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Reversal of neuronal and cognitive consequences of amphetamine sensitization following chronic treatment with a D1 antagonist

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ABSTRACT

Neuroplasticity is a key factor in restoration of brain function following neuropathology associated with disease or drug exposure. Here we examined the potential for chronic treatment with the selective D1 receptor antagonist SCH39166 to reverse the profound and enduring cognitive impairment associated with amphetamine (AMPH) sensitization in the nonhuman primate and to stimulate re-growth of atrophied pyramidal dendrites in the dorsolateral prefrontal cortex of these animals. Four rhesus monkeys with sustained cognitive impairment (>1 year following AMPH sensitization) were treated for up to 8 months with SCH39166. Cognitive testing was performed before, during, and for up to 1½ year following treatment. Significant improvement in working memory performance was observed only after cessation of the D1 antagonist treatment but then was sustained for the duration of the post-treatment testing period. Postmortem quantitative assessment of Golgi-impregnated pyramidal neurons in BA9 showed that apical dendritic length and trunk spine density were increased in D1 antagonist treated monkeys relative to AMPH-sensitized and AMPH-naïve monkeys. These findings, which suggest that the deleterious consequences of AMPH sensitization can be reversed by modulation of D1 receptor signaling, have implications for treating the underlying neural basis of cognitive deficits in both schizophrenia and substance abuse.

1. Introduction

Repeated amphetamine (AMPH) administration induces a form of long-lasting behavioral sensitization in which behavioral responses to subsequent AMPH exposure are significantly augmented (Robinson and Becker, 1986; Castner and Goldman-Rakic, 1999; Castner et al., 2000a; Strakowski and Sax, 1998). This persistent behavioral sensitization to AMPH is accompanied by equally long-lasting alterations in brain neurochemistry and morphology (Paulson et al., 1991; Robinson and Kolb, 2004; Boileau et al., 2006; Selemon et al., 2007). It is of particular interest that repeated AMPH exposure in rodents produces increased dopamine turnover in medial prefrontal cortex (PFC) and an increased number of spines on pyramidal neurons in this region while reducing spine density in several other cortical areas (Robinson and Kolb, 2004). D1 receptors are preferentially localized to the spines of pyramidal neurons in PFC (Bergson et al., 1995) and both behavioral and neurophysiological studies have shown that an optimal level of stimulation at these sites is required for maximizing working memory performance (Williams and Goldman-Rakic, 1995; Cai and Arnsten, 1997; Castner et al., 2000b; Mattay et al., 2003). On this evidence, we have hypothesized that even brief periods of AMPH exposure, akin to recreational psychostimulant use in humans, may disrupt prefrontal cortical function and impair cognitive performance. Support for this hypothesis was demonstrated in Castner et al. (2005), in which a 6-week AMPH sensitization regimen profoundly impaired both acquisition and performance on working memory tasks in the nonhuman primate. Specifically, animals that were not pretrained were markedly impaired in their ability to acquire the spatial delayed response and delayed nonmatch-to-sample (DNMS) tasks (see Castner et al., 2005 for details) and these deficits persisted for an additional 6–12 months of testing beyond the initial acquisition period prior to pharmacological intervention with a D1 antagonist (see below). In the years following sensitization, in addition to marked cognitive impairment, we have also observed significant decreases in dopamine turnover and degradation of spinodendritic architecture in dorsolateral PFC (dlPFC; Selemon et al., 2007; Castner et al., 2005). As reported in Selemon et al. (2007), overall dendritic complexity and spine density on the apical dendritic trunk were reduced in layer II/IIIa pyramidal neurons, and basilar dendritic length was reduced in pyramidal cells across all cortical layers. These deficiencies in the integrity of dlPFC were found more than 3 years after repeated AMPH administration and are suggestive of a severe compromise of neuronal signaling that is critical to executive function and working memory.

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We posited that the state of reduced dopamine turnover and dendritic atrophy induced by AMPH sensitization might be associated with D1 receptor hypersensitization and therefore would be amenable to modification via chronic blockade with a D1 antagonist. This supposition was supported by the evidence that dopamine depletion leads to an elevated D1 binding potential and a loss of spine density in PFC (Guo et al., 2003; Wang and Deutch, 2007) as well as the finding that D1 antagonists can block the induction of AMPH sensitization (Vezina, 1996). In addition, our previous experience in using repeated, intermittent treatment with a D1 agonist to ameliorate persistent deficits induced by chronic haloperidol exposure (Castner et al., 2000b) indicated that long-term alterations in dopamine receptor signaling could be modified by selective targeting of D1 receptors. With regard to the AMPH-sensitized state, we hypothesized that chronic treatment with a selective D1 antagonist might enhance expression of mRNA for the D1 receptor, suppress the hypersensitive D1 receptor signaling, and in so doing, restore the normal dopaminergic regulation of synaptic efficacy in the prefrontal cortical circuits that subserve working memory.

2. Materials and methods

2.1. Cognitive studies

Four young adult rhesus monkeys (*Macaca mulatta*; two male, two female) received their normal biscuits, fruit, and peanuts each day. Food intake during AMPH was closely monitored and the animals' diets were supplemented as necessary. They were housed individually and maintained in accordance with federal regulations and Yale Animal Use and Care Committee guidelines for nonhuman primates.

This within-animal study utilized four young adult monkeys that were previously AMPH-sensitized (AMPH-sens). In brief, they had received intermittent, escalating low doses of S(+)-amphetamine sulfate (RBI, Natick, MA) over six weeks (0.1–1.0, mg/kg; IM; b.i.d. with weekends off). Behavioral sensitization was confirmed by comparing the animals' responses to a challenge dose of AMPH (0.4 mg/kg; IM) before and after AMPH. Behavioral sensitization to AMPH was documented for more than 6 months before initiation of cognitive testing and these animals continued to demonstrate sustained deficits on working memory tasks for a further 6–12 months after the initial acquisition periods for each task prior to the pharmacological intervention used in this study (see Castner et al., 2005).

