Differential Effects of 8-OH-DPAT on Two Forms of Appetitive Pavlovian Conditioning in the Rat

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Rats were trained on an appetitive Pavlovian conditioning task in which the conditioned stimulus (CS) was either localized (a light in the food tray) or nonlocalized (an increase in the general level of illumination). The conditioned response (CR) of approaching the site of food delivery in the presence of the CS was monitored. Presession treatment with the 5-HT$_{1A}$ agonist 8-OH-DPAT (subcutaneous injections at a dose of 0.15 mg/kg) retarded acquisition of the CR, but only when the localized CS was used. The results confirm the general proposal that serotonergic processes are involved in learning. The selective effect of the drug is not to be explained in terms of its motor effects and is consistent with the specific suggestion that systemic administration of 8-OH-DPAT is especially effective in disrupting learning tasks mediated by hippocampal mechanisms.

It is now widely accepted that the serotonergic system plays a role in learning and memory (see Meneses, 1999, 2002, for reviews). Lesions and drugs that affect serotonergic function have been found to influence cognition in both animals and humans (e.g., McEntee & Crook, 1991; Woolley & Van der Hoeven, 1963), and degeneration of 5-HT neurons has been found in patients with Alzheimer's disease (Curcio & Kemper, 1984). 5-HT$_{1A}$ receptor subtype activity may be responsible for at least some of these effects, as a reduction in 5-HT$_{1A}$ receptor binding in the temporal lobe has been found in Alzheimer's patients (Bowen et al., 1983) and also in the cortex and hippocampus of aged rats (Nyakas et al., 1997).

Consistent with this notion is the observation that selective 5-HT$_{1A}$ agonists, such as 8-OH-DPAT, can affect performance on at least some learning tasks. In particular, it has been shown that systemic administration of 8-OH-DPAT can impair performance in tasks involving the processing of spatial information, such as delayed nonmatching to position (Warburton, Harrison, Robbins, & Everitt, 1997), the radial arm maze (Winter & Petti, 1987), the Morris water maze (Carli, Luschi, Garofalo, & Samanin, 1995; Carli & Samanin, 1992; Kant et al., 1996; Kant, Wylie, Chu, & Ghosh, 1998), and contextual conditioning (Stiedl, Misane, Spiess, & Ogren, 2000). Finally, deficits in autoshaping in rats (the conditioned stimulus [CS] being the presentation of a response lever) have also been observed (Meneses & Hong, 1994). However, researchers have more commonly found that systemic 8-OH-DPAT has no effect on learning in classical conditioning preparations, such as the conditioned emotional response paradigm (Stanhope & Dourish, 1996) and the rabbit nictitating membrane preparation (Welsh, Kachelries, Romano, & Harvey, 1998).

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This pattern of deficits is remarkably reminiscent of that produced in rats by lesions of the dorsal hippocampus. First, it is well established that such lesions produce a marked disruption of spatial learning (see Macphail, 1993, for a review). Second, although dorsal hippocampal lesions are normally considered not to affect classical conditioning (see Macphail, 1993), deficits have been reported in the autoshaping procedure (Good & Honey, 1991). The marked similarity of the behavioral profile produced by these two treatments, combined with the fact that the dorsal hippocampus contains a high concentration of postsynaptic 5-HT$_{1A}$ receptors (Pazos, Hoyer, Dietl, & Palacios, 1988), prompts the suggestion that the effects of systemic 8-OH-DPAT result, at least in part, from disruption of hippocampal function. The proposal that this effect of 8-OH-DPAT is hippocampally mediated is supported by the observations that systemic and intrahippocampal 8-OH-DPAT have similar effects in the delayed nonmatching to position task (Warburton et al., 1997) and that the effects of systemic 8-OH-DPAT on the water-maze task can be reversed by infusions of 5-HT$_{1A}$ antagonists, such as spiroxatrine, into the dorsal hippocampus (Carli et al., 1995). Similarly, the deficit in context conditioning reported by Stiedl et al. (2000) was reversed by intrahippocampal WAY 100635, a selective 5-HT$_{1A}$ receptor antagonist.

