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RAPID COMMUNICATION

Scopolamine Selectively Disrupts the Acquisition of Contextual Fear Conditioning in Rats

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Muscarinic cholinergic antagonism produces learning and memory deficits in a variety of hippocampal-dependent tasks. Hippocampal lesions produce both acquisition deficits and retrograde amnesia for contextual fear conditioning, but do not impact fear conditioning to discrete cues. In order to examine the effects of muscarinic antagonism in this paradigm, rats were given scopolamine (1 mg/kg) either before or for 3 days after a Pavlovian fear-conditioning session in which tones were paired with aversive footshocks. Fear to the context and the tone was assessed by measuring freezing in separate tests. It was found that pretraining, but not posttraining, scopolamine severely impaired contextual fear conditioning; tone conditioning was not affected under either condition (cf., Young, Bohenek, & Fanselow, *Neurobiology of Learning and Memory*, **63,** 174–180, 1995).

Cholinergic systems are known to be important for learning and memory (e.g., Deutsch & Rocklin, 1967). In many behavioral paradigms, the deficits produced by muscarinic cholinergic antagonism are similar to the effects produced by hippocampal lesions (Watts, Stevens, & Robinson, 1981), while in others they are more general (e.g., Rudy, 1995). Although the precise mechanism for muscarinic antagonistic effects on memory is unknown, it may involve the disruption of hippocampal theta rhythm (Vanderwolf & Robinson, 1981).

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Recent studies indicate that the hippocampus has an important role in Pavlovian fear conditioning. During fear conditioning, a conditional stimulus (often a tone) is paired with an aversive unconditional stimulus (usually an electrical shock) in a novel context. With repeated pairings, the animal learns to fear both the tone and the training context. Hippocampal lesions produce an acquisition deficit (Phillips & LeDoux, 1992) and a time-limited retrograde amnesia that is selective for contextual fear (Kim & Fanselow, 1992). Moreover, there is a high correspondence between hippocampal theta rhythm and the acquisition of contextual fear (Maren, DeCola, Swain, Fanselow, & Thompson, 1994).

As such, one would expect that contextual fear conditioning should be disrupted by muscarinic antagonism. However, the available data are unclear. We recently reported that pretraining scopolamine produced an acquisition deficit that was selective for tone conditioning, whereas posttraining administration enhanced contextual conditioning (Young, Bohenek, & Fanselow, 1995). On the other hand, Rudy (1995) reported that both tone and contextual fear conditioning were impaired by scopolamine. Therefore, we reexamined the effects of scopolamine on fear conditioning with procedures that eliminated some of the concerns (see discussion below) about our previous report. Rats were trained using behavioral parameters that produce high levels of fear and are similar to those for which we have reported hippocampal-lesion deficits, a vigorous retrograde treatment was used, and animals were tested drug-free 1 week after drug administration.

Subjects. Thirty female Long–Evans rats (250–300 g, 100–107 days) bred at UCLA were used in

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this experiment. They were individually housed in metal cages located in a colony maintained on a 14:10 light:dark cycle. They had unrestricted access to food and water and were handled prior to testing.

Drugs. Scopolamine-HBr (Research Biochemicals International, Natick, MA) was prepared fresh daily in cold 0.9% saline and stored in a light-proof container. The drug was given in doses of 1 mg/kg (salt, 1 ml/kg), ip, or equivalent saline injections were given.

Training. Fifteen minutes prior to training, anterograde ($n = 10$) animals received scopolamine in their home cages and retrograde ($n = 10$) and saline control ($n = 10$) animals received saline. The animals were then transported to the laboratory and immediately placed into conditioning chambers. After two min, a 30-s, 2-kHz, 85-dB (A scale) tone was presented that coterminated with a 2-s, 1-mA footshock. Sixty seconds later animals received another tone-shock pairing and this was repeated until the animals had received five pairings. One minute after the last trial, the animals were removed, and retrograde animals received scopolamine, while anterograde and saline control animals received saline. The animals were then returned to their home cages. Twenty-four and 48 h after training, retrograde animals again received scopolamine in their home cages (others received saline).

Testing. One week after the final injection all animals were placed back into the original training context for a 16-min context test. Freezing behavior, an index of conditional fear in the rat (Fanselow & Bolles, 1979), was scored blind using an 8-s time sampling procedure. Sixteen observations per 2-min interval for each animal were converted into percentage of time freezing measures. Two days later the animals were brought to a novel context (described below) for a 10-min tone test. The animals were placed in this context and after a 2-min baseline interval the tone (as before) was presented for 8 min.

