D2 Dopamine Receptor Blockade Immediately Post-training Enhances Retention in Hidden and Visible Platform Versions of the Water Maze

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Considerable evidence shows that post-training administration of dopamine agonists can enhance memory through actions on consolidation processes, but relatively little is known regarding the effects of dopamine antagonists on consolidation. These experiments investigated the effects of post-training systemic administration of the D2 receptor antagonist sulpiride on consolidation of memory for two versions of the Morris water maze task. Rats trained in either the hidden (spatial) or visible (cued) platform version received a subcutaneous injection of sulpiride or vehicle immediately following training. Retention testing 48 hr later revealed that relative to vehicle controls, sulpiride reduced platform latencies in both task versions, suggesting that like dopamine agonists, sulpiride can also have memory-enhancing effects.

There is a well-documented role for central dopamine systems in memory consolidation processes. Immediate post-training systemic administration of direct and indirect dopamine agonists in rodents can enhance retention in several tasks, including inhibitory avoidance, Y-maze visual discrimination, and several versions of the radial-arm and Morris water mazes (Packard and White 1989; Castellano et al. 1991; Gasbarri et al. 1993; Packard and McGaugh 1994), although in mice, dopamine agonists can also impair retention, depending on the strain of mice used (Castellano et al. 1991). Administration of these drugs directly into several brain regions has similar effects in these and other tasks, supporting a central action of the systemically administered treatments (Haycock et al. 1977b; Packard and White 1991; White and Viaud 1991; Hitchcott et al. 1997; Packard and Teather 1998). In mice, these retention-enhancing effects are thought to be mediated by both D1 and D2 dopamine receptors (Castellano et al. 1991). In rats, however, D2 receptors have been more strongly implicated in early memory consolidation processes (Packard and White 1989, 1991; White et al. 1993), whereas D1 receptors may play a more important role in later stages of consolidation (Bevilaqua et al. 1997).

Comparatively speaking, there have been many fewer studies of dopaminergic involvement in memory consolidation using dopamine antagonists. In mice, as is the case with dopamine agonists, post-training systemic administration of D1 and D2 antagonists can either enhance or impair retention in an inhibitory avoidance task, depending on the strain of mice used (Castellano et al. 1991). In one of the few

experiments to employ post-training systemic administration of dopamine antagonists in rats, the nonselective antagonist haloperidol impaired later retention of a two-way avoidance task (Gozzani and Izquierdo 1976). In the few studies using post-training intracerebral administration of dopamine antagonists, microinjection of the D2 antagonist sulpiride into either the nucleus accumbens or posteroventral caudate-putamen impaired different measures of retention in the hidden platform version of the Morris water maze (Setlow and McGaugh 1999a,b), whereas the same manipulation of the perifornical region of the lateral hypothalamus enhanced acquisition of a Pavlovian discriminative approach task (Phillips and Morutto 1998).

To study further the effects of dopamine antagonists on memory consolidation, we examined the consequences of immediate post-training systemic sulpiride administration in rats on retention in hidden platform (spatial) and visible platform (cued) versions of the Morris water maze. The water maze task and a D2 antagonist were chosen to allow comparison with previous work showing that post-training systemic administration of the D2 agonist quinpirole enhanced retention in both versions of this task (Packard and McGaugh 1994) and because the two versions of the water maze allow examination of the effects of systemically administered treatments on different brain systems (Packard and Teather 1998). Based on these findings, as well as previous findings of retention-impairing effects of dopamine antagonists (including sulpiride) in rats (Gozzani and Izquierdo 1976; Setlow and McGaugh 1999a), it was expected that sulpiride would impair retention in both versions of the task.

The subjects were 106 male Sprague-Dawley rats (C.

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River, San Diego, CA) weighing 250–275 g upon arrival. They were individually housed in a temperature-controlled colony room (22°C) and maintained on a 12-hr light/12-hr dark cycle (6:00–18:00 lights on) with ad libitum access to food and water. The rats were handled several times prior to training. The guidelines set forth in “Principles of laboratory animal care” (NIH publication no. 85-23, revised 1985) were followed throughout the experiments.

The water maze was located in a room (3 × 3.6 m) containing several extra-maze cues and consisted of a circular black-painted metal tank (1.83 m in diameter, 0.58 m in height) filled with water (25 ± 1°C). Four start positions (labeled N, S, E, and W) were located equidistantly around the edge of the maze, dividing it into four equal quadrants. During training and testing in the hidden platform version, a transparent Plexiglas platform (25 × 20 × 19.5 cm) was submerged 2 cm below the surface of the water. During training and testing in the visible platform version, a black and white striped plastic ball (7 cm in diameter) was mounted on a Plexiglas platform (11 × 12 × 19 cm), which was submerged 1 cm below the surface of the water.

Behavioral procedures were carried out between 10:00 and 15:00. Training consisted of a single session of four trials, with the start positions ordered pseudorandomly such that each was used once during training. On each training trial, the rats were placed into the water facing the wall of the maze, and their latencies to swim to the platform were recorded. Rats that failed to find the platform within 60 sec were guided to it by hand.