Bonardi (2001) has argued that the reason hippocampal lesions produce deficits in autoshaping but not in other conditioning preparations is that the CS used in autoshaping is localized in space so that it may be regarded as a type of spatial task. There may be some common processes involved in learning about localized cues, both in classic spatial learning paradigms and in more orthodox classical conditioning procedures. If hippocampal damage were to impair these common processes, then this would produce both a spatial learning deficit and a selective inability to condition to localized CSs. To verify this proposal, Bonardi examined the effects of small electrolytic lesions of the rat dorsal hippocampus in a conditioning procedure in which presentations of a CS signaled the delivery of a food pellet, and the conditioned response (CR) was approaching the food tray. She reported that rats with lesions conditioned as readily as sham-operated controls

when the CS was diffuse (an auditory cue or a change in chamber illumination), but that a deficit was observed when the CS was localized (the illumination of a small bulb located inside the food tray). She argued that this finding confirms the proposal that animals with hippocampal damage show a selective deficit in classical conditioning to localized cues.

In the present experiment, we investigated the effects of systemic administration of 8-OH-DPAT on the behavioral task used by Bonardi (2001). If the cognitive impairment produced by this treatment is mediated (at least in part) by way of a disruption of hippocampal function, then we might expect to find a selective deficit on conditioning with a localized cue as the CS.

The experiment used four groups of subjects, all trained on an appetitive Pavlovian conditioning task in which the CR measured was approach to the food tray. For two groups, the CS was the illumination of a small light (localized; L) positioned inside the tray to which the unconditioned stimulus (US; a food pellet) was delivered; for the other two groups, the CS was an increase in the general illumination of the chamber (nonlocalized; N). One group from each pair (the localized–drug group [L-D] and the nonlocalized–drug group [N-D]) received a subcutaneous injection of 8-OH-DPAT (at a dose of 0.15 mg/kg) before each training session; the two remaining groups (the localized–vehicle groups [L-V] and the nonlocalized–vehicle group [N-V]) received an injection of vehicle. If the effects of treatment with 8-OH-DPAT parallel those found in Bonardi's (2001) study of the effects of hippocampal lesions, we should find that Group N-D will learn as well as Group N-V, but that Group L-D will be retarded with respect to its control Group L-V.

Method

Subjects

The subjects were 48 naive male hooded Lister rats with a mean ad-lib weight of 462 g (range = 305–660 g). They were housed in pairs in a colony room lit daily from 8 a.m. to 8 p.m. Experimental sessions occurred during the light portion of the cycle. The rats had free access to water, but a schedule of food deprivation was introduced prior to the start of training, reducing them to 80% of their ad-lib feeding weights. They were maintained on this schedule for the duration of the study.

Apparatus

The apparatus consisted of four Campden Instruments (Loughborough, UK) operant chambers. The chambers had three walls of sheet aluminum, a transparent plastic door as the fourth wall, and a white translucent ceiling. Each of the boxes contained a recessed food tray to which 45-mg mixed composition food pellets could be delivered; this was situated in the center of one of the walls adjacent to the door. Access to the food tray was by means of a rectangular aperture 6 cm high × 5 cm wide, which was covered by a transparent plastic flap of the same dimensions. Pushing the flap inward allowed access to the food tray and operated a microswitch; this was recorded as a single response. The flap automatically returned to its resting position when the rat removed its snout from the tray. The floor was composed of stainless steel rods 0.5 cm in diameter and 1.5 cm apart. The boxes were dimly illuminated throughout training by a 2.8-W house light (rated for 24 V but operated at 15 V) located high on the front wall of the chamber. A similar light (operated at full intensity) located inside the food tray was used as the localized CS. A 60-W strip light, rated for 240 V, was fixed above the translucent ceiling of the box. When operated at 100 V, this light produced a general increase in the illumination of the

chamber (the nonlocalized CS). The chambers were housed in sound- and light-attenuating shells; masking noise was provided by the operation of ventilating fans contained in these shells. The apparatus was controlled by a microcomputer programmed in a version of BASIC.

