Reversal of Antipsychotic-Induced Working Memory Deficits by Short-Term Dopamine D1 Receptor Stimulation

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Chronic blockade of dopamine D2 receptors, a common mechanism of action for antipsychotic drugs, down-regulates D1 receptors in the prefrontal cortex and, as shown here, produces severe impairments in working memory. These deficits were reversed in monkeys by short-term coadministration of a D1 agonist, ABT 431, and this improvement was sustained for more than a year after cessation of D1 treatment. These findings indicate that pharmacological modulation of the D1 signaling pathway can produce long-lasting changes in functional circuits underlying working memory. Resetting this pathway by brief exposure to the agonist may provide a valuable strategy for therapeutic intervention in schizophrenia and other dopamine dysfunctional states.

The efficacy with which typical neuroleptics block D2 receptors is positively correlated with their ability to alleviate positive symptoms in schizophrenia (*1*). Chronic D2 antagonism, which is characteristic of neuroleptic therapy, up-regulates these receptors in both the striatum and prefrontal cortex (*2*). However, long-term D2 receptor blockade also induces a coincident down-regulation of D1-type receptors in the prefrontal cortex in nonhuman primates (*3*). D1 receptors are present in high concentrations in this region (*4*), and optimal stimulation at these sites potentiates signaling in neurons that are essential to the working memory process (*5*). Thus, we hypothesized that chronic haloperidol treatment in monkeys should induce working memory impairments due to insufficient stimulation at D1 receptors in the prefrontal cortex and, if so, that these deficits might be reversed by a D1 receptor agonist.

Six young adult female monkeys (*6*) were trained on delayed response (*7*) and delayed nonmatch-to-sample (DNMS) (*8*) tasks in order to assess spatial and object working memory, respectively. After establishing a consistent baseline performance, haloperidol, a typical neuroleptic commonly used in the treatment of schizophrenia, was administered twice daily [0.07 to 0.20 mg per kilogram of body weight (mg/kg) per day] throughout all phases of the experiment (*9*). These doses approximate clinically effective doses (~5 to 15 mg/day) used in patients and compensate for the more rapid metabolism of the drug in monkeys. Performance on the tasks was assessed in alternate test sessions 3 to 5 days per week.

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By the end of the pre-drug baseline testing period of 6 to 12 or more months, all six monkeys reached a consistent (~75%) level of performance on each task (Table 1 and Fig. 1, A and E; delayed response: $R = 0.01$, $F[1,159] = 0.03$, $P = 0.86$; two-object DNMS: $R = 0.19$, $F[1,97] = 3.47$, $P = 0.07$). After 1 to 4 months of chronic haloperidol administration, performance decrements emerged in five monkeys. A sixth monkey refused to leave the home cage at the lowest dose of haloperidol and had to be dropped from the group analysis. Regression analysis on data obtained from the five other monkeys revealed a highly significant impairment in performance on both working memory tasks (Fig. 1, B and F; delayed response: $R = -0.2$; $F[1,195] = 8.73$; $P = 0.003$; DNMS: $R = -0.48$; $F[1,103] = 29.82$; $P = 0.0000004$) (also see Table 1, Fig. 2, A and B, and Fig. 3A). In the spatial task, there was a significant effect of delay across all conditions; that is, monkeys made more errors at longer delays, independent of drug treatment (delay: $F[4,80] = 13.369$, $P = 0.0001$; treatment: $F[3,80] = 0.088$, $P = 0.9664$).

To determine the cognitive specificity of the haloperidol-induced deficits, we also tested the monkeys on two control tasks—object retrieval and fine motor performance—which required sensorimotor integrative skills but lacked a working memory requirement (*10*). Neither chronic haloperidol nor D1 agonist treatment affected the monkeys' performance on these tasks {fine motor: $F[3,19] = 0.35$, $P = 0.7906$, Fig. 3B; object retrieval: $F[3,15] = 1.36$, $P = 0.3032$ (*11*)}, which indicates that the working memory deficits were not due to the sensory or motor components required to perform these tasks. With the exception of the monkey that refused to test, the monkeys showed no change in appetite, motivation, or sedation, nor did they display any extrapyramidal symptoms during haloperidol treatment.