Contexts. The training and context test environment consisted of aluminum (side walls) and Plexiglas (front, back, and top) chambers (28 w × 21 h × 22 d cm; Lafayette Instruments, Lafayette, IN). The floor of each chamber had 18 steel rods (0.2-cm radius, 1.5 cm apart) connected to a shock scrambler and generator (supplying background noise of 70 dB). The chambers were scented with a 5% ammonia solution (in collection pans below the rods). These chambers were in a well-lit room separate from the observers, who viewed the animals on video screens. Tones were presented from a speaker in the wall of each chamber. The tone test context was in a separate room. The chambers (same dimensions as above) in this room had a white rear wall and two white plastic side walls (24 × 21 cm) placed at a 60° angle to the floor forming a triangular enclosure. The floors consisted of 17 staggered rods (two rows 1 cm vertically apart; for each row, 2.6 cm apart). Background white noise (70 dB) was supplied by a noise generator and the chambers were scented with a 1% acetic acid solution. This room was kept entirely dark except for a 60-W red light bulb. The carriers used to transport the animals to each context were also different. Animals show little generalization between these contexts (Fig. 1B, baseline).

Context test. As can be seen in Fig. 1A, a pretraining injection of 1 mg/kg scopolamine (anterograde) severely impaired the acquisition of contextual conditioning, but three posttraining injections (retrograde) had no impact on contextual conditioning. There was a significant group × time interaction [MANOVA: $F(14, 189) = 3.1$, $p < .001$] so each interval was considered separately. There were significant group differences for the 2-min [ANOVA: $F(2, 27) = 11.2$, $p < .001$], 4-min [$F(2, 27) = 6.1$, $p < .01$], and 6- and 8-min [$F(2, 27) > 3.5$, $p < .05$] points, but not for those thereafter [$F(2, 27) < 1.5$, $p > .2$]. Post hoc comparisons revealed that for the first 4 min, anterograde animals showed significantly less freezing than saline control (Fischer's PLSDs: 2 min, $p < .001$; 4 min, $p < .05$) and retrograde animals ($p < .001$ and $p < .01$), which did not differ from each other ($p > .6$). Anterograde animals also differed from retrograde animals for 4–8 min ($p < .05$) but no other group comparisons at any other time points were significant ($p > .1$).

Tone test. Scopolamine did not affect conditioning to the tone (Fig. 1B). There was no group × time interaction for this test [MANOVA: $F(8, 108) = 1.057$, $p > .35$] nor group main effect [$F(2, 27) = 1.8$, $p > .15$]. There were no group differences [ANOVAs: $F(2, 27) < 1.8$, $p > .15$] nor any significant post hoc group comparisons ($p > .10$) at any time point.

Group × test interaction. Figure 1C. Finally, we considered the first 6 min (collapsed) of each test where significant differences occurred for the context test in a two-way (group, test) ANOVA. There was a significant group × test interaction [$F(2, 27) = 3.4$, $p < .05$], indicating that we were justified in considering these two tests separately. For these collapsed tests, there were group differences for the context [$F(2, 27) = 7.3$, $p < .01$], but not for the tone