For the hidden platform version, the platform was located in the NE quadrant of the maze. After reaching the platform, the rats were allowed to remain on it for 20 sec and were then removed to a holding cage for a 30 sec inter-trial interval. For the visible platform version, the location of the platform was moved on each training trial, such that it was located in each of the four maze quadrants once. The position of the platform relative to the start position was varied, such that it was located to the left or right and distal or proximal to the start position with equal frequency. In this version, the rats were allowed 10 sec on the platform, followed by a 30 sec inter-trial interval in a holding cage.

Rats were assigned to drug treatment groups and given injections immediately following the last training trial. Sulpiride (10, 30, or 100 mg/kg) or vehicle was injected subcutaneously at a volume of 2 ml/kg body weight. (–)Sulpiride (Sigma, St. Louis, MO) was dissolved initially in one drop each of 100% EtOH and 0.1 M acetic acid and brought up to volume in 0.9% saline. Vehicle control injections consisted of one drop each of EtOH and 0.1 M acetic acid in 0.9% saline. The rats were returned to their home cages immediately following the injections.

At 48 hr following training, the rats were returned to the water maze for a retention test. No drugs were admin-

istered during the retention test. In both versions, the rats were placed into the water at a start position distal to the platform, and their latencies to swim to the platform (60 sec maximum) were recorded. All training and testing procedures were performed blind to drug treatment condition.

Training latencies were analyzed using one-factor, one-repeated measure analyses of variance (ANOVAs), with training trial as the within-subjects variable and drug treatment as the between-subjects variable. Retention latencies were analyzed using one-factor ANOVAs, with drug treatment as the between-subjects variable, and post-hoc comparisons between groups used Fischer's post-hoc test. In all cases, *P* values <0.05 were considered significant. Outliers, defined as scores outside the range of ±2 s.d. from the group mean, were excluded from each group prior to statistical analysis. Retention data from one rat in the 10 mg/kg group in the hidden platform version and one in the 30 mg/kg group in the visible platform version were excluded for this reason.

Acquisition data from both the hidden and visible platform task versions are shown in Figure 1. One-factor, one-repeated measure ANOVAs revealed no between-group differences in acquisition latencies prior to drug treatment in either the hidden platform (Fig. 1A), $F(3, 54) = 0.96$, n.s., or visible platform (Fig. 1B), $F(3, 44) = 0.08$, n.s., versions. Data from the retention test 48 hr after training are shown in Figure 2. In the hidden platform version (Fig. 2A) a one-factor ANOVA (with drug treatment as the between-subjects variable) revealed a significant effect of drug treatment condition, $F(3, 53) = 3.67$, $P < 0.05$. Post-hoc comparisons between groups showed that rats in the 10 mg/kg sulpiride group had significantly shorter platform latencies than rats in the vehicle control group ($P < 0.05$). Additionally, rats in the 30 mg/kg group had significantly longer platform latencies than rats in both the 10 and 100 mg/kg groups ($P < 0.01$, $P < 0.05$, respectively). In the visible platform version (Fig. 2B) a one-factor ANOVA revealed a significant effect of drug treatment condition, $F(3, 43) = 3.34$, $P < 0.05$. Post-hoc comparisons between groups showed that rats in the 30 mg/kg sulpiride group had significantly shorter platform latencies than rats in the vehicle control group ($P < 0.01$), as well as significantly shorter latencies than rats in both the 10 and 100 mg/kg groups ($P < 0.05$ for each).

The results of these experiments show that, in contrast to the expected outcome, immediate post-training systemic administration of the D2 dopamine receptor antagonist sulpiride enhanced retention in both the hidden and visible platform versions of the water maze. Given that dopaminergic transmission in the hippocampus and nucleus accumbens in the hidden platform version and in the caudate-putamen in the visible platform version are involved in consolidation of memory in these tasks as assessed by the platform latency measure (Packard and Teather 1998; Setlow and McGaugh 1999a), it is likely that the systemic ma-

Figure 1 Mean (in sec $\pm$ S.E.M.) platform latencies of rats during training in the hidden platform (A) and visible platform (B) versions of the water maze prior to sulpiride or vehicle injection.

nipulations in the present experiments affected memory consolidation at least in part through actions in these structures.

It is important to note that the use of post-training drug administration rules out interpretations of the results in terms of effects on attention, motivation, sensory perception, or other non-mnemonic functions critical to task acquisition. Furthermore, the long (48-hr) retention interval and brief half-life of sulpiride in the rat (Segura et al. 1976) suggest that the drug was not present during the retention test. Thus, it is highly likely that the effects of sulpiride on retention resulted from actions on the consolidation phase of memory. The fact that sulpiride (at doses that enhance retention when given immediately post-training) is ineffective when given two hours post-training in mice (Cestari et al. 1992) supports this contention. It is also unlikely that sulpiride's effects on retention can be attributed to rewarding or reinforcing properties of the drug (e.g., post-training sulpiride administration making escape onto the platform a more rewarding experience, thus increasing motivation to escape to the platform during the retention test). Although

there is some evidence that systemic administration of low doses of sulpiride can enhance responding for conditioned reinforcement, it is ineffective or impairs responding at the doses effective in the present experiment (Smith et al. 1997). Moreover, although sulpiride microinjections into the perifornical region can produce a conditioned place preference (Morutto and Phillips 1997), systemically administered sulpiride appears to have an impairing, if any, effect on this behavior (Meisel et al. 1996; Noda et al. 1998).

