will not be described here.

cFos Immunohistochemistry

Two Weeks after the completion of the learning battery, the subjects were subdivided into two groups with equal representation of animals exhibiting high GCA and low GCA (see results). One of these groups received an intraperitoneal injections (1 mg/kg) of a full D1 agonist, SKF82958 (Chloro-APB-hydrobromide), and the other which received a 0.09% saline solution. One hour after injections, the subjects were deeply anesthetized with Nembutal (150 mg.kg), i.p.) and perfused transcardially with a 4% paraformaldehyde solution. The brains were then extracted and allowed to post-fix for a 12 hour period in 4% paraformaldehyde. After the 12 hour post-fixation period the brains were then transferred to a 30% sucrose solution (in 0.05M KPBS) and allowed to equilibrate. The brains were the sectioned in a 1:3 series at 30µm sections on a freezing microtome (Thermo Scientific Microtome Cryostat Micron HM 525) and stored in a cryprotectant at 4^{o}C until ready to be stained.

Free floating sections were washed 5 times for 5 minutes in 0.05M KPBS (pH 7.4) and blocked for endogenous peroxidase binding activity in 1% H_2O_2 for a period of 30 minutes. Following a series of five washes the sections were then incubated in rabbit anti-Fos antiserum (CalBiochem Ab-5; 1:15,000) diluted in 0.4% Triton-X, 1% bovine serum albumin, in KPBS for 48 hours. After a series of five 10 min washes the sections were then incubated for two hours with biotinylated goat anti-rabbit IgG (1:500; Vector

Laboratories, Burlingham, CA, USA) in KPBS, .4% Triton-X at room temperature. An avidin-biotin peroxidase procedure (Vectastain Elite ABC Kit; Vector Laboratories) with 3,3'-diaminobenzidine (Sigma Fast DAB tablets D4293, Sigma) as the chromogen was then used to visualize c-Fos positive cells.

Cell counts

Sections of the medial prefrontal cortex and dorsolateral prefrontal cortex located 5.9 mm from the interaural line, as well as the dorsal striatum located 4.48 mm from the interaural line were examined and photographed using a Sony DFW SX900 Nikon Eclipse E400 digital camera. Cell counts were performed by an observer that was naive to the experimental conditions. Fos-immunoreactive nuclei were counted on the captured images using Image J software (NIH). The number of immunoreactive nuclei were then averaged for each subject.

Results

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. 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 were influenced by a single source of variance (consistent directionality of variable loadings). That factor

(eigenvalue 1.79) accounted for roughly 30% of the variance in performance. From that analysis, a general cognitive ability factor score was 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 tasks' loading on the primary (general cognitive ability) factor. Thus an animal's factor score is a quantification of that animal's position in the distribution of general cognitive abilities.

Once each animal's factor score was obtained (where higher factor scores = higher general cognitive abilities), we then compared the factor scores of the animals which received the D1 agonist to their average cFos immunoreactive nuclei in the medial prefrontal cortex $r(22) = .48$, $p < .02$ (Fig 1A), dorsolateral prefrontal cortex $r(22) = .29$, n.s., (Fig 1B), as well as the striatum $r(22) = .06$, n.s (Fig 1C). These results indicate that animals which have higher general cognitive abilities also expressed an increased level of neuronal activation in the mPFC induced by the D1 agonist. There was no significant correlation for any of the above regions in the animals that were treated with saline (ps < .20).

In a subsequent analysis, groups of animals were compared based on their aggregate performance across all learning tasks. This was accomplished by separating animals into groups comprised of high, intermediate, and low factor scores (based on the principal components analysis presented above). 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 general cognitive abilities. Marked differences in the average number of cFos immunoreactive nuclei in mPFC induced by the D1 agonist were observed in animals of high,

intermediate, and low general cognitive abilities (Fig 2). The average number of cFos immunoreactive nuclei in the mPFC (see Fig 3A) was then compared between groups using an ANOVA which revealed a main effect of group, $F(2,21) = 3.59$, $p<.05$. An LSD post hoc analysis revealed a significant difference between animals of high GCA and low GCA, $p<.05$. No significant differences were observed for the dlPFC or striatum (Fig 3B-C).

Experiment 2

While the results of Experiment 1 indicated there was a differential level of Fos immunoreactive nuclei in animals characterized as having high GCA compared to animals of low GCA, those results are not able to determine whether the differential expression level is due to an increase sensitivity of dopamine D1 receptors or whether there is an increased density of dopamine D1 receptors in animals of High GCA when compared to animals of Low GCA. To this end, prior observations from our laboratory should be considered where a microRNA analysis revealed that animals of High GCA exhibited an up-regulation in DRD1a mRNA levels (22). This increase in the mRNA level could potentially lead to an increased *number* of D1 receptors in the mPFC of animals exhibiting high GCA, and this increase in number may mediate the D1 agonist-induced neuronal activation that we observed in Experiment 1.

Here we aimed to elucidate whether the overall density or the sensitivity of D1 receptors in the PFC was responsible for the differential expression level of Fos immunoreactive nuclei in response to the D1 agonist. For this purpose animals were

again characterized for their general cognitive abilities (described above) and the density of D1 receptors was assessed using Western blotting procedures.

Material and Methods

Subjects: A sample of 32 CD-1 outbred mice were obtained from Harlan Laboratories (Indianapolis, IN) at 45-50 days of age which their weight varied between 25-30 grams. Housing and maintenance conditions were identical to those previously described in Experiment 1.