2.2. Chronic D1 antagonist treatment

Animals were administered the selective D1 antagonist, SCH39166 (0.001–0.01 mg/kg; IM; Schering-Plough, Kenilworth, NJ), twice daily over a 5–32 week period with cognitive testing at 1.5 h post-treatment. Details of the duration of testing for pretreatment, treatment and post-treatment epochs are provided in Table 1, including the elapsed time between sensitization and D1 antagonist treatment. The doses of SCH39166 were chosen on the basis of previous studies with SCH23390 which indicated that doses as low as 0.001–0.01 mg/kg had little or no effect on cognition themselves but were capable of attenuating the delayed response deficit induced by physiological stress or pharmacological manipulation (Murphy et al., 1996; Arnsten and Goldman-Rakic, 1998; Roberts et al., 2010). The dose and/or duration of this experimental treatment were increased in some animals in the anticipation that an alteration in cognitive performance might be seen during administration. Performance on spatial delayed response (DR) and/or delayed nonmatch-to-sample tasks (DNMS; see Castner et al., 2005 for details) was assessed 3–5 days per week before, during, and following D1 antagonist administration (one animal would not test on DR in the post-treatment period but continued to test on DNMS for up to 7 weeks). To assess DR performance, one of two wells (left or right) was baited in the monkey's view and both wells were covered with identical plaques. An opaque screen was lowered and immediately raised, corresponding to a <1 s delay after which the animal had to move the correct plaque to obtain reward. If an animal reached criterion at this delay it was then tested on varied delays of 0, 5, 10, 15, and 20 s that were randomized across a given test session (see Castner et al., 2005 for further details). In DNMS, each trial was initiated by displacing a novel object (sample) to retrieve a food reward. The opaque screen was then lowered for a 10 s delay and after it was raised the animal chose between the sample and a "novel" (rewarded) object. Animals were tested with trial unique objects over 20 trials per session. Post-treatment data used in the analyses were collected for DR for the first 4–9 months following D1 antagonist treatment and the animals continued to be tested out to more than 1–1.5 years after cessation of treatment (up until the time of euthanasia where possible). Cognitive data were analyzed using one-way ANOVA with Scheffe post-hoc comparisons using StatView (Abacus Concepts, Inc.).

Table 1
Duration of cognitive testing for assessment of performance over each epoch (approximate time in weeks).

Animal	Pretreatment period[a]	Time post-sensitization[b]	Treatment period[c]	Post-treatment period[d]
MK1	19	82	19	40
MK2	19	81	5	18
MK3	31[e]	117	7	32
MK4	9	114	32	7[f]

[a] Includes data immediately up until treatment.
[b] The time between the end of AMPH-sens and start of treatment.
[c] Testing over the entire time of treatment.
[d] Testing from immediately after cessation of treatment.
[e] Testing in one bout of 10 weeks followed by another bout of 21 weeks (up until treatment), interleaved by 30 weeks of testing on other tasks.
[f] Including one week delay after cessation of treatment.

2.3. Golgi processing

After cognitive assessment, the AMPH-sens/D1 receptor antagonist treated (AMPH-sens/D1x) monkeys were euthanized and perfused with ice-cold Ringer's solution as described in Selemon et al. (2007). Euthanasia occurred 4 years ± 2 months following sensitization and more than 1 year following D1 antagonist treatment. An additional male animal was included for morphological analysis that had undergone a twelve-week AMPH sensitization period some 3 years earlier and that had received chronic treatment with the less selective D1 antagonist SCH23390 (Sigma-Aldrich, St. Louis, MO) during the 3-month period prior to this analysis. Note that *in vitro* and *in vivo* assays have demonstrated that both SCH23390 and SCH39166 bind with high affinities to D1/D5 receptors, while both antagonists show affinity only in the micromolar range for D2 receptors (Iorio et al., 1983; Chipkin et al., 1988). However, unlike SCH39166, SCH23390 shows significant affinity at 5-HT2 receptors (~ 300 nM for the former, and ~30 nM for the latter; see Taylor et al., 1991; Alburges et al., 1992).

Cortical blocks were trimmed to ~1 cm^2 on face and 0.5 cm thick and processed with a modified Rapid Golgi method. Tissue blocks were postfixed for 2 h in 4% paraformaldehyde in phosphate buffered saline. The blocks were then transferred to a freshly prepared aqueous solution of 2.5% potassium dichromate and 0.2% osmium tetroxide for 3–5 days in the dark. The blocks were washed several times with 0.75% silver nitrate and reacted in this same solution for 24–48 h in the dark and then dehydrated through increasing concentrations of ethanol, embedded in celloidin and sectioned at 120 μm. Sections were mounted on slides with Permount, coverslipped, and air-dried on a flat surface. All analyses were performed by one observer (AB).

2.4. Dendritic drawings

Dendrites were drawn using Neurolucida software (ver. 5.05.4) on a computer aided microscope system (MicroBrightfield, Williston,

VT). At low power (350×), Golgi-impregnated neurons were identified and marked on an outline of the whole section. Pyramidal neurons were identified by their characteristic somal shape and by the pattern of their dendritic arborization, i.e., one primary apical dendrite extending outward from the apex of the soma and multiple basilar dendrites emanating from the base of the soma. The depth of the cell soma from the pial surface was measured, and cells were categorized as residing in one of three layers based on previous cytoarchitectural analyses in monkey PFC (Selemon et al., 1999): layer II/superficial layer III (IIIs) (250–650 μm), deep layer III (IIId)(700–1000 μm), or layers V/VI (>1100 μm). At higher magnification (1500×), 10 pyramidal cells in each of these 3 layers were drawn for Sholl analysis and measurement of dendritic length. Another 10 were chosen for analysis of spine density along the apical trunk, on a single apical branch and on a single basilar dendrite. The following criteria were used to select cells: (1) The apical trunk could be traced for at least 150 μm from the soma. (2) At least two basilar dendrites were present. (3) Basilar and apical branch dendritic processes were well impregnated and extended to a natural tapered ending. (4) Dendrites were not obscured by overlying glial processes, other neuronal processes, or artifact. (5) In addition, for analysis of apical trunk spine density, cells must have an impregnated apical trunk that extended for at least 100 μm distal to the peak density.