Because the emergence of cognitive deficits falls within the time frame in which the D1 receptor in the prefrontal cortex has been shown to be down-regulated in previous studies (3), it seemed highly probable that the monkeys in the present study also experienced similar down-regulation of D1 receptors, and that their working memory impairments could be due to suboptimal stimulation at these prefrontal receptors.

To examine this possibility, the selective full D1 agonist ABT 431 [0.00001 to 0.0001mg/kg, intramuscular (i.m.)] was coadministered for five to six blocks of five consecutive days with a minimum washout period of 2 weeks between blocks (*9*). Across 3 to 7 months of this intermittent coadministration of D1 agonist, all monkeys displayed a significant improvement in their performance on the spatial working memory task (Fig. 1C; $R = 0.27$, $F[1,241] = 18.50$, $P =$

Table 1. Spatial and object working memory performance across all test conditions. Shown are the mean ± SEM for all five monkeys across baseline, the "impaired" haloperidol dose, the last two rounds of D1 agonist coadministration, and the post-D1 testing periods. Symbols indicate that performance under a particular condition differs significantly from that under another condition at an alpha level of 0.05, as indicated by factorial analysis of variance with Scheffe post-hoc comparisons.

Monkey	Baseline	Haloperidol	Haloperidol +D1 agonist	Haloperidol post-D1
		Delayed response		
ROS	66.00 ± 3.23	48.86 ± 2.30*	61.82 ± 2.70†	67.77 ± 1.14†
NOE	74.29 ± 2.24	56.47 ± 2.45*	65.46 ± 1.86	72.82 ± 1.26†
AUD	88.75 ± 1.58	74.35 ± 1.87*	80.00 ± 1.68*	83.81 ± 1.10†
DOR	76.94 ± 2.29	64.80 ± 2.13*	72.33 ± 2.43	78.00 ± 1.38†
RUP	81.55 ± 1.89	68.42 ± 2.42*	71.17 ± 1.45*	73.48 ± 1.11*
		Two-object DNMS		
ROS	78.00 ± 3.00	66.50 ± 3.58	78.75 ± 2.80	75.63 ± 1.97
NOE	66.88 ± 3.27	58.33 ± 2.36	61.88 ± 3.27	65.16 ± 2.08
AUD	76.25 ± 2.02	73.09 ± 2.99	74.17 ± 4.56	82.80 ± 2.14
DOR	74.09 ± 2.32	63.07 ± 2.16	69.00 ± 2.08	63.28 ± 3.25
RUP	72.86 ± 2.30	58.85 ± 2.54*	68.33 ± 1.74†	73.33 ± 1.55†

*Significant versus baseline. †Significant versus haloperidol.

