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SHORT COMMUNICATION

Posttrial Administration of Cholinergic Drugs does not Affect Consummatory Successive Negative Contrast in Rats*

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Posttraining administration of cholinergic drugs modulates the consolidation of memory processes in several learning tasks. We studied the effect of the administration of atropine (cholinergic antagonist, Experiment 1) and physostigmine (acetylcholinesterase inhibitor, Experiment 2) immediately after the first session of reward downshift, and immediately after the last preshift session (Experiment 3) on a consummatory successive negative contrast procedure. Animals were given access to a high-value reward (32% sucrose solution), and surprisingly shifted to a low-value reward (4% sucrose solution) in a second phase. The results indicate that atropine and physostigmine have no effects on contrast. The role of cholinergic neurotransmission in the memory of surprising reward changes is discussed.

There is a growing body of evidence indicating that some drugs administered immediately after training influence memory consolidation processes. In general, inhibitors of acetylcholinesterase, like physostigmine, and nicotinic or muscarinic

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agonists facilitate the storage of the information and the consolidation processes, whereas antagonists, like atropine and scopolamine, induce amnesia (Baratti et al., 1979; Baratti & Kopf, 1996). For example, the posttraining administration of physostigmine and oxotremorine enhance memory in a passive avoidance task in mice (Taylor, 1990), while the administration of scopolamine and oxytocine (negative modulators of cholinergic neurotransmission) produce deficits on acquisition and extinction of the same response (Prado-Alcalá et al., 1994; Boccia & Baratti, 2000).

When animals that receive a high-value reward in a first phase suddenly receive a low-value reward, their instrumental or consummatory behaviors is transiently diminished in comparison to controls that always receive the low reward. This effect, called successive negative contrast (SNC) effect, has been observed in instrumental paradigms (e.g., running speed in a runway, iSNC) and in consummatory procedures (e.g., consumption of sucrose solutions, cSNC; see Flaherty, 1996). It has been postulated that the effects of surprising reward downshifts are equivalent to those of aversive stimulus administration (e.g., Gray, 1987, 1998).

There are only a few reports in the literature about the effect of cholinergic drugs on SNC. Salinas et al. (1997) showed, using an instrumental runway procedure, that intraamygdala oxotremorine infusions immediately after the first session of reward downshift, extended the amount of sessions in which the contrast effect was evident. However, the effects of cholinergic drugs on consummatory SNC (cSNC) are quite different. Flaherty and Meinrath (1979) have shown that systemic administration of scopolamine does not affect cSNC. Importantly, in this study the drug was administered 20 min before the first shift session, and not immediately after the change of reward as in Salinas et al.'s (1997) study. The divergent results between these two studies might be due to differences in the timing of drug administration, in the conditioning paradigm (i.e., instrumental vs. consummatory), or both.

The aim of the present work was to study the potential involvement of cholinergic neurotransmission in memory of reward changes in the cSNC paradigm. Two experiments explored the effects of atropine (cholinergic antagonist, Experiment 1), and physostigmine (acetylcholinesterase inhibitor, Experiment 2), administered immediately after the first postshift trial. In Experiment 3, the same drugs were administered immediately after the last preshift trial.

Experiment 1

Method

Subjects. The subjects were 48 adult male Wistar rats, all experimentally naive and approximately 90 days old at the beginning of the experiment. The average ad libitum weight for the rats used in this experiment was 266 g. They were housed in individual cages two weeks before the experiment and exposed to a 12:12 h cycle of light:darkness (lights on from 06:00 to 18:00 h). Along this period, the daily amount of food was gradually reduced until weights were lowered to an 85% of individual ad libitum values.

Apparatus. Rats received training in 4 similar MED Associates conditioning boxes enclosed in a sound-attenuating cubicle that provided masking white noise. Each box measured 24.1 cm in

length, 29.2 cm in width, and 21 cm in height. The floor was made of aluminum bars 0.4 cm in diameter and separated by gaps measuring 1.1 cm. In the center of one of the lateral walls there was a squared, 5 cm hole, 3.5 cm deep, and 1 cm above the floor level in which a sipper tube (introduced from the outside) protruded 2 cm. Goal tracking was measured by detecting the insertion of the head into the hole by means of photocells. A diffuse house light was located above the sipper tube, 18 cm above the floor.