2.5. Sholl analysis

The number of intersections with concentric circles placed 50 μm, 100 μm and 150 μm from the cell soma was counted as a measure of dendritic complexity.

2.6. Analysis of dendritic length

The length of all apical dendritic segments within 150 μm of the cell soma was summed. As very few apical dendritic trees were present in entirety within the 100-μm thick section, analysis of dendritic length was limited to a 150 μm radius from the soma in order to avoid skewing by apical trunk dendrites that remained within the section for longer distances. The sum of all basilar dendritic segments regardless of distance from the cell soma was calculated.

2.7. Analysis of spine density

Spine density is expressed as number of spines per micron of dendritic segment. For apical dendritic trunks, spine density was averaged over 25 μm lengths of the trunk starting at the somal origin and extending distally. As spine density varied considerably along the length of the dendritic trunk, the points of peak spine density for the 10 neurons in each layer were aligned. Spine densities 50 μm proximal to the peak, at peak, 50 μm distal to the peak, and 100 μm distal to the peak were then averaged for each case. For apical dendrite branches, spine densities were measured along secondary (2°), tertiary (3°), and 4th order (4°) branches. Likewise, spine densities along all primary (1°), 2°, and 3° branches of basilar dendrites were measured. Spine density from 10 neurons in each layer was then averaged for each case.

2.8. Statistical analysis of dendritic parameters

Note that because of the within-animal design of the behavioral experiment, the dendritic parameters in this study were compared with similarly collected data from our previous report of AMPH-naïve and AMPH-sens monkeys (Selemon et al., 2007; see Discussion). Morphological data used for statistical analysis represented mean values for each animal from 10 neurons per layer. All measurements are expressed as mean ± standard error of the mean. As in the previous study, data were checked for normality prior to analysis using normal probability plots and Kolmogorov–Smirnov tests. No transformation was necessary. Effects were considered significant at the 0.02 level (with a Bonferroni adjustment for multiple comparisons) or at the 0.01 level for group by layer and group by circle radius effects.

For each dendritic parameter examined, a mixed model was fitted with fixed effects of group (AMPH-naïve controls, AMPH-sens, AMPH-sens/D1x), layer (II/IIIs, IIId, or V/VI) and dendritic measurement of interest (e.g., for Sholl analysis, intersections at circles of 50 μm, 100 μm, and 150 μm; for dendritic length, apical dendritic length, basilar dendritic length). Although neither somal size nor somal depth differed between groups for any of the layers, cell depth and somal size were included as covariates in the model. Least square means were then computed (e.g., for each radius averaged over all other factors), and all possible comparisons between least square means were performed.

As a primary analysis of spine density, a mixed model with fixed effects of group, layer and spine density across dendritic region (apical dendritic trunk, apical branch, or basilar dendrite) and all possible interactions was fitted. A secondary analysis was undertaken with a separate mixed model for each dendritic region with fixed effects of group, layer, position within each dendritic region (i.e., distance from peak along apical trunk or branch order for apical branches or basilar dendrites), and all possible interactions were fitted. The analysis of spine density along the apical trunk was performed using cell depth and somal size as covariates; cell depth and somal size were not available for apical branch and basilar dendritic analyses. Finally, a mixed model with fixed effects of group, layer and the interaction was fitted only to the peak spine density of the apical trunk.

3. Results

3.1. Cognitive performance

Prior to treatment with the D1 antagonist, the animals showed consistently poor performance on the spatial delayed response task with a group average of 58.29 ± 4.02% correct for six or more months following the initial acquisition period (Castner et al., 2005) and prior to pharmacological intervention. One-way analysis of variance (ANOVA) on working memory performance revealed a significant group effect across baseline, treatment and post-treatment conditions ($F(2,8) = 7.511$; $p = 0.0146$). However, group performance (66.85 ± 3.07%) was not significantly improved during the period of D1 antagonist treatment as compared to their pretreatment baseline (Fig. 1; Scheffe post-hoc comparison of treatment vs. baseline, $p = 0.3284$) with only one animal (MK1) showing significant improvement during the actual treatment period (see Table 2). Remarkably, following cessation of D1 antagonist administration, a pronounced and sustained cognitive improvement over the animals' pretreatment baseline to an average of 80.62 ± 4.98% became evident (Scheffe post-hoc comparison post-treatment vs. baseline, $p < 0.02$). The animal that would not perform DR following D1 antagonist treatment did however show the same pattern of improvement in cognition post-treatment on DNMS (see Table 2). In more than 8 months of treatment, including use of the higher dose of the D1 antagonist, this animal showed an increase in performance of little more than 1%, whereas a significant improvement in DNMS performance of more than 9% was observed in the 7 weeks following cessation of treatment, starting after the first week post-treatment. Across the four animals, we saw no relationship between treatment duration/dose and any improvement in cognitive performance during or following D1 antagonist treatment. The ameliorative effects of the D1 antagonist on cognitive performance persisted for more than 1–1½ years post-antagonist treatment in three animals and in the other individual on DNMS for as long as we were able to test him.

