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Posttraining handling facilitates memory for auditory-cue fear conditioning in rats

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Abstract

A large number of studies have indicated that stress exposure or the administration of stress hormones and other neuroactive drugs immediately after a learning experience modulates the consolidation of long-term memory. However, there has been little investigation into how arousal induced by handling of the animals in order to administer these drugs affects memory. Therefore, the present study examined whether the posttraining injection or handling procedure per se affects memory of auditory-cue classical fear conditioning. Male Sprague–Dawley rats, which had been pre-handled on three days for 1 min each prior to conditioning, received three pairings of a single-frequency auditory stimulus and footshock, followed immediately by either a subcutaneous injection of a vehicle solution or brief handling without injection. A control group was placed back into their home cages without receiving any posttraining treatment. Retention was tested 24 h later in a novel chamber and suppression of ongoing motor behavior during a 10-s presentation of the auditory-cue served as the measure of conditioned fear. Animals that received posttraining injection or handling did not differ from each other but showed significantly less stimulus-induced movement compared to the non-handled control group. These findings thus indicate that the posttraining injection or handling procedure is sufficiently arousing or stressful to facilitate memory consolidation of auditory-cue classical fear conditioning.

Keywords: Auditory fear conditioning; Emotion; Learning; Memory consolidation

Extensive evidence indicates that exposure of animals or humans to either a physical or psychological stressor immediately after a learning experience can influence memory consolidation (Cahill, Gorski, & Le, 2003; Holahan & White, 2002; Jodar, Takahashi, & Kaneto, 1996; Liu, Tsuji, Takeda, Takada, & Matsumiya, 1999). The effects likely depend on the activation of an arousal state or stress response that strengthens memory consolidation processes in a retrograde fashion. Such findings are consistent with extensive evidence indicating that posttraining administration of stress hormones, such as epinephrine and corticosterone, enhances memory consolidation on a variety of emotionally arousing learning tasks (Buchanan & Lovallo, 2001; Cordero & Sandi, 1998; Gold & van Buskirk, 1975; Liang, Juler, & McGaugh, 1986; McGaugh & Roozendaal, 2002; Roozendaal & McGaugh, 1996; Sandi & Rose, 1997). It has also been reported that posttraining systemic administration of corticosterone enhances the consolidation of memory of auditory-cue classical fear conditioning (Hui et al., 2004; Zorawski & Killcross, 2002). Importantly, even though systemic or local drug administration requires handling of the animal and acute handling is known to be a mild stressor that induces the release of stress hormones as well as the stimulation of stress-activated neurotransmitters (de Boer, Koopmans, Slangen, & van der Gugten, 1990), little attention has been given to determine the role of this posttraining handling as an experimental variable that might influence memory consolidation. Therefore, in this study we investigated the effect

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of a posttraining subcutaneous injection of a vehicle solution or handling for a brief period without injection on memory for auditory-cue classical fear conditioning.

Adult male Sprague–Dawley rats ($n = 52$; 343 ± 67 g at time of training) from Charles River Laboratories (Wilmington, MA) were individually housed in a temperature-controlled (22 °C) vivarium on a standard 12/12-h light/dark cycle (lights on at 07:00 h) and given food and water ad libitum. Training and testing were performed between 10:00 and 15:00 h. All experimental procedures were performed in compliance with NIH guidelines and were approved by the University of California, Irvine's Institutional Animal Care and Use Committee.