Learning Battery: These animals were subjected to our standard five task learning battery as previously described in Experiment 1 in order to characterize their general cognitive abilities. The procedures for implementing the learning battery were identical to Experiment 1 as well as the order that the animals were subjected to each individual task.

Drd1a Western Blot

Two weeks following the completion of the learning battery all animals were sacrificed and their brains were rapidly extracted. Following brain extraction, their brains were placed in a brain blocker (Knopf Instruments) in order to ensure near identical sections were cut. Once the brain sections were cut, tissue punches from the medial prefrontal cortex were taken. Each tissue section yielded roughly three mg of tissue.

Once the intended tissue samples were collected, the samples were suspended in ice cold lysis buffer. Protein extracts were then purified by sonication and centrifuged at 12,000 rpm for 10 minutes at 4^{o}C. Supernatents were then extracted, flash frozen in

liquid nitrogen, and stored at -70^{o}C until for use. Protein concentrations were then determined in duplicates using a Bradford Assay Kit (Bio-Rad). Samples of 25µg of protein were separated by SDS-PAGE using 10% gels and blotted onto 0.45µm pore size PVDF membrane using the mini-Proteon tetra cell electrophoresis system (Bio-Rad). Immunohistochemistry was then performed using D1a receptor 1:250 (#MAB5290 Chemicon International) primary antibodies in 20mM Tris HCL pH 7.5 containing 0.9% w/v NaCl, 0.1% v/v Tween-20 and 5% w/v non-fat dry milk (Carnation). Antibody binding was detected using goat anti-mouse-IgG-HRP conjugated secondary antibody 1:500 (#AP124P, Millipore) and visualized by chemiluminescence (Millipore). Protein bands were visualized using a BioRad Fluor-S multi-imager and analyzed for their density using Image J software provided by NIH.

Results

A sample of 32 genetically heterogenous mice were assessed for their learning performance in the five learning tasks, which was once again subjected to a principal components analysis in order to derive each individual animal's factor score. A primary factor was extracted with an eiganvalue of 1.94, which accounted for 32 percent of the variance (Table 2). From this primary factor, factor scores were extracted to represent animals' general cognitive abilities. Since the scope of this experiment was to elucidate whether there was a differential level in the density of D1 receptors between animals of high GCA compared to low GCA animals, eight animals with the highest GCA and eight animals with the lowest GCA were further assayed for their levels of D1 receptor protein by Western Blotting procedures (Figure 4A-B). By doing so we ensured that we were

sensitive to any differences in cognitive ability as well as to the density of D1 receptor levels.

Once the density of D1 protein in the mPFC was quantified for each subject, we then assessed whether there were differences in the density of D1 receptors 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 and compared that to the average density of the eight animals with the lowest GCA using an independent samples t-test. Results indicate that there were no significant differences in the average density of each group, $t(14) = -.15$, n.s. In a subsequent analysis we compared individual subjects D1 receptor density level to their factor scores $r(14) = .004$, n.s (Figure 4C) since we were interested in determining whether differences occurred at an individual level. These results indicate that the number of D1 receptors does not differ between animals of high GCA and low GCA and that the increases in neuronal activation induced by the D1 agonist seen in Experiment 1 was not merely the result of a differential number of D1 receptors.

Experiment 3

Prior studies in our laboratory have shown that the imposition of a working memory traiing regimen with a high selective attention demand promoted an increase in the general cognitive abilities of animals (6). While this cognitive/behavioral training seems to have a modulatory effect on GCA, the underlying mechanisms which are responsible for the modulation of GCA are unknown. Here we aimed to ascertain if

working memory training targeted the same dopaminergic signaling mechanisms (D1 receptors) that underlie innate cognitive abilities.

Material and Methods

Subjects: A sample of 85 CD-1 outbred mice were obtained from Harlan Laboratories (Indianapolis, IN) at 45-50 days of age and weighed between 25-30 grams. Housing and maintenance conditions were identical to those previously described in Experiment 1 (above).

Working Memory Training

The mice were segregated into three groups. One group which received working memory training (WMT n=30), one which received an equivalent amount of time EXPOSED to the training apparatus without being trained (EXP n=28), and one which would remain in their homecages and received only an equivalent amount of handling and reinforcers (HOME n=26).

A complete description of these procedures appears in (6). Briefly, mice in the WMT group were trained to asymptote on two distinct (one black with walls around the center hub, on grey) radial arm mazes, 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 extramaze visual cues (patterns of lights, pictures, and architectural details that are used by the animal to guide its search). After performance in each of the two mazes stabilized, animals then continued training in each of the two mazes each day (with a four hour intertrial interval) for 4 days (the order of testing in the two mazes alternated across days). Subsequent to this initial training, the animals then performed concurrently on

both mazes once a day for 12 days (constituting working memory training). During this training, mice alternated choices (i.e., were allowed to find food: three pellets in the black maze, three pellets in the gray maze, three pellets in the black, three pellets in the gray, find two pellets in the black, find two pellets in the gray), in the two mazes and consequently were required to maintain a memory of the choices in each maze and to segregate those memories despite the overlapping extramaze visual cues. Thus, this training taxed both the maintenance of information as well as working memory capacity and selective attention.

During each of the training periods described above, subjects in the EXP group were placed in the apparatus for the average amount of time that an animal in the WMT group was in the maze. The EXP group also received their 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 the animal would not tax its working memory system (since the animal would not have to actively maintain the locations that it has previously been to) to locate the food. This group ensured that the effects seen from 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.

cFos Immunohistochemistry

Two weeks following the completion of the working memory training regimen, the three groups of animals (WMT, EXP, HOME) were further subdivided into three groups (resulting in a total of nine groups). Three groups received (via i.p. injection; 1

mg/kg) the D1 agonist, SKF82958 (Chloro-APB-Hydrobromide), three received an injection of 0.09% saline, and three were assessed for performance in the battery of learning tasks (previously described in (41). Further histological procedure/analysis for the expression of c-Fos was performed identically to that of Experiment 1.