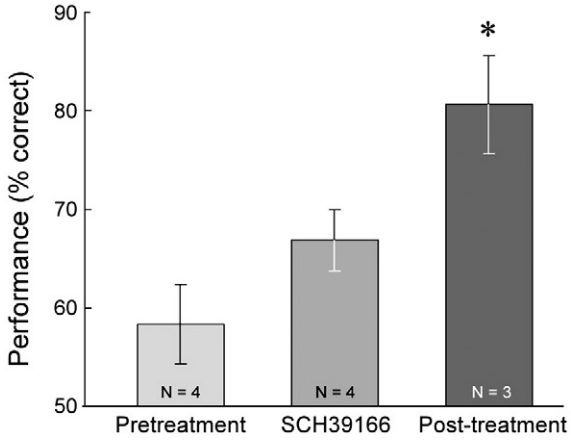

Fig. 1. Effects of chronic administration of SCH39166 on cognitive performance in AMPH-sensitized monkeys. During the pretreatment period, more than one year following AMPH sensitization, monkeys were significantly impaired in performance on the DR task and remained impaired for more than six months prior to pharmacological intervention. During the period of D1 antagonist administration, there was no significant improvement in performance across the group. However, *after* the period of chronic treatment, there was a significant and enduring improvement in performance of all monkeys (one individual did not continue to perform DR but his performance on DNMS showed a trend similar to that of the other monkeys on DR, see Table 2). * indicates significant difference ($p<0.02$) versus pretreatment by Scheffe post-hoc comparison.

3.2. Dendritic morphology

3.2.1. Sholl analysis

The groups did not differ overall ($F(1,2)=2.39$, $p-0.133$) in the number of intersections with the three circles (50 μm, 100 μm, and 150 μm), but there was a significant group by layer effect ($F(1,4)=4.22$, $p-0.011$). These findings reflected the fact that AMPH-naïve animals tended to show more intersections than AMPH-sens animals in layer II/IIIs (10.0 ± 1.3 vs. 6.1 ± 1.4; $t(1,15.8)=2.74$, $p-0.015$) and in layer IIId (10.7 ± 1.0 vs. 7.1 ± 1.0; $t(1,15.7)=2.52$, $p-0.023$). AMPH-sens/D1x animals had intersections that were intermediate in number for layer II/IIIs (7.0 ± 1.5) and layer IIId (9.0 ± 1.6) and did not differ significantly from either the AMPH-naïve or the AMPH-sens groups. Although a significant group by radius (of circle) effect ($F(1,4)=3.67$, $p-0.009$) was found, comparison of all possible pairs of least square means revealed only trend differences. AMPH-naïve monkeys tended to have more intersections with the 50 um circle (13.38 ± 0.98) compared with AMPH-sens/D1x animals (10.10 ± 0.98; $t(1,14.7)=2.35$, $p-0.033$) and more intersections with the 100 μm circle (11.17 ± 0.98) than AMPH-sens animals ($7.97+0.97$, $t(1,14.3)=2.32$, $p-0.036$).

Table 2
Cognitive performance on working memory tasks[a].

Animal	Baseline	Chronic SCH39166	Post-treatment
Spatial delayed response			
MK1	49.81 ± 0.57	73.94 ± 1.98[b]	86.50 ± 1.27[b]
MK2	68.65 ± 1.72	70.00 ± 2.32	84.64 ± 1.56[b]
MK3	59.93 ± 1.15	62.33 ± 2.18	70.71 ± 2.15[b]
MK4	$54.77+2.41$	$61.13+3.11$	–
Mean	58.29 ± 4.02	66.85 ± 3.07	80.62 ± 4.98[c]
Delayed nonmatch-to-sample			
MK4	$59.36+2.03$	$60.9+3.19$	$69.12+1.60$[d]

[a] Data expressed as mean percent correct ± SEM; statistical comparisons represent Scheffe post-hoc comparison vs. baseline following one-way ANOVAs.
[b] $p<0.003$; MK1: $F(2,127)=146.301$; MK2: $F(2,121)=26.179$; MK3: $F(2,124)=6.168$.
[c] $F(2,8)=7.511$; $p-0.0146$.
[d] $F(2,64)=5.913$; $p<0.01$.

3.2.2. Dendritic length

Group differences in overall dendritic length, the sum of apical and basilar dendrites, were observed ($F(1,2)=7.6$, $p-0.010$). A significant group by dendrite (apical or basilar) effect was also present ($F(1,2)=30.89$, $p<0.001$). As reported in our previous study (Selemon et al., 2007), AMPH sensitization did not significantly alter apical dendritic length. However, AMPH-sens/D1x animals exhibited significantly longer apical dendrites (1171.93 ± 72.93 μm) compared to both AMPH-sens monkeys (582.57 ± 71.89 μm, $t(1,9.09)=5.75$, $p-0.0003$) and AMPH-naïve animals (697.08 ± 72.71 μm, $t(1,9.36)=4.55$, $p-0.0012$; Figs. 2, 3d–f). In contrast, basilar dendrites were longer in AMPH-naïve animals (1239.35 ± 82.53 μm) compared to AMPH-sens animals (816.30 ± 81.81 μm, $t(1,21.5)=3.64$, $p-0.0015$) and marginally longer compared to AMPH-sens/D1x animals (968.08 ± 82.69 μm, $t(1,21.8)=2.30$, $p-0.031$; Fig. 2). Basilar dendritic length did not differ between AMPH-sens and AMPH-sens/D1x groups ($t(1, 21.5)=1.30$, $p=0.206$). The group by layer interaction was not significant ($F(1,4)=2.28$, $p-0.122$).