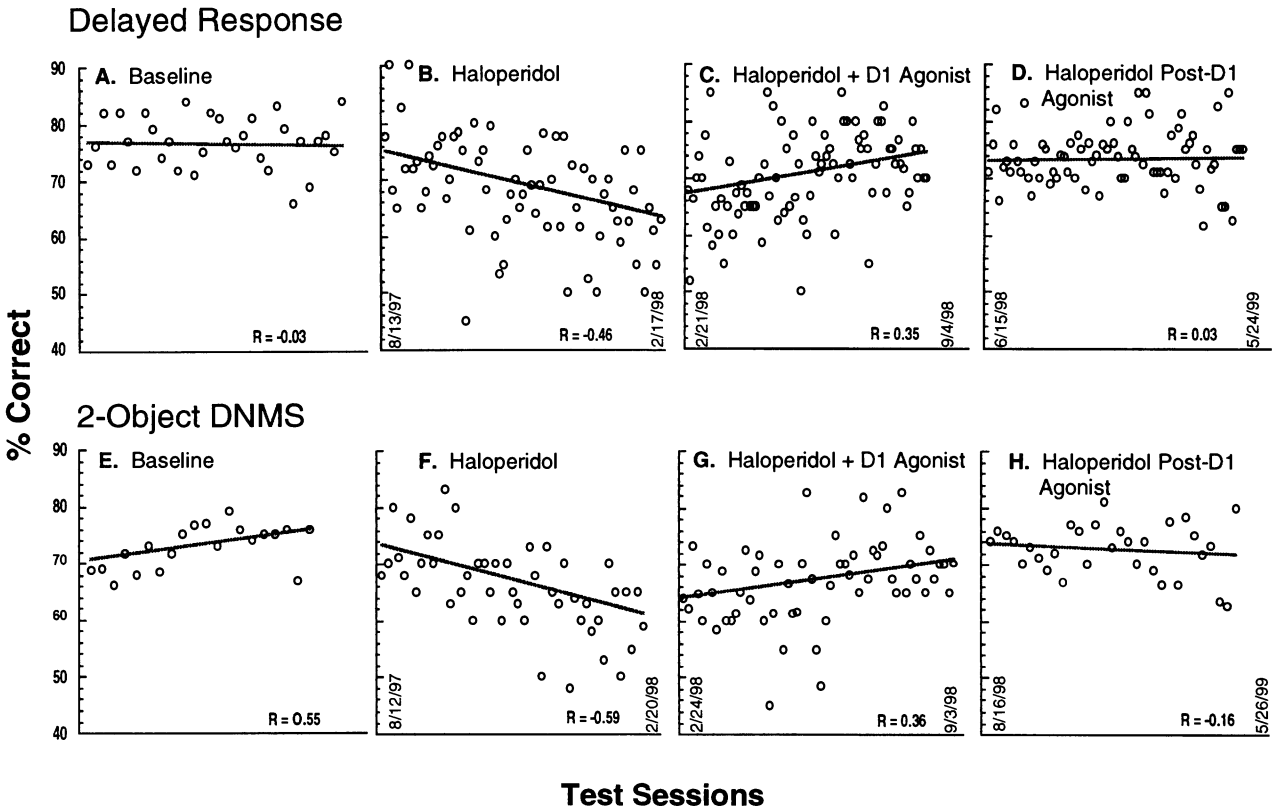

Fig. 1. Circles represent the average performance of five monkeys on the spatial (top) and object working memory (bottom) tasks for individual test sessions across baseline, haloperidol, D1 agonist, and post-D1 testing periods. Baseline performance was stable before haloperidol treatment (**A** and **E**). Performance declined on haloperidol for both tasks (**B** and **F**). D1 agonist coadministration produced a significant (**C**) or near-significant (**G**) reversal of deficits. After D1 coadministration, the monkeys' performance on both tasks has remained stable for periods of up to 1 year (**D** and **H**). *R* values in the graphs reflect the mean performance of all five monkeys on given test sessions.

0.000025) and a nonsignificant but strong trend for improvement on the object working memory task (Fig. 1G; $R = 0.15, F[1,139] = 3.22, P = 0.075$) (see Table 1 for individual means). Upon repeated exposure to the agonist, the monkeys showed increasingly extended periods of sustained improvement on the working memory tasks, which carried over into the washout periods between coadministrations (Fig. 1, C and G, and Fig. 2, C and D), despite continued haloperidol treatment. Indeed, after the final treatment with D1 agonist, performance did not differ significantly from pre-haloperidol baseline for all monkeys on the object working memory task and for four monkeys on the spatial task (Table 1). This reversal has persisted, in some cases, for more than 1 year (Fig. 1, D and H; delayed response: $R = 0.03, F[1,293] = 0.32, P = 0.57$; DNMS: $R = -0.07, F[1,134] = 0.57, P = 0.45$). Further, the sixth monkey that stopped testing when given chronic haloperidol treatment began to test again on the first day of D1 agonist coadministration and has continued to test for more than a year since.