Results

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). 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 (as described above). 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 single factor with an eigenvalue of 1.70, which accounted for 36% of the variance in performance of individual animals across all tasks. From this analysis, factor scores were derived that represented the general cognitive abilities of individual animals. Factor scores were then segregated according to the treatment that the animals had previously received (i.e., WMT, EXP, HOME). When factor scores from the three treatment conditions were compared (see Fig 4) a main effect of treatment was observed, $F(2, 37) = 6.23$, $p < .01$. 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), $p < .05$, and between Group WMT and the HOME cage control condition, $p < .01$. No significant difference was observed between Groups EXP and HOME. These results indicate that 12 days of

composite working memory training promoted an increase in the general cognitive abilities of treated animals.

Subgroup of animals received working memory training (WMT n=16), simple exposure to the maze (EXP n=14), or remained in their home cages (HOME n=14) and underwent no behavioral testing. These groups were further subdivided (resulting in a total of six groups) such that half of each group received an intraperitoneal injection of a D1 agonist (SKF82958) and the remaining half received a saline injection. One hour post injections, levels of cFos immunoreactive nuclei were assessed in the mPFC, dlPFC, and striatum. Marked differences in the average number of cFos immunoreactive nuclei (in response to the D1 agonist) were observed in the mPFC between animals that received working memory training compared to animals which were either exposed to the maze or remained in their home cages throughout the experiment (Fig 5A-B). The average number of cFos immunoreactive nuclei, induced by the D1 agonist, was compared between groups using an ANOVA which revealed a main effect of group, $F(2,19) = 5.11$, $p<.05$. A Tukey HSD revealed a significant difference between animals that had undergone working memory training and animals that had been exposed to the apparatus for an equivalent amount of time ($p<.05$) as well as animals that remained in their home cages ($p< .05$). No significant differences were observed in the dlPFC $F(2,19) = .911$, n.s., (Fig 5C), but there was a trend towards significance for a main effect of group when differences in cFos immunoreactive nuclei in the striatum was assessed, $F(2,19) = 3.5$, $p = .051$ (Fig 5D). Further post hoc analysis revealed a marginally significant difference between working memory trained animals and animals that were exposed to the apparatus for an equivalent amount of time ($p = .075$).

General Discussion

The present experiments provide evidence that there is a differential endogenous level of neuronal activation induced by a D1 agonist in the mPFC of animals that express high general cognitive abilities relative to animals of low cognitive abilities. It was hypothesized that this difference in activation was the result of an increase in the amount of D1 receptors since a previous study performed in our laboratory showed that there was an up-regulation in DRD1 mRNA levels in animals characterized as having high GCA compared to low GCA (22). While that was a plausible hypothesis it was not substantiated here as the results of Experiment 2 indicated that there was no correlation between the density of D1 receptors and animals' GCA. Also, it was determined that the mechanisms that may modulate an animal's innate cognitive ability are targeted by the imposition of a working memory training regimen. 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 prefrontal cortex (11) as well as facilitating the execution of behaviors that in aggregate are indicative of fluid intelligence (7).

The question of whether or not increases in mRNA levels should correlate with protein expression has been a central dogma in the field of biology. Some researchers have shown that an increase in mRNA does highly correlate with levels of protein expression (24), whereas others have found no direct correlation between the two (25). The central supposition that DNA is transcribed into RNA which in turn is translated into a protein is often assumed to occur without considering other rate limiting factors. While the results of mRNA analyses aid in the elucidation of how specific phenotypes may

become manifested, a myriad of factors mediating these processes need to be taken into account since an increase in mRNA simply means that there is an increased 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 whether there are differences in the number of transporter mechanisms regulating the trafficking of proteins between the endoplasmic reticulum, the golgi apparatus, and ultimately the cell membrane (26). One such transporter mechanism which has the ability to regulate trafficking of the D1 receptor protein is Drip78. Overexpression of the Drip78 protein has been shown to inhibit the trafficking of the D1 receptor from the endoplasmic reticulum to the golgi apparatus (27). This inhibition would ultimately lead to a reduction in ligand binding which would correlate with a reduced level of neuronal activation.

While the present study is unable to ascertain whether any of these extraneous factors impacted the level of D1 receptor expression in high GCA animals it seems likely that the rate of receptor turnover may be increased in animals of high GCA which would account for the increase in DRD1 mRNA levels that we have previously reported (22). An increase in receptor turnover rates would also correspond to an increase in the sensitization of the neurons signaling process (28). When a D1 agonist binds to the receptor it facilitates specific signaling cascades and once that signaling cascade becomes activated, the receptor is then removed from the membrane through sequestration. It has been long thought that sequestrations 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 (29, 30). Therefore an

enhanced rate of receptor turnover would thus enhance neuronal signaling. That increase in a neurons signaling process may be facilitating the increase in neuronal activity observed in animals of high GCA compared to low GCA.