3.2.3. Spine density

In the primary analysis of spine density, group ($F(1,2)=2.59$, $p-0.115$) and group by layer ($F(1,4)=0.77$, $p-0.544$) effects were not significant; however, the group by dendritic region effect was significant ($F(1,4)=16.36$, $p<0.001$). Comparison of least square means indicated that spine density on the apical dendritic trunk was significantly higher in AMPH-sens/D1x monkeys across all layers (0.82 ± 0.03 spines/μm) compared to spine density in AMPH-sens (0.60 ± 0.03 spines/μm, $t(1,16.9)=5.38$, $p<0.001$) and AMPH-naïve animals (0.68 ± 0.03 spines/μm, $t(1,17)=3.68$, $p-0.0019$).

In the secondary analysis in which each dendrite (apical branch, basilar dendrite, and apical dendritic trunk,) was modeled separately, there were no significant group or group by layer effects for the apical branch or the basilar dendrite (all $p>0.467$). As reported in our previous study (Selemon et al., 2007), the basilar dendrite showed a significant effect of branch order ($F(1,2)=24.39$, $p<0.001$) that

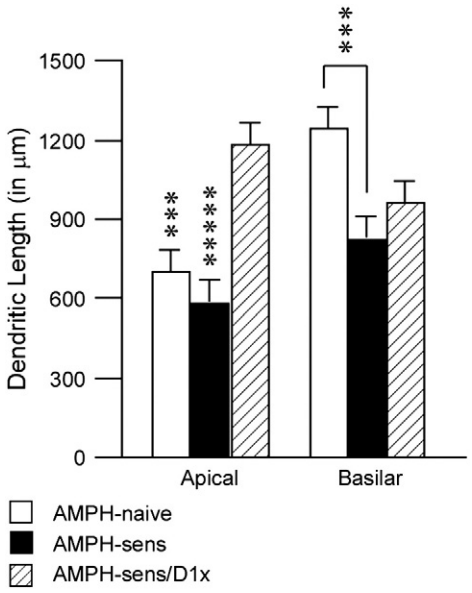

Fig. 2. Apical and basilar dendritic length represented as least square means averaged across all layers. Apical dendrites in D1 antagonist treated animals (striped bars) were longer than apical dendrites in either AMPH-sens (black bars) or AMPH-naïve groups (white bars). However, basilar dendrites in D1 antagonist treated monkeys were not significantly longer than those of AMPH-sens animals and still marginally shorter than basilar dendrites in drug naïve animals. Asterisks indicate significant differences between AMPH-sens/D1x animals and the other two groups for apical dendrites and significant differences between AMPH-naïve and AMPH-sens groups for basilar dendrites (***<0.005, *****<0.0005).

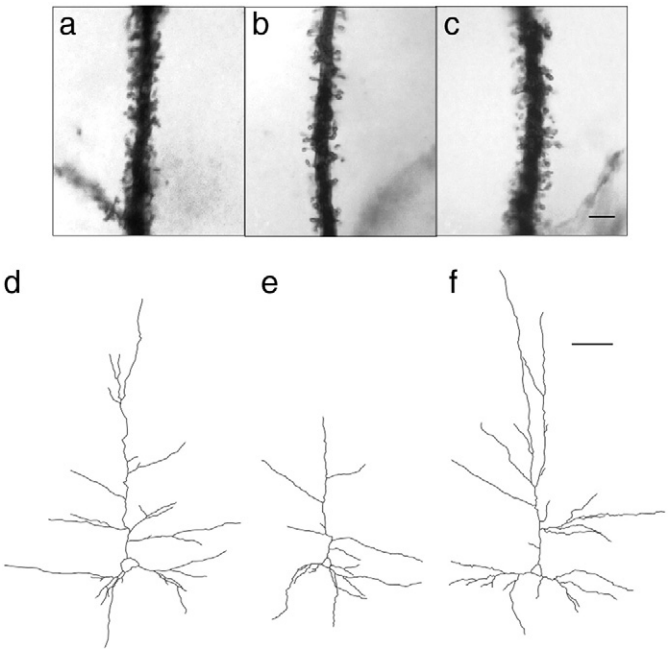

Fig. 3. Illustration of increased spine density and dendritic length on the apical dendrite of layer II/IIIs prefrontal pyramidal cells following D1 antagonist treatment. Photomicrographs (above) illustrate peak spine density on the apical dendritic trunk (scale bar = 5 um), and drawings (below) show dendritic arbors of layer II/IIIs pyramidal neurons (scale bar = 50 um) for (a, d) AMPH-naïve, (b, e) AMPH-sens and (c, f) AMPH-sens/D1x monkeys.