The present findings provide evidence that chronic haloperidol treatment can induce cognitive impairment and that these impair-

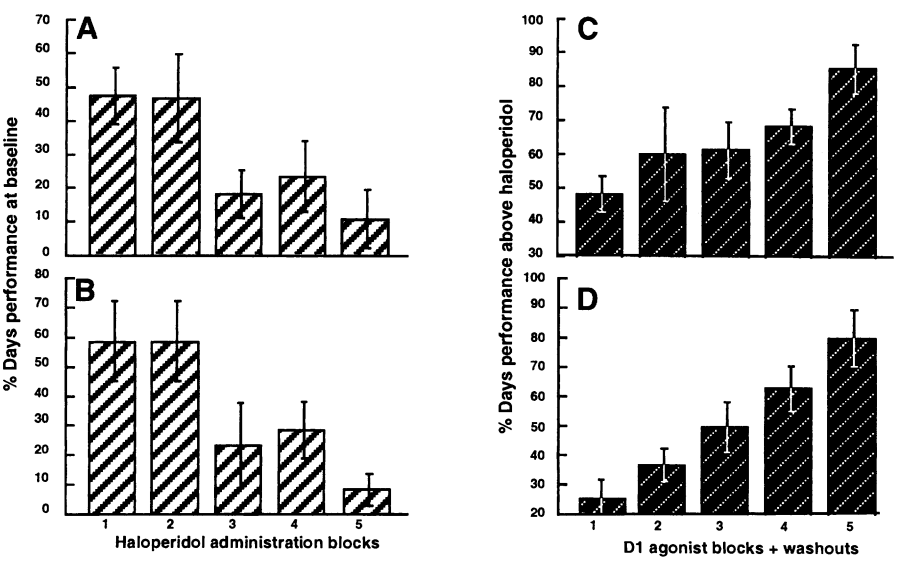

Fig. 2. (**A**) The spatial task and (**B**) the object task show the gradual emergence of haloperidol-induced cognitive deficits in five monkeys across 6 months. The period has been divided into five blocks of approximately equivalent numbers of test sessions per block. Data are expressed as the percent of test days on which cognitive performance did not differ significantly from baseline. (**C**) The spatial task and (**D**) the object task reveal that D1 coadministration progressively reverses the cognitive deficits. Each D1 agonist block includes test performance under D1 coadministration and during the subsequent washout. Data are expressed as the percentage of days on which cognitive performance was significantly above that shown under haloperidol treatment alone.

ments can be reversed by short-term D1 stimulation. This result is consistent with previous evidence about the behavioral effects of D1 agonists, both in monkeys and in patients with schizophrenia (*12*). However, because D2 up-regulation is also produced by chronic haloperidol treatment (*3*), the possibility exists that this change alone or in combination with D1 could also contribute to the cognitive deficits observed.

The developing pattern of cognitive enhancement evoked by intermittent D1 agonist administration is reminiscent of the phenomenon of behavioral sensitization to drugs that elevate dopamine release, such as amphetamine and cocaine (*13*). However, the present findings suggest a potentially beneficial role for selective stimulation of this receptor in altered dopaminergic states, such as we produced by blocking the D2 receptor (*14*). It remains to be determined whether a similar state exists in patients with schizophrenia treated with a variety of neuroleptic drugs.

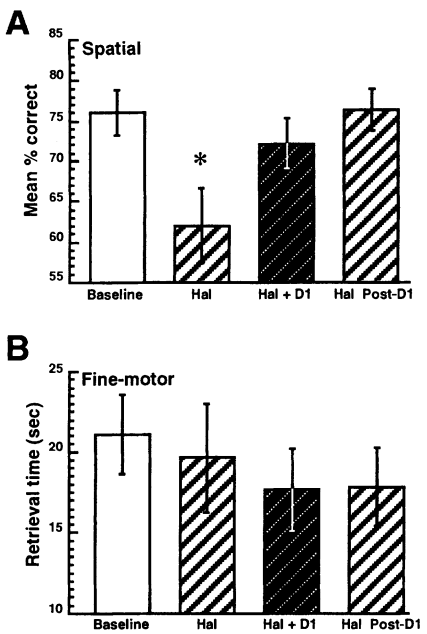

Fig. 3. Average performance of five monkeys on the spatial working memory (**A**) and fine motor (**B**) tasks for baseline (open bars), haloperidol (Hal) alone (white cross-hatched bars), D1 agonist coadministration (black cross-hatched bars), and up to 1 year of continued haloperidol after the final D1 washout period (white cross-hatched bars at right). Chronic haloperidol produced a significant impairment in delayed response performance [(A); $F[3, 20] = 4.36$, $P = 0.0189$; asterisk indicates $P < 0.05$ by Scheffe post-hoc comparison]. This deficit was improved during the 3 to 6 months of D1 agonist coadministration. In the 8- to 12-month period after D1, the monkeys exhibited sustained enhancement of cognitive performance as compared to the 6-month period of haloperidol alone ($P < 0.05$ by Scheffe post-hoc comparison). Conversely, all monkeys displayed only continued improvement, as indicated by decreased retrieval times, on the fine motor task (B) across treatment conditions (see text for statistics).