Rats were initially habituated to human contact by placing them on the experimenter's lap and lightly stroking them on three consecutive days for a period of 1 min. On the day of conditioning, 24 h after the last handling session, they were transported to the laboratory and placed in a quiet room (resting room) for 1 h. The home cages were covered with a mantle to reduce contextual cues before taking them to the conditioning room. Rats were placed in the conditioning chamber within a larger acoustically dampened isolation chamber (Coulbourn Instruments, Allentown, PA; model #E10-16SC modified into a $51 \times 29 \times 25.5$ cm single chamber). The floor of the chamber consisted of 4.8-mm diameter steel rods spaced 18.0 mm apart, wired to a precision-regulated shock generator (Coulbourn model #E13-14) for the delivery of scrambled footshock. A calibrated open-field speaker and tone generator (Coulbourn model #E69-20) delivered the auditory stimulus. A small houselight provided indirect ambient light. After an acclimation period of 4 min in this chamber, the subjects were given three trials consisting of a single-frequency tone (6.0 kHz, 70 dB, 5 s) as the conditioned stimulus (CS) co-terminating with a mild footshock (.5 mA, 1 s, 40 Hz bipolar pulse) as the unconditioned stimulus (US). The interval between onsets of the CS and US was 4 s and the intertrial interval was about 4 min. The rats were removed from the conditioning chamber immediately after the last tone–shock pairing and placed inside their home cages, which were covered again, before returning them to the resting room. The rats immediately received different treatments according to one of the three groups to which they were randomly assigned. Rats from the injection group ($n = 19$) were gently picked up from their home cages and placed on the experimenter's lap and a vehicle solution (5% ethanol in 95% saline, 2.0 ml/kg of body weight) was injected subcutaneously in the nape of the neck within 30 s of being placed back in their home cages. This control solution was selected on the basis of a previous experiment in which it served as the vehicle for corticosterone administration (Hui et al., 2004). The handled group ($n = 18$) underwent the same handling procedure except that no injection was given. The control group ($n = 15$) did not receive any posttraining treatment. After the treatment, the rats remained in the resting room for 1 h before being returned to the vivarium.

After 24 h, retention was tested in a novel chamber that had different dimensions than the conditioning chamber ($29 \times 29 \times 24$ cm, Coulbourn model #E10-10). The floor of the chamber consisted of 6.4-mm diameter steel rods spaced 17.4 mm apart, placed within a small sound-proof isolation cabinet in the same experimental room. The chamber contained small objects and toys (e.g., wooden blocks, rubber and fuzzy balls, plastic tubing, etc.) to facilitate the rat's natural tendency to explore and to further differentiate it from the conditioning chamber. The testing chamber was equipped with an infrared activity monitor (Coulbourn model #E24-61), tone generator (Coulbourn model #E69-20), calibrated open-field speaker, and a small houselight to provide indirect ambient light. Approximately 3 min after the subject was placed in the test chamber, the rat was given a 10-s presentation of a single CS (6.0 kHz, 70 dB). The Coulbourn *WinLinc* program recorded and quantified movement detection units in consecutive 1-s bins during the testing phase. Movement during the testing phase was quantified for the 10 s immediately preceding the tone and the 10 s during the tone presentation. Subjects that ceased their exploration of the chamber prior to the time of tone presentation were eliminated from further analysis because assessment of to-be-elicited reduction of movement could not be determined in a background of no movement. To ascertain whether learning had occurred, paired t tests were used to compare the two time periods within each individual. One-way ANOVAs were used to determine differences between the three experimental groups both before and during CS presentation. Post hoc analyses with Fisher's PLSD were used to detect differences between groups. A probability level of less than .05 was considered statistically significant for all comparisons.

Fig. 1 illustrates the effects of the different posttraining treatments on motor activity before and during tone presentation, 24 h after conditioning. The injected, handled, and non-handled groups did not differ in mean movement during the 10-s period immediately prior to the presentation of the CS ($F_{2,49} = 1.92$, $p = .16$; Fig. 1A). These findings indicate that the handling/injection procedure did not induce any generalized fear or non-specific effects on locomotor activity 24 h after conditioning. Furthermore, all groups showed a significant reduction of movement during tone presentation compared to the time period immediately preceding CS presentation (paired t tests: all $p \leqslant .0001$), indicating that all three groups had acquired the task. However, one-way ANOVA for movement during tone presentation revealed a significant group effect ($F_{2,49} = 3.58$, $p = .04$; Fig. 1B). Post hoc analysis indicated that the injected and handled groups showed significantly less movement during tone presentation than the non-handled group ($p = .029$ and $p = .018$, respectively). The injected and handled groups did not differ significantly from each other in movement during tone presentation ($p = .81$).

As many findings indicate that acute handling affects endocrine and neural mechanisms (de Boer et al., 1990; Nilsson, Kalén, Rosengren, & Björklund, 1990; Stein, Hiller, & Simon, 1992; Thiel, Huston, & Schwarting, 1998), this study investigated the effect of handling stress as an experimental variable in influencing memory consolidation.