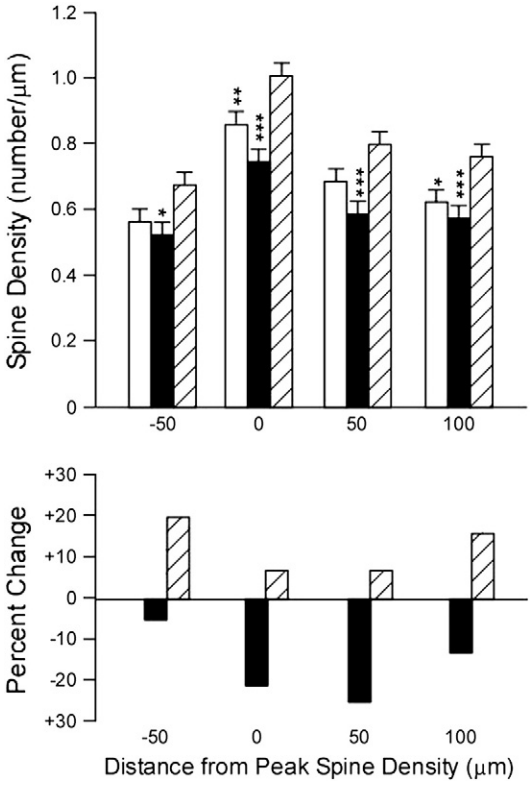

Fig. 4. Graph (above) shows least square means of spine density averaged across all layers at 4 points along the apical dendrites in AMPH-naïve (white bars), AMPH-sens (black bars), and AMPH-sens/D1x monkeys (striped bars). Note that spine density was greater in AMPH-sens/D1x monkeys relative to AMPH-sens monkeys at all locations and was higher in AMPH-sens/D1x monkeys compared to AMPH-naïve monkeys at peak (0) and 100 μm distal to peak spine density. Asterisks indicate significant differences between AMPH-sens/D1x monkeys and the other two groups (*<0.02, **<0.01, ***<0.005). Graph (below) illustrates the percent change in spine density in apical dendritic trunks in AMPH-sens (black bars) and AMPH-sens/D1x monkeys (striped bars) compared to AMPH-naïve monkeys. The deficits in spine density resulting from AMPH-sens were reversed by treatment with a D1 antagonist and resulted in higher than normal spine density in limited regions of the apical trunk.

correlated with increasing spine density on higher order branches, but the group by branch order effect was not significant ($F(1,2) = 1.83$, p − 0.143). In contrast, for the apical dendritic trunk, significant group ($F(1,2) = 8.2$, p − 0.005), location, i.e. distance from peak ($F(1,3) = 276.98$, $p<0.001$), and group by location ($F(1,6) = 3.38$, p − 0.008) effects were observed. As described previously, spine density exhibited an inverted "V" pattern with a sharp rise from the cell soma to peak density and a more gradual decline distal to the peak accounting for the location effect (Selemon et al., 2007). Overall, spine density on the apical dendritic trunk across all layers was higher in the AMPH-sens/D1x animals (0.81 ± 0.03 spines/μm) in comparison to AMPH-sens (0.61 ± 0.03 spines/μm, $t(1,12.8) = 4.01$, p − 0.0015) and to AMPH-naïve monkeys (0.68 ± 0.03 spines/μm, $t(1,12.7) = 2.64$, p − 0.021). Spine density was significantly higher along the apical trunk in AMPH-sens/D1x compared to AMPH-sens animals at all locations (Fig. 4) : 50 μm proximal to peak (AMPH-sens/D1x = 0.68 ± 0.04 spines/μm, AMPH-sens = 0.53 ± 0.04 spines/μm, $t(1,18.8) = 2.65$, p − 0.016), peak (AMPH-sens/D1x = 1.01 ± 0.03 spines/μm, AMPH-sens = 0.75 ± 0.04 spines/μm; $t(1,13.9) = 5.14$, p − 0.0002), 50 μm distal to peak (AMPH-sens/D1x = 0.79 ± 0.04 spines/μm, AMPH-sens = 0.59 ± 0.04 spines/μm, $t(1,16.4) = 3.87$, p − 0.0013), 100 μm distal to peak (AMPH-sens/D1x = 0.76 ± 0.04 spines/μm, AMPH-sens = 0.57 ± 0.04 spines/μm, $t(1,15.3) = 3.52$, p − 0.003). Spine density in the AMPH-sens/D1x group also was significantly higher than that of AMPH-naïve animals at peak density (AMPH-naïve = 0.86 ± 0.03 spines/μm, $t(1,13.9) = 3.07$, p − 0.008) and 100 μm distal to peak (AMPH-naïve = 0.62 ± 0.03 spines/μm, $t(1,15.4) = 2.75$, p − 0.015; Fig. 4). Differences in apical trunk spine density were further confirmed when a model was fitted specifically to peak density on the apical dendritic trunk. A significant effect of group ($F(1,2) = 20.05$, p − 0.0002) was observed with AMPH-sens/D1x monkeys exhibiting higher peak density than AMPH-sens or AMPH-naïve animals (Fig. 3a–c).

3.2.4. Distance to peak (DTP) density on the apical dendritic trunk

Finally comparison of DTP revealed a significant group effect ($F(1,2) = 29.19$, $p<0.001$) but not a significant group by layer effect ($F(1,4) = 1.04$, p = 0.406). DTP across all layers was marginally longer in AMPH-naïve animals (158.72 + 8.25 μm) in comparison to AMPH-sens animals (127.69 ± 8.98 μm, $t(1,16.5) = 2.49$, p = 0.024). DTP in AMPH-sens/D1x animals (225.78 + 8.79 μm) was significantly longer than that of the other two groups (vs. AMPH-sens, $t(1,14.4) = 7.48$, $p<0.001$; vs. AMPH-naïve, $t(1,15.8) = 5.31$, $p<0.001$).

3.2.5. Summary of morphologic findings

D1 antagonist treatment increased spine density on the apical dendrite trunk across all layers. AMPH-sens/D1x monkeys exhibited greater spine density along the apical dendritic trunk relative to the AMPH-sens animals at all measured locations. Moreover, spine density was not just restored to pre-sensitization levels; chronic D1 antagonist treatment resulted in higher spine density at both peak and 100 μm distal to peak relative even to AMPH-naïve controls (Fig. 2). DTP was also greater in the D1 antagonist treated animals; therefore if spine density had been compared on the basis of distance from the soma rather than by matching of peak densities, the apparent increase in spine density with D1 antogonist treatment would have been even more marked. In addition, D1 antagonist treatment exerted a trophic effect on apical dendrites as apical dendritic length was greater in AMPH-sens/D1x animals compared to both other groups. Notably, the reduction in basilar dendritic length associated with AMPH sensitization was not restored by D1 antagonist treatment.