The enduring improvement in performance suggests that the agonist treatment induced a fundamental change in the circuits involved in working memory. These long-lasting changes may involve alterations in the D1 signal transduction pathway. Cyclic adenosine monophosphate (cAMP) production is stimulated by D1 agonists but inhibited by D2 agonists, particularly from the down-regulated state (*15*). It could be the case that cognitive improvement requires synergistic interactions between D1 stimulation and D2 blockade to achieve a high degree of activation of the cAMP cascade. An additional pathway may involve the novel protein calcyon, which has recently been shown to enable D1 potentiation of intracellular calcium release (*16*). Morphological changes could also be involved. Chronic haloperidol treatment in rodents decreases the density of dendritic spines in prefrontal neurons (*17*), whereas indirect dopamine agonists have been shown to increase the density of dendritic spines (*18*). Another strong possibility is that D1 agonist treatment may normalize D2 receptor sensitivity, and this may be the mechanism that reinstates and maintains normal cognitive performance.

The present findings have potential relevance for the treatment of cognitive deficits and/or negative symptoms in a variety of conditions, including schizophrenia, Parkinson's disease, and age-related memory decline. D1 down-regulation in the prefrontal cortex has recently been reported in both drug-naïve and medicated schizophrenic patients (*19*). Thus, the persistent recovery produced by brief periods of coadministration of D1 agonist revealed here suggests that schizophrenic patients now treated with D2 antagonist drugs may show substantial improvements in their cognitive abilities from a limited adjuvant exposure to a D1 agonist.

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6. Monkeys received a maintenance number of biscuits, fruit, and peanuts each day. They were housed individually and maintained in accordance with Yale Animal Use and Care Committee guidelines for nonhuman primates.
7. Delayed response was measured as follows: A well was baited in the monkey's view, then all the wells (two to four) were covered with identical plaques and an opaque screen was lowered for a randomized delay. After the delay, the animal had to move the correct plaque to obtain the reward. Performance was maintained at 75% by increasing the delays or the number of wells. The maximal delay set for a given number of wells included 0-, 10-, 20-, 30-, and 40-s delays. Monkeys were required to perform 20 trials.
8. Monkeys were trained on a traditional DNMS task (*20*). Next, they were trained to 75% performance on two-object DNMS, where only two objects were randomly selected as the "original" and "novel" stimuli for 20 trials during a given test session.
9. Haloperidol (in powder form) was obtained from RBI (Natick, MA). Daily doses were weighed out from a haloperidol:sucrose (1:99) mixture. Haloperidol was always administered (twice daily, 8 to 12 hours apart) after testing and disguised in fruit. The selective D1 agonist ABT 431 (provided by Hoechst Marion Rousell) was dissolved in nitrogen-percolated double-distilled water under dark conditions with 0.2% ascorbic acid. Individual aliquots were stored in light-protected containers at −70°C. This drug was always administered (i.m.) 30 min before testing.
10. For the object retrieval task, a three-dimensional clear Plexiglas box (4 inches by 4 inches) with an opening on one side was used. Animals performed nine trials per session, with the location of the opening (left, right, or front) randomized across trials. All trials were baited in the view of the monkey. The retrieval time and the number of barrier reaches were recorded. The fine motor task required the monkeys to use only the thumb and index finger to retrieve 20 treats from recessed wells on a clear Plexiglas board. The retrieval time and the number of drops were recorded.
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21. We thank T. A. Trakht and H. A. Findlay for their expert technical assistance and Hoechst Marion Rousell for funding this work and for their kind gift of ABT-431. Additional funding was provided by grants from the National Institutes of Mental Health (P50MH44866) and the National Institute of Drug Abuse (P01DA10160).

8 November 1999; accepted 25 January 2000