Two acrylic barriers (20 x 18 x 1 cm, length x height x thickness) were inserted into the boxes, at 5 cm from each lateral wall. One of the barriers was put against the back wall and the other against the frontwall of the box, describing a zigzag path. All animals began each session from the opposite site of the sipper tube in the zigzag path. The reason for introducing these barriers was to record the latency time to consumption; previous unpublished research conducted in our laboratory had shown that this procedure could be used to measure latency. The purpose of this procedure was to introduce an instrumental (anticipatory) measure (e.g., Salinas et al., 1997) to explore a potential dissociation between consummatory and instrumental responses (Flaherty & Caprio, 1976).

Procedure. Rats were matched according to their weight and each pair member was then randomly assigned to one of two groups: 32-4 (n = 26) and 4-4 (n = 22). A single trial per day was administered throughout the experiment.

One day prior to the beginning of training, rats were preexposed during 20 min to sucrose solution, according to their later experimental condition, into their individual cages in the vivarium. Preshift started on the following day and lasted for 12 daily trials, during which animals received 5 min of access to either a 32% or a 4% sucrose solution (w/v, weight/volume). The 32% and 4% solutions were prepared by mixing 32 g or 4 g, respectively, of commercial sugar with tap water until reaching 100 ml of solution. A computer recorded the time a rat spent with their head inserted into the drinking hole (goal tracking time), measured with a 0.01-s accuracy. There were 8 postshift trials. During postshift trials, all the animals received access to the 4% sucrose solution. At the end of trial 12, the animals were matched according to their mean goal tracking time during the last three trials of preshift training. Each pair member was randomly assigned to one of four groups depending on the dose of atropine: 0 (saline), 0.1, 1, or 3 mg/kg. Groups were labeled 32-4/3 (n = 6), 32-4/1 (n = 7), 32-4/0.1 (n = 7), 32-4/0 (n = 6), 4-4/3 (n = 6), 4-4/1 (n = 5), 4-4/0.1 (n = 6), and 4-4/0 (n = 5). Injections were administered intraperitoneally (i.p.) immediately after trial 13 (i.e., first postshift trial). The complete experiment took a total of 20 daily trials of training.

The dependent measure was the goal tracking time, in seconds. Previous studies showed that this measure correlates positively and significantly with the amount of solution consumed by the animals (Mustaca, Freidin, & Papini, 2002). Furthermore, the latency time to consumption was also recorded in seconds. Data were subjected to analysis of variance. In all the statistical results reported in this paper, the alpha value was set at the 0.05 level.

Results and Discussion

Goal tracking times of Groups 32-4 were higher and increased more rapidly than in Groups 4-4 in the preshift phase. An analysis of variance made on acquisition data with Sucrose (32%, 4%) and Trial (1 to 12) as factors (the latter as a repeated-measure factor), revealed the following effects. Groups 32% performed significantly above Groups 4%, $F(1, 46) = 11.20$; and there was a significant acquisition effect, $F(11, 506) = 60.22$. The interaction between factors was not significant, $F < 1$. Another analysis of variance computed on the data from trials 10 to 12 revealed that Groups 32% performed significantly below Groups 4%, $F(1, 46) = 7.47$. No significant trial effect was found, $F(2, 92) = 2.17$. The interaction was not significant, $F < 1$.

Figure 1 shows the mean goal tracking time of the last preshift trial and each

postshift trial. During postshift trials, goal tracking times dropped substantially for the groups switched from 32% to 4% solution. This cSNC effect was followed by recovery of performance; within eight trials, the level of all the groups was approximately the same.

An overall mixed model analysis of the postshift performance with Contrast (32-4%, 4-4%), Drug (atropine 0, 0.1, 1, or 3 mg/kg), and Trial (13 to 20) as factors indicated the following effects. There was a significant contrast effect, $F(1, 40) = 7.88$, indicating that goal tracking scores were lower for groups shifted from 32% to 4% solution than for unshifted controls. There was a significant main effect for trial, $F(7, 280) = 21.87$. The contrast by trial interaction was also significant, $F(7, 280) = 9.03$, reflecting the decreasing differences between groups across postshift trials. But neither the drug nor the drug by trial interaction were significant, Fs < 1.17. Separate analysis for each postshift trial indicated significant contrast effects for trials 13, 14, and 15, Fs$(1, 40) > 10.57$.