Procedure

Behavioral training sessions occurred once daily and lasted 40 min. In the first two sessions, food pellets were delivered according to a variable-time 60-s schedule, allowing the rats to learn to retrieve them from the food tray. Each of the next four conditioning sessions contained eight presentations of a 10-s CS, the termination of which was followed immediately by the delivery of a food pellet. For Groups L-D and L-V, the CS was the illumination of the tray light; for Groups N-D and N-V it was the presentation of the overhead light. Operations of the tray flap were measured separately during CS presentations and also during the 10-s period that immediately preceded each CS (the pre-CS period). The first trial (measured from the start of the pre-CS period) occurred after 250 s, and the same interval intervened between the end of one CS and the onset of the pre-CS period for the next.

Rats in Groups L-D and N-D received a subcutaneous injection of 0.15 mg/kg 8-OH-DPAT (hydrobromide, Sigma-Aldrich, UK; dissolved in 0.9% saline with an injection volume of 1 ml/kg) 20 min before each conditioning session. Rats in Groups L-V and N-V received an equivalent injection of the saline vehicle at this time. In order to equate the experience of the various groups, all rats received a further injection 4 hr later, with Groups L-D and N-D receiving saline and Groups L-V and N-V receiving 8-OH-DPAT.

The measure of conditioning was a corrected score, obtained by subtracting the total number of responses made by a given subject during all the pre-CS periods in each session from the total number of responses made during all the CS periods. This gave a measure of the degree to which CS presentation elevated the rate of food-tray entry over that occurring in the absence of the CS. A significance level of $p < .05$ was adopted in all the analyses that follow.

Results

The group mean corrected response rates, pooled over all trials for each session of training, are presented in Figure 1. All four groups showed acquisition, in that the rate of response increased across sessions, but they did so at different rates, depending both on the nature of the CS and the drug treatment. Conditioning occurred readily in both control groups (i.e., in those injected with the vehicle), with those trained with the localized CS showing a consistent advantage over those trained with the diffuse CS. No such difference was evident in subjects that were given the drug. Those trained with the diffuse CS learned almost as well as the controls, whereas those trained with the localized CS showed a marked deficit, with only a marginal increase in rate of response from the first to the last session of training.

This description of the data was supported by an analysis of variance with treatment (drug or vehicle), CS type (tray light or overhead light) and session (1–4) as the variables. This revealed a significant main effect of session, $F(3, 132) = 22.79$, confirming that the rate of responding increased over the course of training. There was also a significant main effect of drug treatment, $F(1, 44) = 8.31$, and of CS type, $F(1, 44) = 4.54$, and, critically, a significant interaction between these two factors, $F(1, 44) = 5.31$. Nothing else was significant, largest $F(3, 132) = 1.77$. Analysis of this significant interaction with a simple main effects analysis revealed a significant main effect of the drug in subjects condi-
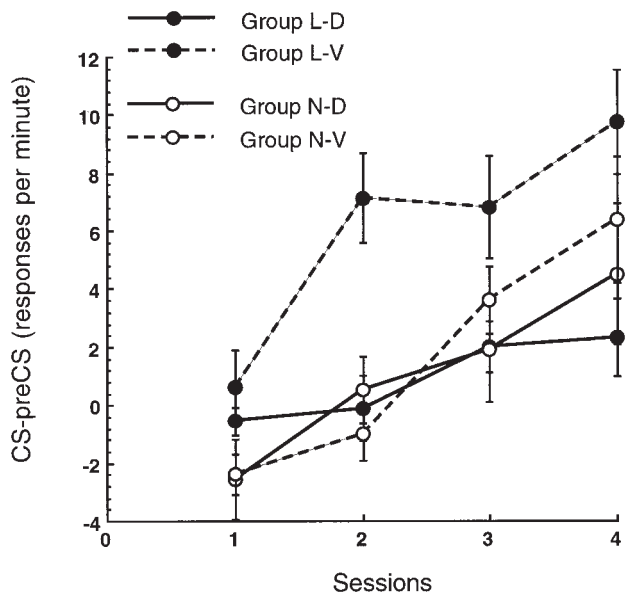
Figure 1. Group mean corrected response rates (± *SEM*) for the four conditioning sessions. Group L-D = rats using a localized conditioned stimulus (CS) and given a presession injection of 8-OH-DPAT; Group L-V = rats using a localized CS and given an injection of vehicle only; Group N-D = rats using a nonlocalized CS and given a presession injection of 8-OH-DPAT; Group N-V = rats using a nonlocalized CS and given an injection of vehicle only.

tioned to the localized cue, $F(1, 44) = 13.45$, but not in those trained with the overhead light ($F < 1$).