Simulations of PFC firing patterns have led to the hypothesis that D1 modulation of the mPFC implements a gating function which serves to regulate the access of information to 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, D1 receptors in the mPFC underlie the maintenance of relevant information by increasing the tonic activity (via increasing the gain) of dopaminergic neurons, and thus protecting the memory from interference (31, 32). 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 towards a goal, dopamine D2 receptors are activated in the striatum which "opens the gates", by increasing the phasic activity of dopaminergic neurons and allowing the memory to be updated (32, 33). This model of attentional regulation of information fits well with the present results in that animals with a higher level of general cognitive abilities exhibited more robust neuronal activation in response to the administration of the D1 agonist. 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. It also explains the observed effect of Experiment 3 in that animals which underwent a working memory training regimen with a high

selective attention demand exhibited consequent increases in D1-mediated neuronal activation. Since working memory training required animals to actively maintain a memory of locations in the face of interfering external stimuli, that taxation may have increased the sensitivity of D1-targeted cells. 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 (23; for review, see 34).

One pathway that could incorporate the current findings with the model presented above may arise from the D1 receptor's ability to inhibit protein phosphatase 1's (PP1) negative regulation of downstream proteins and kinases. PP1's inactivation is the result of stimulated D1 receptors activating 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 phosyphorylated by PKA it becomes a potent inhibitor of PP1 (35, 36). 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 (36, 37, 38, 39).

The discussion above is congruent with the hypothesized role that D1 receptors in the mPFC may play a role in the modulation of general cognitive abilities 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 level of the D1 receptors 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 though an increased gain in receptor excitability. While the current experiment cannot ascertain what the direct cause is of the increase in receptor sensitivity, it does suggest that behavioral training regimens and/or pharmacological manipulations could potentially serve to increase an individual's general intelligence.

References

1. Conway AR, Kane MJ, Engle RW 2003 Working memory capacity and its relation to general intelligence. Trends Cogn Sci, 7, 522-547
2. Engle RW, Tuholski SW, Laughlin W, Conway AR 1999 Working memory, short-term memory, and general fluid intelligence: A latent variable approach. J Exp Psychol Gen, 128, 309-331
3. Colom R, Rbello I, Palacios A, Jaun-Espinosy M, Kyllonen M 2004 Working memory is (almost) perfectly predicted by g. Intelligence, 32, 277-296
4. Jensen, AR 1998 The g factor: The science of mental ability. New York Praeger
5. Kolata S, Light K, Grossman H, Hale G, Matzel LD 2007 Selective attention is a primary determinant of the relationship between working memory and general learning ability in outbred mice. Learn Mem, 14, 22-28
6. Light K, et al. 2010 Working memory training promotes general cognitive abilities in genetically heterogeneous mice. Curr Biol,
7. Jaeggi SM, Buschkuehl M, Jonides J, Perrig WJ 2008 Improving fluid intelligence with training on working memory. Proc Natl Acad Sci U S A, 105, 6829-6833
8. Gray JR, Chabris CF, Braver TS 2003 Neural mechanisms of general fluid intelligence. Nat Neurosci, 6, 316-322
9. Jung RE, Haier RJ 2007 The parieto-frontal integration theory (P-FIT) of intelligence: Converging neuroimaging evidence. Behav Brain Sci, 30, 135-154
10. Rowe JB, et al. 2000 The prefrontal cortex: Response selection or maintenance within working memory? Science, 2, 1656-1660

11. McNab F, et al. 2009 Changes in cortical dopamine D1 receptor binding associated with cognitive training. Science, 323, 800-802
12. Olesen PJ, Westerberg H, Klingberg T 2003 Increased prefrontal and parietal activity after training of working memory. Nat Neurosci, 7, 75-79
13. Fischer H, et al. 2010 Simulating neurocognitive aging: Effects of a dopamiergic antagonist on brain activity during working memory. Bio Psychiatry, 67, 575-580
14. Chudusama Y, Passetti F, Rhodes SEV, Lopian D, Robbins TW 2003 Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic, and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. Behav Brain Res, 146, 105-119
15. Puumala T, Sirvio J 1997 Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. Neuroscience, 83, 489-499
16. Castner SA, Williams GV, Goldman-Rakic PS 2000 Reversal of antipsychotic induced working memory deficits by short term dopamine D1 receptor stimulation. Science, 287, 2020-2022
17. Pezze MA, Dalley JW, Robbins TW 2009 Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra accumbens administration of dopamine receptor antagonist sulpiride. Psychopharmacology, 202, 307-313
18. Passetti F, Dalley JW, Robbins TW 2003 double dissociation of serotonergic and dopaminergic mechanisms on attentional performance using a rodent five-choice reaction time task, Psychopharmacology, 165, 136-145

19. Muir JL, Everitt BJ, Robbins TW 1996 The cerebral cortex of the rat and visual attentional function: Dissociable effets of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions of a five-choice serial reaction time task. Cereb Cortex, 6, 470-480
20. Paine TA, Tomasiewicz HC, Zhang K, Carlezon WA, Jr 2007 Sensitivity of the five choice serial reaction time task to the effects of various psychotropic drugs in sprague dawley rats. Biol Psychiatry, 62, 687-693
21. Paine TA, Neve RL, Carlezon WA, Jr 2009 Attention deficits and hyperactivity following inhibition of cAMP dependent protein kinase within the medial prefrontal cortex of rats. Neuropsychopharmacology, 34, 2143-2155
22. Kolata S, et al. 2010 A dopaminergic gene cluster in the prefrontal cortex predicts performance indicative of general intelligence in genetically heterogeneous mice. PLoS ONE, 5, e14036
23. Matzel LD, et al 2011 Longitudinal attentional engagement rescues mice from age-related cognitive declines and cognitive inflexibility, Learn Mem, 18, 345-356
24. Futcher B, Latter GI, Monardo P, McLaughlin CS, Garrels JI 1999 A sampling of the yeast proteome. Mol Cell Biol, 19, 7357-7368
25. Gygi SP, Rochon Y, Franza BR, Aebersold R 1999 Correlation between protein and mRNA abundance in yeast. Mol Cell Biol, 19(3), 1720-30
26. Greenbaum D, Colangelo C, Williams K, Gerstein M 2003 Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol, 4, 117