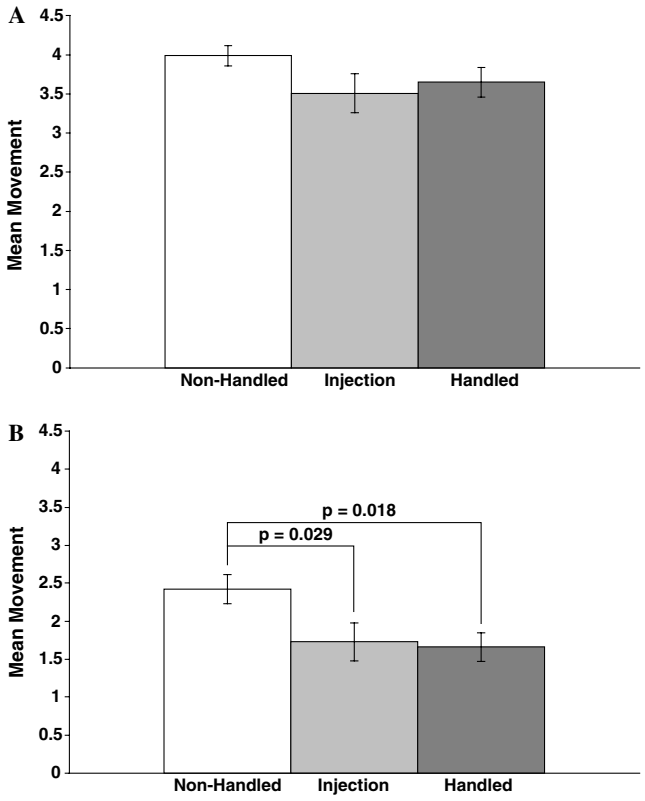

Fig. 1. Mean movement (±SEM) in number of beam breaks/second measured immediately before (A) or during (B) the 10-s presentation of the conditioned auditory stimulus 24 h after conditioning. Rats had received a subcutaneous injection of a vehicle solution ($n = 19$), were handled for a brief period without injection ($n = 18$), or were left undisturbed (non-handled; $n = 15$) immediately after the last of three tone–shock pairings. Both the injected and handled groups showed significantly less movement during the presentation of the tone than the non-handled group.

Our finding that the posttraining injection or handling procedure facilitated memory of auditory-cue classical fear conditioning indicates that even a minimal stressor is capable of influencing memory consolidation. Furthermore, the finding that both the injected and handled groups exhibited a greater conditioned responding than the non-handled group indicates that the act of injecting an animal per se is not necessary for facilitation of memory. Obviously, such findings also exclude the possibility that under these conditions the low concentration of ethanol in the vehicle solution had influenced memory consolidation. All groups showed a reduction of movement during tone presentation, indicating that an association between tone and shock was acquired. In a previous study (Hui et al., 2004), we found that conditioning was specific to the pairing of the tone and footshock, as the tone did not elicit conditioned suppression of movement on a retention test in animals that had received unpaired presentations of tone and shock, or tone or shock alone, on the training session.

The present findings may be of significance as handling stress seems an inherent part of experimental drug administration. However, as rats readily habituate to repeated handling, the efficacy of the posttraining handling procedure to influence memory consolidation likely depends on the amount of pre-handling. In the present study, animals received moderate pre-handling (i.e., 3 times 1 min) but more extensive handling prior to training may thus attenuate the arousal induced by posttraining handling. Furthermore, emotional arousal associated with the handling or injection procedure may shift or even reverse drug effects on memory consolidation, as extensive evidence indicates that the effect of posttraining drug treatments on memory consolidation depends not only on the dose administered but that this interacts with the level of stress or arousal induced by the training procedure (Gold, van Buskirk, & Haycock, 1977).

Several early findings have indicated that exposing animals to different stressors such as tail shock, water deprivation, or swim stress shortly before classical conditioning enhances conditioned responses such as eye blink (Berry & Swain, 1989; Shors, Weiss, & Thompson, 1992) or heart rate (Wilson, Wilson, & DiCara, 1975). Our finding that *posttraining* handling or injection can influence memory of auditory-cue fear conditioning suggests an effect on memory consolidation, not confounded by possible effects on attentional, motivational or sensory-perceptual mechanisms at the time of conditioning, or test. These findings are consistent with our previous report that posttraining corticosterone administration facilitates memory consolidation of auditory-cue fear conditioning (Hui et al., 2004) and are thus very similar to a wealth of data finding enhancement of memory consolidation with posttraining stress exposure or drug administration on other emotionally arousing tasks, including inhibitory avoidance (McGaugh & Roozendaal, 2002; Roozendaal, 2000).

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