A similar general analysis was computed on latency time to consumption. However, nonsignificant differences were found for the main factors and their interactions, Fs < 1.60.

These results indicate that the administration of atropine had no effects on cSNC under the present conditions, using a range of concentrations that proved effective in other training situations (e.g., instrumental runway procedure, Salinas et al, 1997), and using the same timing for drug administration (immediately after the first session of reward downshift). These findings suggest that atropine may not modulate the aversive memory of the experienced shift solution or interfere with the memory of the preferred reinforcement (32%) evoked by contextual stimuli. Therefore, the present results are not in agreement with those reported in the literature indicating that atropine interferes with memory consolidation.

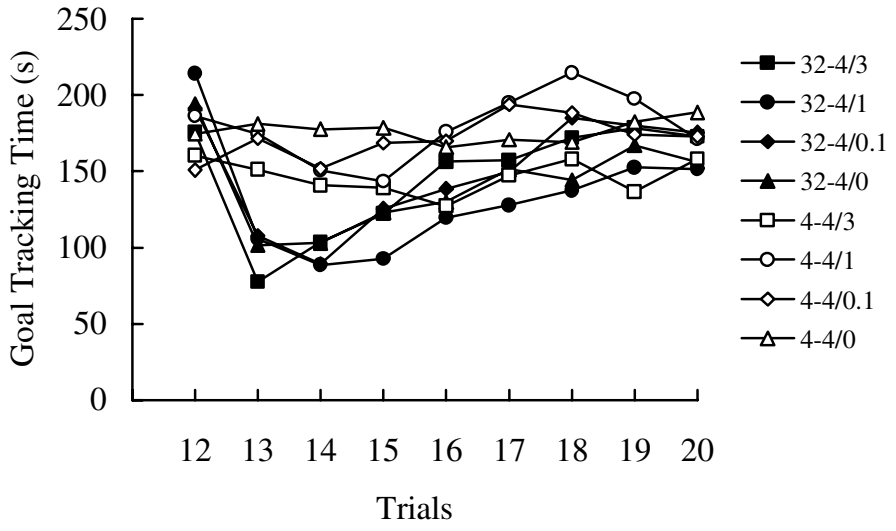

Figure 1. Mean goal tracking time in shifted (34-4) and unshifted (4-4) rats to different sucrose solutions. Animals were injected with atropine (0, 0.1, 1, or 3 mg/kg) after the first postshift trial.

Experiment 2

Method

Subjects and Apparatus. The subjects were 69 adult male Wistar rats, with a mean ad libitum weight of 284 g. The experimental conditions and the apparatus were as described in Experiment 1.

Procedure. The procedure was similar to that of Experiment 1, except that animals were injected with physostigmine (i.p.): 0 (saline), 50, 250, or 500 µg/kg. Groups were labeled 32-4/500 (n = 10), 32-4/250 (n = 11), 32-4/50 (n = 11), 32-4/0 (n = 9), 4-4/500 (n = 7), 4-4/250 (n = 8), 4-4/50 (n = 6), and 4-4/0 (n = 7).

Results and Discussion

Goal tracking times of Groups 32-4 were higher than in Groups 4-4 on preshift trials. An analysis of variance with Sucrose (32%, 4%) and Trial (1 to 12) as factors, revealed the following effects. The Groups 32% performed significantly above the Groups 4%, $F(1, 66) = 24.44$. There was a significant acquisition effect, $F(11, 726) = 94.98$. There was also a significant interaction between contrast and trial, $F(11,726) = 2.88$. An analysis of variance made on the goal tracking times from trials 10 to 12 revealed that Groups 32% performed significantly below Groups 4%, $F(1, 67) = 9.15$. There was a significant effect for trial, $F(2, 134) = 3.35$. The interaction between factors was not significant, $F < 1$.