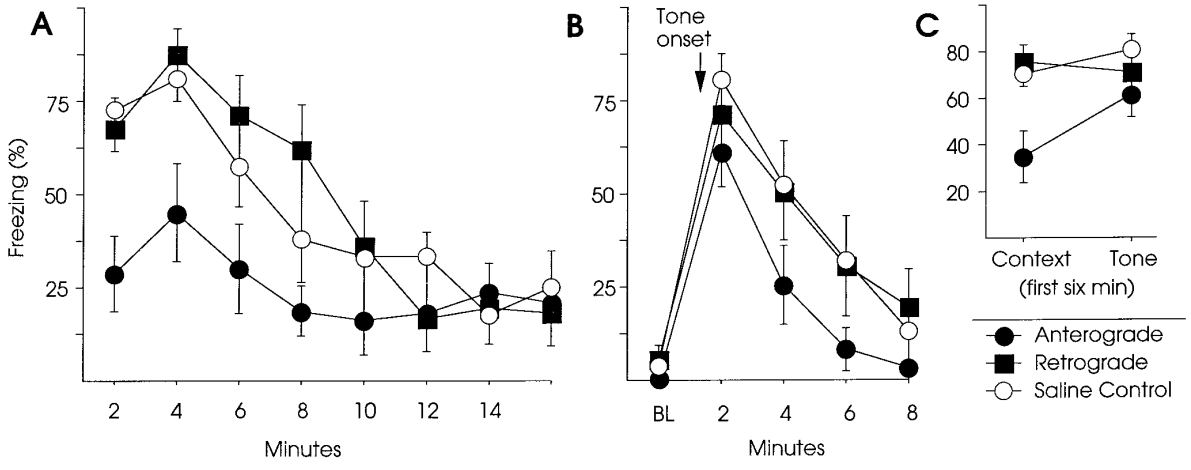

FIG. 1. (A) Context test. One week after the final injection, the animals were placed in the original training context and freezing behavior (percentage time, mean ± SEM) was scored for 16 min and is reported in 2-min intervals. Anterograde animals, which received 1 mg/kg scopolamine 15 min prior to initial training, showed significantly less freezing than saline control animals or retrograde animals (which received the drug three times after training), which did not differ significantly. There was a significant group × time interaction: the impairment was evident only in the first 8 min. (B) Tone test. Two days after the context test, the animals were placed in a novel context, and after a 2-min baseline (BL) period, the original training tone was played continuously for 8 min. Freezing is depicted for the entire 10 min. The groups did not differ significantly for the entire test or at any time point, indicating that neither drug treatment produced a deficit in tone conditioning. (C) Group × test interaction. Drug treatment differentially affected the context and tone tests, as is indicated by the significant group × test interaction. The drug produced an acquisition (anterograde), but not a consolidation (retrograde), deficit and did so only for contextual conditioning.

test [$F(2, 27) = 1.4$, $p > .25$]. Anterograde animals were significantly impaired only for contextual conditioning when compared with saline control or retrograde animals ($p < .01$).

In this study, 1 mg/kg ip scopolamine given 15 min prior to training was sufficient to severely disrupt contextual, but not tone, fear conditioning. Three daily treatments with this dose beginning immediately after training had no effect on conditioning. These results are in contrast with those we previously reported (Young et al., 1995); however, several aspects of the procedures in Young et al. are problematic. (1) Many of the behavioral measurements were taken while the animals were under the influence of scopolamine, which is known to produce a motor hyperactivity (Campbell, Lytle, & Fibiger, 1969; Hooks, Jones, Smith, Neill, & Justice, 1991) that may interfere with freezing. (2) The animals were trained on the drug repeatedly (across several days), which is problematic because both the drug's motor hyperactivity effects and its ability to disrupt learning may show tolerance (Hooks et al., 1991). (3) The levels of freezing were low, making deficits difficult to detect, (4) the training parameters were different from those that show hippocampal deficits, and (5) behavior was scored during a "recruitment" period when freezing has not yet reached optimal levels (Fanselow & Bolles, 1979).

In a similar line of experiments, Rudy (1995) has reported that scopolamine was sufficient to block the acquisition of both tone and contextual fear conditioning and produce retrograde amnesia for contextual conditioning if given up to 3 h after training. However, Rudy used young (23-day-old) rats and it is likely that their drug dose was effectively higher than that in adults. Thus, it is plausible that a higher dose of scopolamine in adult rats would also produce tone conditioning deficits and/or retrograde amnesia. Indeed, in our study tone conditioning appears to be slightly, but nonsignificantly, impaired. Because contextual fear conditioning was also more readily impacted by scopolamine than tone conditioning in the studies reported by Rudy, it is plausible that the dose–effect curve for tone conditioning was shifted to the right relative to contextual conditioning. As such, the hippocampus, which mediates contextual conditioning, may be more readily impacted by scopolamine than the amygdala, which mediates fear more generally (Davis, 1986). Alternatively, scopolamine may have disrupted the basic sensory–perceptual processes through which an animal evaluates its environment. Contextual conditioning may be more readily impacted because it requires the integration of multiple conditional cues, while tone conditioning requires the sampling of a single conditional cue (see, e.g., Rudy, 1995; Kim & Fanselow, 1992). In either case, there are probably few conditions under which scopolamine disrupts

tone, but not contextual fear conditioning (cf., Young et al., 1995).

In summary, 1 mg/kg scopolamine selectively disrupted the acquisition, but not the consolidation, of contextual fear conditioning, a hippocampal-dependent form of learning. Further study is necessary to determine the dose–effect relationships for tone and contextual conditioning and the specific muscarinic receptor populations necessary for these types of conditioning.

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