The groups showed very similar levels of response in the absence of the CS, suggesting that the reported effects were not a consequence of differences in general levels of responsiveness. The mean pre-CS rates, pooled over all pre-CS periods, were 2.87 responses per minute for Group L-D, 3.05 per minute for Group L-V, 2.50 per minute for Group N-D, and 2.27 per minute for Group N-V. An analysis of variance with type of CS (tray light or overhead light) and treatment (drug or vehicle) as factors revealed no significant effects or interactions, largest $F(1, 44) = 1.05$.

Although these results suggest that 8-OH-DPAT selectively impaired learning about the localized cue, it is important to note that this substance may also produce what has been called the serotonergic syndrome (Hjörth & Carlsson, 1982)—a set of motor disturbances that can include hyperactivity, forepaw treading, tremor, and head shaking. A failure of rats treated with 8-OH-DPAT to perform the CR in our training situation could, in principle, reflect dysfunction in the motor system rather than an effect on the associative learning process. Even though we observed no obvious behavioral effects, this does not eliminate the possibility that subtle motor deficits were interfering with the CR. There are, however, a number of reasons to reject such an interpretation. First, in the present experiment we showed that rats treated with 8-OH-DPAT displayed a deficit in conditioning with the localized CS but not with the diffuse CS. It is difficult to see how a nonspecific motor dysfunction could produce such selectivity. The second comes from a further study, conducted in our laboratory, that treated two groups of subjects identically to

Groups L-D and L-V of the present experiment. After acquisition training, both groups received two further test sessions in which CS–US presentations continued as before. On the first of these tests, which immediately followed the last day of acquisition, half the subjects in each group received an injection of 8-OH-DPAT 20 min before the session, while the remainder received vehicle. On the second test session, those that had received the drug now received the vehicle and vice versa. The resulting data are shown in Figure 2. The left panel shows response in Groups L-D and L-V when they were tested under the same (S) conditions as those in which they were trained (i.e., Group L-D with the drug and Group L-V without), and the right panel shows response in these two groups when they were tested under the alternative (A) condition (i.e., Group L-D without the drug and Group L-V with the drug). In the S test, subjects in Group L-D responded less than those in Group L-V, consistent with the idea that the drug impaired acquisition of the CR. However, it is also consistent with the possibility that this difference was no more than a performance effect—that the presence of the drug produced a motor deficit that prevented subjects from performing the CR. The data from the A test speak against this possibility. If the effect seen in the S test were no more than a deficit in performance, then the effect should be reversed in the A test, such that the group experiencing the drug (Group L-V) should now respond less than the group that is drug free (Group L-D). But if the effect seen in the S test was really the result of an effect on learning, then Group L-D should continue to show less of a CR than Group L-V in the A test. It is clear that Group L-D responded less than Group L-V in both tests, consistent with the proposal that the effect in the S test indeed reflected an acquisition deficit. This description was supported by the results of an analysis of variance with Group (L-D or L-V) and test (S or A) as factors,
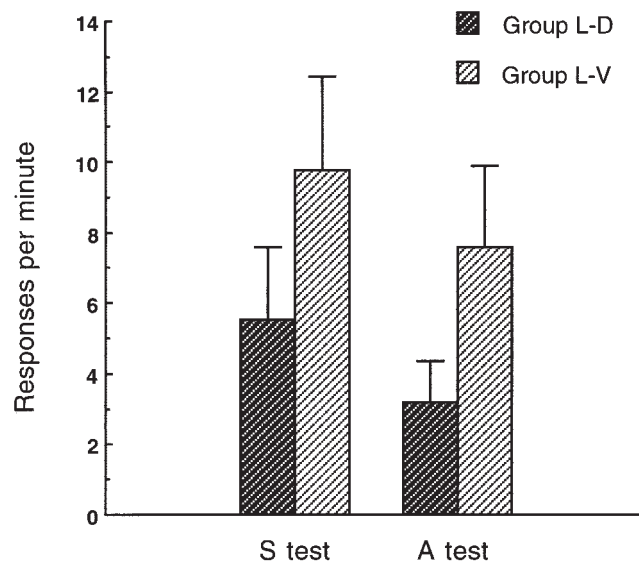