Figure 2 shows the mean goal tracking time of the last preshift trial and each postshift trial. As in Experiment 1, performance dropped abruptly for the groups switched from 32% to 4% solution, and then recovered gradually. An overall analysis of the postshift performance with Contrast (32-4%, 4-4%), Drug (physostigmine 0, 50, 250, 500 µg/kg), and Trial (13 to 20) as factors showed the following effects. There were significant effects of contrast $F(1,60) = 8.97$, trials $F(7,420) = 19.90$, and of the contrast by trial interaction, $F(7,420) = 16.01$. Importantly, neither the drug effect nor the trials by drug interaction were significant, Fs < 1. Separate analyses for each postshift trial showed significant contrast effects for trials 13, 14, and 15, Fs$(1, 61) > 18.06$.

The analysis of latency time to consumption indicated nonsignificant differences in all the main factors and their interactions, Fs < 1.62.

These findings indicate that the administration of several doses of physostigmine does not affect cSNC under these conditions. Unlike in Flaherty and Meinrath's (1979) study, the drug was administered immediately after the change of reward, and not 20 min before the first shift session. Thus, physostigmine has no effects on cSNC independently of the moment of drug administration (before or after the reward downshift). Additionally, the present results failed to support the hypothesis that physostigmine facilitates memory consolidation of the preferred reward, as suggested by previous works from other training situations (Micheau, Destrade, & Jaffard, 1985).

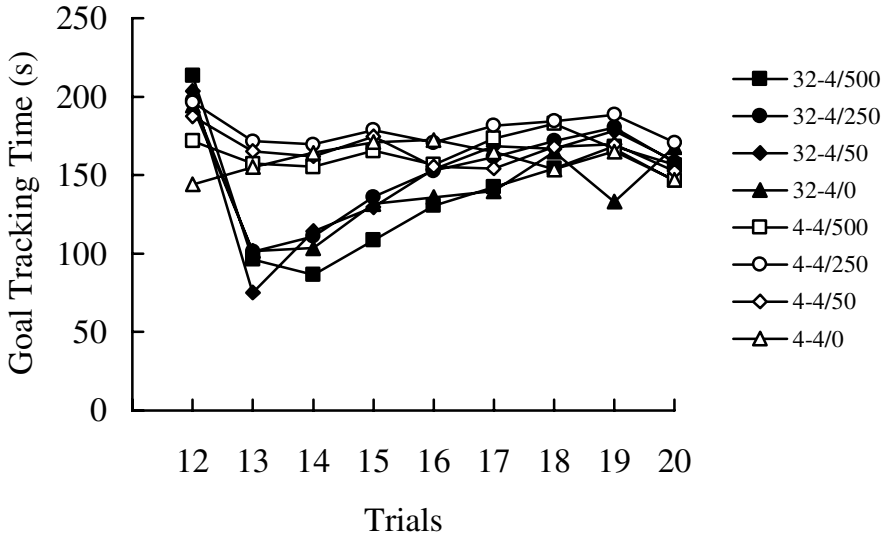

Figure 2: Mean goal tracking time in shifted (34-4) and unshifted (4-4) rats to different sucrose solutions. Animals were injected with physostigmine (0, 50, 250, or 500 µg/kg) after the first postshift trial.

Experiment 3

Method

Subjects and Apparatus. The subjects were 45 adult male Wistar rats, with a mean ad libitum weight of 335 g. Other experimental conditions and the apparatus were as described in Experiment 1.

Procedure. Training parameters used in this experiment were similar to those used in the previous experiments, except that: (1) animals were injected (i.p.) immediately after the last preshift trial with atropine (3 mg/kg), physostigmine (500 µg/kg), or saline; and (2) there were 10 preshift trials and 5 postshift trials. Groups were labeled 32-4/A (n = 8), 32-4/P (n = 8), 32-4/S (n = 7), 4-4/A (n = 8), 4-4/P (n = 7), and 4-4/S (n = 7), depending on the concentration of the sucrose solution received in preshift and postshift trials (32%, 4%) and the drug administered (atropine, A; physostigmine, P; or saline, S).