27. Bermak JC, Li M, Bullock C, Zhou Q 2001 Regulation of transport of the dopamine D1 receptor by a new membrane-associated ER protein. Nat Cell Biol, 3, 492-498

28. Alberts B et al. 1994 Molecular biology of the cell. 3rd edition. Garland Science

29. Pierce KL, Premont RT, Lefkowitz RJ 2002 Seven-transmembrane receptors. Nat Rev, 3, 639-650

30. Ferguson SS, 2001 Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev, 53, 1-24

31. Costa RM 2007 Plastic corticostriatal circuit for action learning: What's dopamine got to do with it? Ann NY Acad Sci, 1104, 172-191.

32. Cohen JD, Braver TS, Brown JW 2002 Computation perspective on dopamine function in prefrontal cortex. Curr Opin Neurobiol, 12, 223-229.

33. Wickens JR, Horvitz JC, Costa RM, Killcross S 2007 Dopaminergic mechanisms in actions and habits. J Neuro, 27, 8181-8183

34. Matzel LD, Kolata S 2010 Selective attention, working memory, and animal intelligence. Neurosci Biobehav Rev, 34, 23-30

35. Williams GV, Castner SA 2006 Under the curve: Critical issues for elucidating D1 receptor function in working memory. Neuroscience, 139, 263-276

36. Neve KA, Seamans JK, Trantham-Davidson H 2004 Dopamine receptor signaling. J Recept Signal Transduct Res, 24, 165-205

37. Allen PB, et al. 2006 Distinct roles for spinophilin and neurabin in dopamine mediated plasticity. J Neurosci, 140, 897-911

38. Calabresi P, et al. 2000 Dopamine and cAmp-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic placticity. J Neurosci, 20, 8443-8451

39. Genoux D, et al. 2002 Protein phosphatase 1 is a molecular constrain on learning and memory. Nature, 418, 970-975

40. Matzel LD, et al. 2003 Individual differences in the expression of a "general" learning ability in mice. J Neurosci, 23, 6423-6433

41. Wass C, et al. 2012 Co-variation of learning and reasoning in mice: evolutionary conservation of the operations of intelligence. J Exp Psych: Animal Beh. Processes.

TABLE LEGENDS

TABLE 1. Experiment 1's Factor Loading From the Principal Components Analysis (n=48) for performance on the Five Learning Tasks

TABLE 2. Experiment 2's Factor Loading from the Principal Component Analysis (n=32) for performance on the Five Learning Tasks

FIGURE LEDGENDS

Figure 1. Individual animals' average number of Fos immunoreactive nuclei in response to SKF82958. Factor scores for each animal were derived from a principal component analysis of all animals' performance on five learning tasks. These scores reflect animals' aggregate performance across all five tasks (higher scores reflect higher general cognitive abilities). *Panel A*, a significant correlation ($p < .05$) was observed between animals' factor scores and the average number of Fos immunoreactive nuclei in the medial prefrontal cortex indicating that animals which possess higher general cognitive abilities exhibit an increase expression of Fos after the administration of SKF82958 when compared to animals of lower general cognitive abilities. No other comparisons were found to be significant when the number of Fos immunoreactive nuclei in the dorsolateral prefrontal cortex (*Panel B*) or the dorsal striatum (*Panel C*) were compared to the animals' factor scores.

Figure 2. SKF82958-induced expression of Fos immunoreactivity in the mPFC of animals that have been characterized for their general cognitive abilities. *Left*, Schematic illustration of the region of interest (marked by an arrow) in the mPFC in a cross section taken 5.9 mm rostral to the interaural line (Franklin & Paxinos, 1997). *Right*, Marked differences in the expression of Fos immunoreactivity was detected 60 minutes after SKF82958 administration between animals of high general cognitive abilities (Panel A) when compared to animals of low general cognitive abilities (Panel C). No measurable difference was observed between animals of high general cognitive abilities (Panel B) and low general cognitive abilities (Panel D) when administered saline. No Fos immunoreactivity was observed in a positive control (Panel E).

Figure 3. Endogenous levels of SKF82958 induced 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 general cognitive abilities) obtained from the principal component analysis of learning test performance (high factor scores = better general cognitive performance). Values are expressed as the Mean +/- SEM. *Panel A*, comparison of the mean number of Fos immunoreactive nuclei in the mPFC of animals that have been characterized as having High, Intermediate, and Low GCA, revealed a significant difference between animals of High GCA and Low GCA ($p < .05$). *Panel B*, comparison of the mean number of Fos immunoreactive nuclei in the dlPFC of animals characterized for their GCA; no significant differences were observed. *Panel C*, no significant differences between groups was observed when the mean number of Fos immunoreactive nuclei in the Striatum was compared.

Figure 4. Factor scores for each animal were derived from principal components analysis of all animals' aggregate performance on five learning tasks whereas higher scores represent higher general cognitive abilities. *Panels A & B*, Twenty five micrograms of protein from High GCA animals (lanes 1,3,5,7) and Low GCA animals (lanes 2,4,6,8) were loaded into each lane. Anti-Drd1 antibodies were then blotted against PVDF membranes and a single protein band was visualized at roughly 55kDA. *Panel C*, No significant correlation was observed between animals' factor scores and their percent band density ($p > .05$) indicating that there are no differences in the total number of D1 receptors in the mPFC of animals.