4. Discussion

Here, we have found evidence which suggests that chronic treatment with a selective D1 antagonist is associated with a profound and long-lasting improvement of cognitive function in the AMPH-sens nonhuman primate and a marked enhancement of dendritic spine density and apical dendritic length on pyramidal cells in PFC in the same animals. These findings underscore the potential that neuroplasticity of the dopamine system and the circuitry that it regulates may hold promise for the remediation of cognitive deficits consequent to psychostimulant drug abuse and suggest that these same mechanisms could be enlisted in the treatment of working memory deficits associated with dopaminergic dysregulation in schizophrenia. The results of this study further attest to the malleability of prefrontal function in response to long-term alterations in dopamine modulation.

Behavioral assessment of the effects of the D1 antagonist SCH39166 was performed as a longitudinal, within-animal control study. For this purpose, the performance of animals that had shown sustained stagnation of working memory function for more than a year after AMPH sensitization was considered "baseline," and these animals were tested before, during, and following D1 antagonist treatment. The seemingly permanent cognitive deficits observed following the AMPH sensitization were an unexpected outcome of the initial study (Castner et al., 2005) and therefore the decision to treat these animals with a D1 antagonist was made after the fact. Thus we did not have the provision to divide the AMPH-sensitized monkeys into two groups to allow for vehicle-injected controls as there were not sufficient sensitized animals available. Nor was it feasible to retrospectively generate a control group for the D1 antagonist study given the extensive experimental protocol (~3 years duration) necessary to train, AMPH sensitize and evaluate via cognitive testing the long-term consequences of the sensitization. In addition such controls would not have been well matched to the treated animals in terms of environmental/experimental experience, and allocation of additional nonhuman primates to a multiyear study in which they would only receive vehicle injections was considered impractical. Nevertheless, the weight of the evidence sufficiently excludes the possibility that repeated intramuscular injection or just the passage of time might have produced the cognitive and morphologic recovery observed in this study, especially considering that the significant improvement in working memory performance was seen only after cessation of D1 antagonist treatment. We have previously shown that without the intervention of chronic D1 antagonist treatment AMPH sensitization results in reductions in PFC dopamine turnover and deterioration in PFC pyramidal cell spinodendritic morphology that persisted for more than 3 years (Castner et al., 2005; Selemon et al., 2007), nearly as long as the post-sensitization interval of the animals in this study. The possibility that vehicle injection may have resulted in delayed recovery of cognitive function and dendritic integrity is highly remote, especially given that intramuscular injection associated with AMPH sensitization had the opposite outcome. Finally, we have previously reported the findings of a similar within-animal control study where we demonstrated that intermittent treatment with a D1 agonist after just six months of chronic haloperidol administration was capable of reversing deficits in working memory for more than a year after cessation of treatment (Castner et al., 2000b). Hence, we conclude that the recovery of cognitive function and dendritic integrity in the AMPH-sens animals studied here was a direct result of treatment with the D1 antagonist and its withdrawal.

Our morphologic findings are based on analysis of Golgi-impregnated neurons. Although a comparison of spine labeling methods has shown that DiI labeling labels greater numbers of spines, particularly small spine heads, compared to Golgi-impregnation (Shen et al., 2009), it seems unlikely that the robust group differences (e.g. ~25% in peak spine density on the apical dendritic trunk) could be due to simply to an alteration in spine size in the AMPH-sens animals.

4.1. Restoration of working memory performance following chronic treatment with a selective D1 antagonist

Performance on a spatial working memory task was significantly enhanced following cessation of the D1 antagonist treatment whereas notably cognitive performance was not significantly improved across the group during the treatment period. Moreover, despite the fact that the animals were treated for different durations, the profound improvement in working memory was seen after cessation of treatment across all animals. Thus, after more than a year of testing following their initial acquisition training (Castner et al., 2005), these animals performance improved from just under 60% correct to just over 80% in the weeks and months following chronic D1 antagonist administration which lasted a maximum of just over 8 months. In comparison, in our previous study examining three animals that were pretrained on the spatial delayed response task prior to AMPH-sens (Castner et al., 2005), these animals reached an average stable baseline level of performance (~72.5% correct) prior to sensitization. In the second month post-sensitization their performance fell to 62.5% but recovered within the third month following sensitization. The ~20% increase in performance across the group seen in the present study following D1 antagonist treatment would be unprecedented in normal animals and strongly suggests that their performance was improved from a persistent deficit state. These findings suggest that a beneficial long-term neuroadaptive change is induced by a long-term D1 blockade which may be masked by the short term, acute effect of D1 antagonist administration. Indeed the doses of the D1 antagonists studied were based on their ability to modulate dopamine transmission without inducing direct changes in cognitive performance acutely (Duffy et al., 1992; Murphy et al., 1996; Arnsten and Goldman-Rakic, 1998; see also Roberts et al., 2010). We hypothesize that the acute effect resulted from reducing D1 signaling further below a suboptimal level for performance on the tasks and that upregulation of D1 receptor signaling and its synaptic substrates contributed to the net long-term amelioration of the deficit in working memory. Thus, altered D1 receptor sensitivity may be at the heart of changes in prefrontal function that are consequent to long-term changes in D1 stimulation. In this regard, we have shown previously in similar within-animal studies that a sensitizing regimen of D1 agonist treatment leads to an enduring enhancement of cognitive function in aged and presumably dopamine deficient monkeys, as well as in animals on chronic haloperidol treatment which has been shown to induce down-regulation of the D1 receptor (Castner et al., 2000b; Castner and Goldman-Rakic, 2004).