Results and Discussion

As in previous experiments, goal tracking times of Groups 32-4 were higher than in Groups 4-4 during preshift trials. An analysis of variance made on preshift data revealed significant effects for sucrose, $F(1, 43) = 7.53$, and for trial, $F(9, 387) = 26.88$. There was also a significant contrast by trial interaction, $F(9, 387) = 2.58$. An independent analysis made on data from trials 8 to 10 revealed a significant effect for trial, $F(2, 86) = 3.82$, but not for the other factors, Fs < 1.22.

As in Experiments 1 and 2, goal tracking times dropped substantially for the groups switched from 32% to 4% solution during postshift trials, showing a solid

cSNC effect that was followed by a gradual recovery of performance. An overall analysis of postshift performance with Contrast (32-4%, 4-4%), Drug (atropine, physostigmine, or saline), and Trial (11 to 15) as factors indicated the following effects. There was a significant contrast effect $F(1, 39) = 15.90$, indicating that goal tracking scores were lower for groups shifted from 32% to 4% solution than for unshifted controls. There was also a significant effect for trial $F(4, 156) = 5.41$. The main effect for drug and the drug by trial interaction were nonsignificant, Fs < 1. Separate analyses for each postshift trial indicated significant contrast effects for trials 11 to 15, $Fs(1, 39) > 4.69$.

The analysis of latency time to consumption showed nonsignificant differences for all the main factors and their interactions, Fs < 1.

These results suggest that shifted rats consumed less sucrose solution than control rats independently from the drug schedule received. Furthermore, these findings are in agreement with those found above using the same drugs, but administered immediately after the first postshift trial.

General Discussion

The present results indicate that the posttrial administration of atropine and physostigmine has no effects on cSNC. The administration of these drugs immediately after the last training session with the preferred reward or immediately after the reward shift produced no reliable effects on performance. This is a surprising result since several experimental procedures with well-trained animals exposed to the learning environment and subsequently treated with cholinergic drugs showed: (1) amnesia in a subsequent retention test after the injection of anticholinergic drugs, such as atropine (e.g., Solana-Figueroa & Prado-Alcalá, 1990); or (2) enhanced working memory and general performance with a positive modulator of cholinergic transmission, such as physostigmine (Ordy et al., 1988). Furthermore, there is general agreement that when memories are evoked, they are in an active state and they are labile and susceptible to disruption or enhancement by memory modulators (Sara, 2000).

The present results also suggest that the distinction between cSNC and iSNC may be important to understand differences in the effectiveness of cholinergic drugs to modulate behavior following downshifts in reward value. For example, this dissociation is present in terms of more general processes: (1) agonistic behavior is shown after instrumental surprising reward omissions (Papini & Dudley, 1997), but not after a consummatory situation (Mustaca & Martinez, 2000; Mustaca, Martinez, & Papini, 2000); (2) extinction of consummatory behavior is slower with large reinforcer magnitudes and more frequent reinforcement (Mustaca, Freidin, & Papini, 2002), while these factors have the opposite effect on instrumental training situations (e.g., Hulse, 1958); and (3) reinstatement of a latency response in a consummatory extinction procedure is larger with less acquisition sessions (Freidin, Trejo, & Mustaca, 2004), but have an inverse relationship in instrumental situations (e.g., Ison, 1962).

Finally, a similar dissociation between consummatory and instrumental phe-

nomena is also apparent in contrast training situations. Flaherty and Caprio (1976) demonstrated that rats running for 32% sucrose solution failed to show iSNC after a shift to 4% solution in terms of running latency (an instrumental response) but, once in the goal box, they consumed significantly less 4% solution than control rats always reinforced with 4% solution (a cSNC effect). Such a dissociation of cSNC and iSNC effects suggests that these response systems are differentially sensitive to the effects of surprising reward shifts. Another source of evidence regarding a dissociation between iSNC and cSNC is provided by studies involving brain lesions. For example, lesions of the hippocampus and nucleus accumbens affect iSNC, but not cSNC (Flaherty, Coppotelli, Hsu, & Otto, 1998; Leszczuk & Flaherty, 2000).

All together, these findings suggest that cholinergic neurotransmission is not involved in the consolidation of reward memories and the modulation of the consummatory response to a surprising change of reward in a contrast procedure.

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