Figure 5. From principal components analysis of all learning tasks, general cognitive abilities (primary factor score) is plotted as a function of group whereas higher general cognitive abilities are indicated as higher factor score values. The imposition of a working memory training (group WMT) regimen promoted an enhancement of general cognitive abilities compared to animals that were exposed (EXP) to the training apparatus for an equivalent amount of time as the trained group, or remained in their homecages (HOME) throughout the experiment.

Figure 6. Differences in Fos immunoreactive nuclei were observed in animals that had undergone working memory training (*A*), simple exposure to the apparatus (*C*) and those which remained in their homecages (*E*) 60 minutes after the administration of SKF82958. No differences were observed in either group of animals that received working memory training (*B*), exposure to the apparatus (*D*) or those which remained in the homecages (*F*) after the administration of saline. *Panel B*, the mean +/- SEM number of Fos immunoreactive nuclei expressed in the mPFC of animals that have been segregated into groups which received working memory training (WMT), exposure to the apparatus (EXP), or remained in their homecages (HOME). Groups labeled with "D" following their respective grouping received an administration of SKF82958 (1 mg/kg), whereas groups labeled with "S" received saline. Post hoc analysis revealed a significant difference between animals that have undergone working memory training and animals that had been exposed to the apparatus for an equivalent amount of time ($p < .05$) as well as animals that remained in the home cages ($p < .05$). No significant differences between groups were observed in the dorsolateral prefrontal cortex (*Panel C*) or the striatum (*Panel D*).

Table 1

	General Cognitive Ability Factor
Odor Discrimination	0.18
Lashley III Maze	0.63
Passive Avoidance	0.54
Fear Conditioning	0.58
Water Maze	0.56
eigenvalue	**1.79**
Variance Explained	**.30**

Table 2

	General Cognitive Ability Factor
Odor Discrimination	.1
Lashley III Maze	.75
Passive Avoidance	.74
Fear Conditioning	.64
Water Maze	.42
eigenvalue	**1.94**
Variance Explained	**.32**

Figure 1

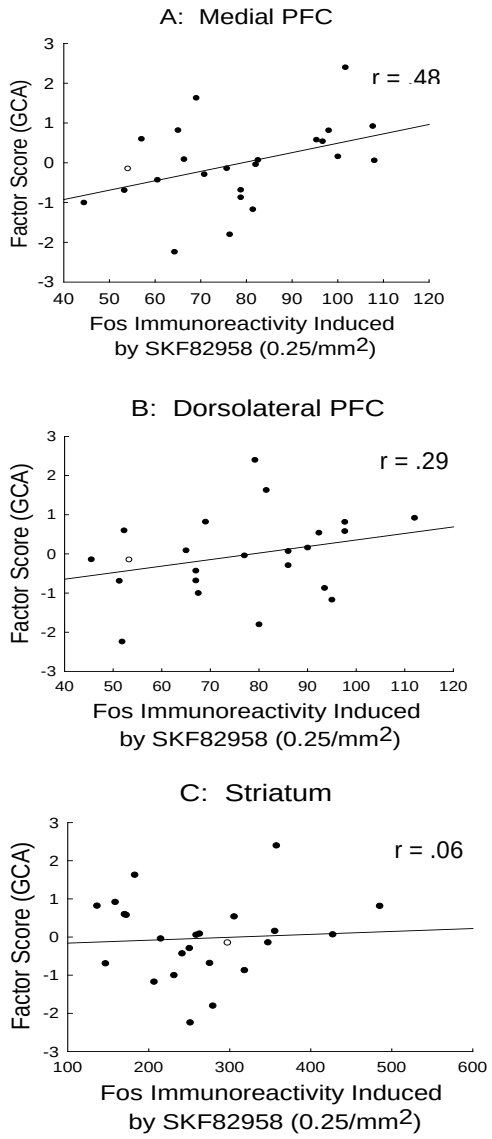

A: Medial PFC
r = .48
Factor Score (GCA)
Fos Immunoreactivity Induced
by SKF82958 (0.25/mm2)
B: Dorsolateral PFC
r = .29
Factor Score (GCA)
Fos Immunoreactivity Induced
by SKF82958 (0.25/mm2)
C: Striatum
r = .06
Factor Score (GCA)
Fos Immunoreactivity Induced
by SKF82958 (0.25/mm2)