4.2. Restitution of spinodendritic integrity in prefrontal pyramidal cells subsequent to D1 antagonist treatment

Our morphologic results suggest that chronic D1 blockade leads to an increase in apical trunk spine density and dendritic length and that reversal of the AMPH-associated morphologic deficits specifically on the apical dendrite may underlie recovery of prefrontal cortical function as manifested in the restoration of working memory performance. We have shown previously that AMPH sensitization produces substantial reductions in both spine density and dendrite length when the brain was examined three or more years after sensitization (Selemon et al., 2007). Coupled with the evidence that these morphologic measures of PFC pyramidal cell integrity were actually increased in the animals studied here compared to AMPH-naïve controls, these results strongly suggest that the pharmacologic intervention had a direct beneficial and enduring impact on prefrontal neuronal circuitry.

These data also provide further evidence that mesocortical dopaminergic modulation can restructure prefrontal pyramidal dendrites bidirectionally, that is, either increasing dendritic length and spine density as illustrated here or decreasing these same parameters in the AMPH-sens nonhuman primate. Our previous study indicated that

repeated intermittent stimulation of dopamine receptors via AMPH administration resulted in atrophic changes in pyramidal dendrites in the PFC (Selemon et al., 2007). The present study in which D1 receptors were specifically targeted with an antagonist also produced alterations in dendritic morphology, this time in the opposite direction with hypertrophy of the dendritic arbor and increased spine density essentially reversing the AMPH-induced deficits. While changes in the function of prefrontal cortical circuitry following dopamine stimulation or blockade may rely on receptor sensitization and/or alterations in intracellular signaling pathways, the present data suggest that the very long-term cognitive consequences of dopamine dysregulation (measured in years) are mediated at least in part by structural modification of pyramidal dendrites and further suggest that even these atrophic changes in dendritic morphology are reversible by pharmacological manipulation of D1 receptor signaling. It is important to note that, while both SCH39166 and SCH23390 are considered to be "selective" D1 antagonists (having very low affinity at D2 receptors), the latter does have significant affinity at 5-HT2 receptors (Alburges et al., 1992). Nevertheless, at the doses administered, we would consider the action of SCH23390 on 5-HT2A and 2C receptors to be minimal.

4.3. Relevance to neuropsychiatric illness and substance abuse

Dopamine dysregulation and cognitive impairment go hand in hand in a number of human conditions. Abi-Dargham and colleagues (2002) discovered an increased D1 receptor binding potential in dlPFC of unmedicated patients with schizophrenia and this increase showed a strong inverse correlation to working memory performance on the N-back task. A similar increase in D1 receptor binding potential was also observed in chronic recreational ketamine users without an apparent cognitive deficit, indicating this increase may reflect an effective compensatory process in this condition (Narendran et al., 2005). The relevance of the status of dopamine transmission to this increase in binding was demonstrated *in vivo* in the rodent where the same effect was obtained after subchronic dopamine depletion (Guo et al., 2003). Thus, it is important to know whether the cognitive impairments in disorders involving the dopamine system are entirely due to neurochemical disturbances or whether dopamine dysregulation might result in structural modification of circuitry. A recent study has demonstrated profound decreases in basal dendritic length and spine density of layer V pyramidal cells in rat prelimbic cortex following targeted dopamine depletion and showed that chronic treatment with an atypical antipsychotic reversed these morphological deficits (Wang and Deutch, 2008). In schizophrenia, reduced spine density on pyramidal cells in PFC has been found in several independent postmortem studies (e.g., Glantz and Lewis, 2000; Broadbelt et al., 2002) which may have parallels with the findings of Lewis and colleagues of diminished dopaminergic innervation in area 9 of PFC (Akil et al., 1999). This reduction of connectivity in the PFC, like that observed following AMPH sensitization in the nonhuman primate, may diminish the flow of information in working memory circuits leading to incoherent processing. Indeed, the association between dopamine imbalance and psychosis, long suspected in disorders like schizophrenia, has been strengthened by recent studies showing that chronic methamphetamine users exhibit an increased morbidity for psychotic disorders in parallel with impaired executive function (Chen et al., 2003; Chung et al., 2007). Some have postulated that the onset of schizophrenia which is often triggered by stressful life events may mimic neurochemical sensitization by inducing changes in the same dopamine-modulated neuronal circuitry that is altered by psychostimulants (Lieberman et al., 1997). One such alteration may be decreased dendritic integrity of prefrontal cortical pyramidal cells. Our finding that treatment with a D1 antagonist can reverse the dendritic atrophy associated with AMPH sensitization, and in so doing restore cognitive function, offers hope that the structural deficits associated with schizophrenia and other psychiatric illnesses might indeed be reversible. In this context, it should be noted that doses of SCH39166 far higher than those tested here were used in a pilot clinical trial designed to treat patients with schizophrenia and resulted in a predominant worsening of symptoms during the course of treatment (Karlsson et al., 1995). This leaves open the question as to whether a D1 antagonist, administered at a lower, physiologically therapeutic dose may yet prove to have significant clinical efficacy for this disorder.

In conclusion, these findings garner further support for the tenet that modulation of D1 dopamine receptor function, either by an antagonist in this study or an agonist in previous studies, can produce a seemingly permanent resetting of signaling through the D1 pathway sufficient to optimize cognitive performance. Thus, the ability to improve cognition can be associated with the normalization of the critical cellular substrate for working memory in PFC. An important component of the restoration of function appears to be structural expansion of the dendritic arbor and its spine complement in pyramidal cells. Here, we show that the restoration of working memory, the cornerstone of cognition, occurs in tandem with the restitution of the structural integrity of dlPFC which may prove to be fundamental for our understanding of how to treat cognitive impairments in dopamine dysfunctional states such as schizophrenia and substance abuse.

Disclosure/conflict of interest

Drs. Selemon, Begovic′, Williams, and Castner have no conflicts of interest with regard to this manuscript. Drs. Castner and Williams would also like to acknowledge research support from Pfizer Global Research and Development, Groton, CT, AstraZeneca Pharmaceuticals LP, Wilmington, DE, and GlaxoSmithKline, UK.

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