Figure 2. Group mean corrected response rates (± *SEM*) for the two test sessions of the pilot study. The left panel shows responding during the same-condition (S) test, and the right panel responding during the alternative-condition (A) test. Group L-D = rats using a localized conditioned stimulus (CS) and given a presession injection of 8-OH-DPAT; Group L-V = rats using a localized CS and given an injection of vehicle only.

which revealed a significant main effect of group, $F(1, 14) = 5.39$, but no effect of test, or Group $\times$ Test interaction ($Fs < 1$).

Discussion

The results of this experiment showed that pretraining administration of 8-OH-DPAT produced an impairment of appetitive Pavlovian conditioned responding in animals trained with a localized light as the CS. No deficit was seen in animals trained with a general increase in illumination as the CS. This effect is unlikely to be due to the motor effects that the drug is known to produce—an inability to perform the CR would be evident with both types of CS. We conclude, therefore, that the drug can act to retard conditioning but does so only when a localized cue is used as the CS. This result exactly parallels that obtained by Bonardi (2001) in a study of the effect of hippocampal lesions on appetitive conditioning. It is therefore consistent with the suggestion that systemic injections of 8-OH-DPAT modify cognitive functioning (at least in part) through an effect on the 5-HT$_{1A}$ receptors of the hippocampus. Although it might be seen as paradoxical that a 5-HT agonist could mimic the effects of a hippocampal lesion, such effects are not without precedent (Yasuno et al., 2003) and could perhaps be explained if receptor activation were to have a net inhibitory effect. It must be acknowledged, though, that our results provide only indirect evidence that our effect was mediated by 5-HT$_{1A}$ receptors in the hippocampus rather than elsewhere. In the absence of more direct evidence for hippocampal mediation of the effect, our conclusions must remain tentative.

But why should the effects of 8-OH-DPAT, and of hippocampal lesions, be selective to the type of CS used? One possible explanation appeals to the proposal that a normally functioning hippocampus is required for the formation or maintenance of short-term memory traces (e.g., Honey & Good, 2000; Rawlins, 1985), based on the observation that although many cases of simple conditioning are unaffected by hippocampal lesions, a disruption is obtained with the trace-conditioning procedure, in which an interval intervenes between presentation of the CS and delivery of the US (e.g., James, Hardiman, & Yeo, 1987; Solomon, Vander Schaaf, Thompson, & Weisz, 1986). Bonardi (2001) pointed out that even when no trace interval is explicitly programmed (as in the conditioning procedures used here), there could still be some delay between the CS as experienced by the subject and US occurrence and that the length of this delay will depend on the nature of the CS. A diffuse CS (like a change in the general level of illumination) will necessarily impinge on the rat throughout its presentation, and the rat will thus be exposed to this event at the time food is delivered. But a localized CS (like the tray light) will be perceived only when the rat orients toward it, and a freely moving rat will likely sample such a cue only from time to time during the formal period of its presentation. With this cue, therefore, there will often be an interval between the receipt of the US and the last occasion on which the CS was sampled, effectively establishing a trace-conditioning procedure and making it susceptible to effects of treatments that disrupt short-term memory.

This analysis prompts the suggestion that 5-HT$_{1A}$ receptors play a role in maintaining short-term memory traces. In the absence of further evidence it would be premature to take this speculation further—it is enough to note that the present results provide further evidence for the notion that treatment with 8-OH-DPAT selec-tively disrupts those instances of learning that depend on the normal functioning of hippocampal mechanisms.

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