Figure 2

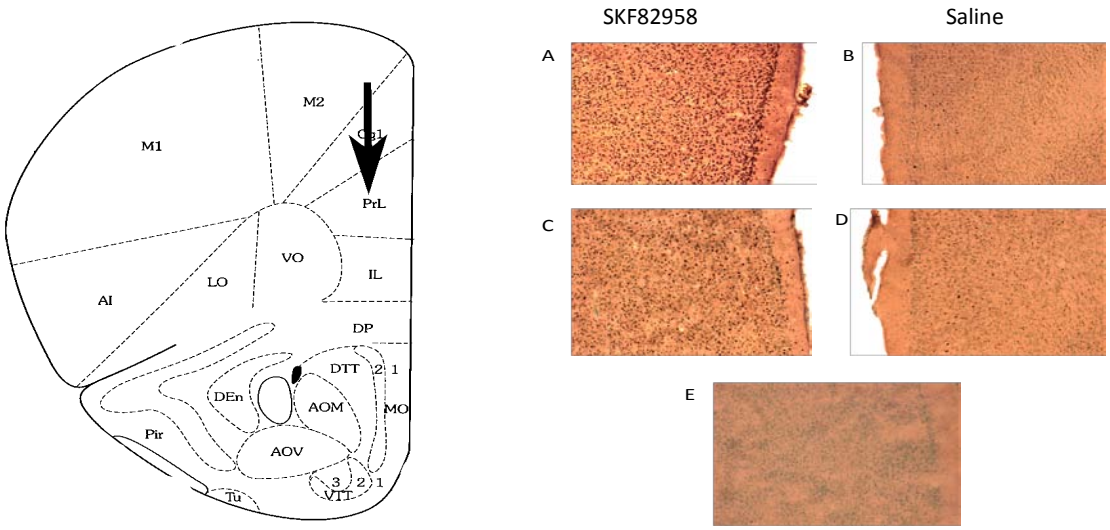

M1
M2
Cg1
PrL
VO
IL
LO
AI
DP
DTT
2 1
DEn
AOM
MO
Pir
AOV
3 2 1
VIT
Tu
SKF82958
Saline
A
B
C
D
E

Figure 3

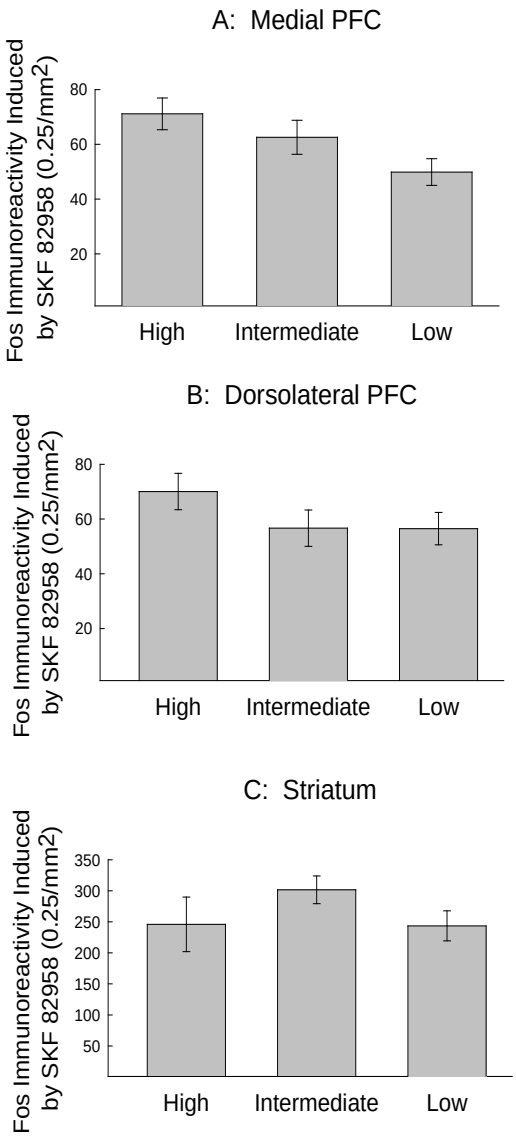

A: Medial PFC
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
80
60
40
20
High
Intermediate
Low
B: Dorsolateral PFC
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
80
60
40
20
High
Intermediate
Low
C: Striatum
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
350
300
250
200
150
100
50
High
Intermediate
Low

Figure 4


A
55 kDa
High
Low
High
Low
High
Low
High
Low
B
55 kDa
High
Low
High
Low
High
Low
High
Low

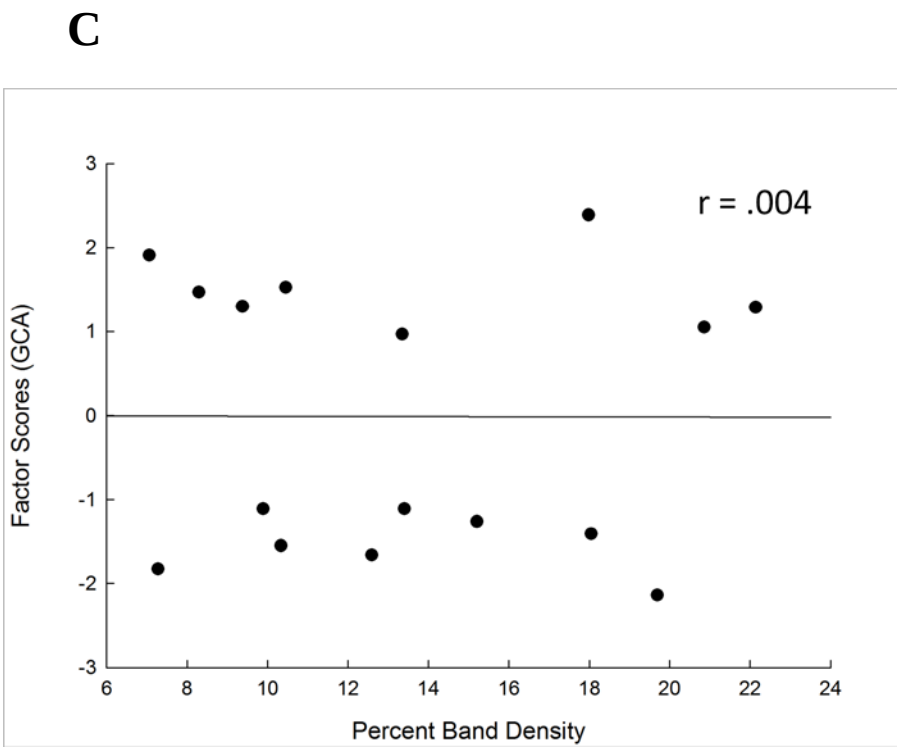

C
r = .004
Factor Scores (GCA)
Percent Band Density
3
2
1
0
-1
-2
-3
6
8
10
12
14
16
18
20
22
24