The finding that a systemically administered D2 antagonist enhances retention in the hidden and visible platform versions of the water maze is somewhat surprising and seems inconsistent with previous findings that D2 agonists enhance retention in these and other tasks (Haycock et al. 1977a; Packard and White 1989; Packard and McGaugh 1994), as well as with the finding that systemically administered haloperidol impairs retention (Gozzani and Izquierdo 1976). However, a resolution of this apparent conflict may be found in the synaptic-level effects of D2 antagonists. As with indirect agonists such as amphetamine, sulpiride

Figure 2 Mean (in sec $\pm$ S.E.M.) platform latencies of rats during the 48 hr retention test given sulpiride (10, 30, or 100 mg/kg) or vehicle immediately following training. (A) Hidden platform version ($n = 14-15$ rats/group). (B) Visible platform version ($n = 11-12$ rats/group). * $P < 0.05$, ** $P < 0.01$ compared with corresponding vehicle control group.

administered systemically at doses within the range of those used here enhances dopamine release in the striatum, presumably through blockade of inhibitory presynaptic autoreceptors (Moghaddam and Bunney 1990; Westerink et al. 1990) and possibly in other dopamine terminal fields (such as the hippocampus) as well. This enhanced dopamine release would lead to increased activation of D2 receptors, although the degree of activation would depend on the relative binding kinetics of dopamine and sulpiride at the relevant D2 receptors. Thus, sulpiride could potentially mimic the effects of both indirect and direct D2 agonists [although the finding that systemically administered dopamine agonists enhance retention at doses thought to occupy preferentially D2 autoreceptors (White et al. 1993) may speak against this account]. Sulpiride may also enhance retention through actions on cholinergic neurons, as systemic injections (in the dose range used here) induce striatal acetylcholine release (Bertorelli and Consolo 1990; Imperato et al. 1994). Support for both dopaminergic and cholinergic mechanisms of sulpiride's action in the present experiments is provided by findings that post-training intra-striatal microinjections of either dopaminergic or cholinergic agonists enhance retention in several tasks, including the visible platform version of the water maze (Prado-Alcalá 1985; Packard and White, 1991; White and Viaud 1991; Packard and Teather 1998; Schilwein et al. 2000). Further support for a cholinergic mechanism is provided by evidence that sulpiride's effects on retention of a Y-maze discrimination task in mice depend on muscarinic cholinergic receptors (Gasbarri et al. 1997). The presence of at least two potential retention-enhancing mechanisms of action for sulpiride, as well as the different brain regions on which it could be acting (Packard and Teather 1998; Phillips and Morutto 1998; Setlow and McGaugh 1999a), may account for the difference in the most effective dose between the two versions, as well as the appearance of a biphasic dose-response curve in the hidden platform version.

In view of our previous findings that post-training intra-accumbens sulpiride administration impaired retention in the hidden platform version of the water maze (Setlow and McGaugh 1999a), it may seem contradictory that systemic administration enhanced retention in the same version in the present study. However, several differences in methodology between our previous and present work must be taken into consideration when comparing the two findings. First, although it is difficult to compare the concentration of sulpiride reaching the brain following systemic and intracerebral injections, it is probable that they differed significantly. Second, the previous experiments with intracerebral microinjections used eight training trials, whereas four were used in the present experiment. The dose-response curves for the effects of many (including dopaminergic) drugs on memory consolidation follow an inverted-U shape, such that they can either enhance or impair retention de-

pending on the dose and intensity of training (Gold and Van Buskirk 1976; Haycock et al. 1977a; McGaugh 1992). Thus, it is likely that the (probable) different doses of sulpiride reaching the brain following systemic and intra-accumbens injections interacted with the different number of training trials used in the two experiments to produce the differential effects of sulpiride seen in the previous and present experiments. Different concentrations of sulpiride reaching the brain in the two experiments also may have acted preferentially at pre- or postsynaptic D2 receptors (Ahlenius 1992; White et al. 1993). Moreover, it is possible that the retention-enhancing effects of systemic sulpiride administration in the hidden platform version are mediated by brain structures other than the nucleus accumbens (Phillips and Morutto 1998). A direct comparison in rats of post-training systemic administration of a wide dose range of D2 agonists and antagonists (including doses thought to act both pre- and postsynaptically) in several different tasks would help to clarify this issue.

In summary, the results presented here demonstrate that systemic administration of the D2 antagonist sulpiride enhanced retention in both the hidden and visible platform versions of the Morris water maze. In view of previous findings, these results highlight the complexity of dopamine involvement in memory consolidation and suggest that multiple factors, including the brain regions and receptor subtypes at which a drug acts, as well as interactions with the conditions of training, may need to be taken into account in explanations of drug effects on consolidation processes.

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