Figure 5

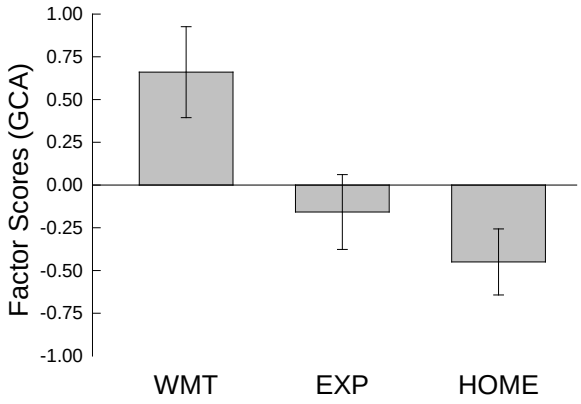
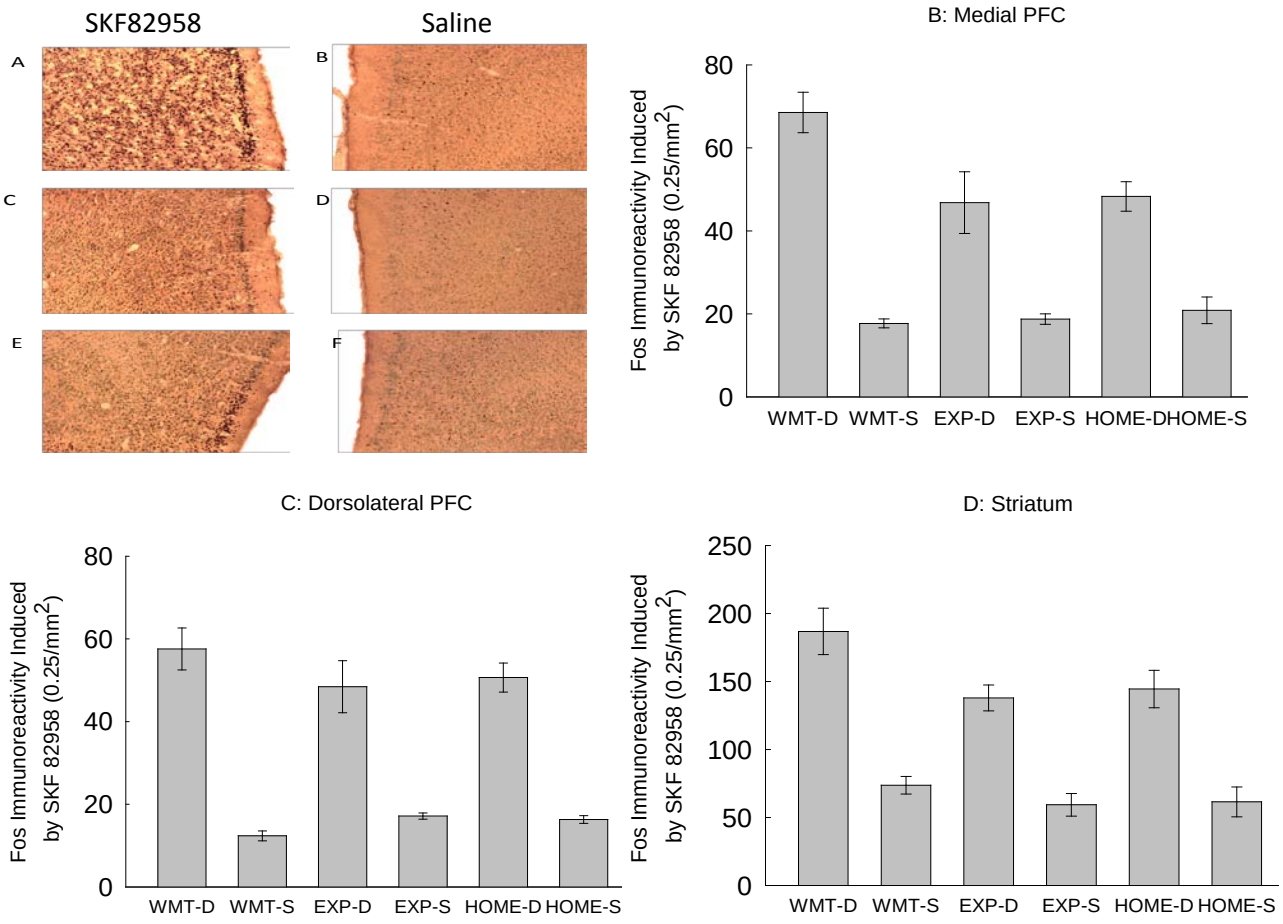

1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
Factor Scores (GCA)
WMT
EXP
HOME

Figure 6


SKF82958
Saline
A
B
C
D
E
F
B: Medial PFC
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
80
60
40
20
0
WMT-D WMT-S EXP-D EXP-S HOME-D HOME-S
C: Dorsolateral PFC
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
80
60
40
20
0
WMT-D WMT-S EXP-D EXP-S HOME-D HOME-S
D: Striatum
Fos Immunoreactivity Induced by SKF 82958 (0.25/mm2)
250
200
150
100
50
0
WMT-D WMT-S EXP-D EXP-S HOME-D HOME-S