Submitted to: Integrative Physiological and Behavioral Science

Stress Effects on Conditioning: Learning or Performance?

David A. Townsend, Tracey J. Shors

Rutgers University
Department of Psychology and Center for Collaborative Neuroscience

Running Head: STRESS EFFECTS ON CONDITIONING

Acknowledgments: This work was supported by NIMH (59970 and 59740), National Alliance for Research on Schizophrenia and Depression (NARSAD).

Correspondence should be addressed to: D.A. Townsend, Dept. of Psychology, Center for Collaborative Neuroscience, 152 Frelinghuysen Road, Rutgers University, Piscataway, NJ 08854-8020 or townsend@rci.rutgers.edu

ABSTRACT:

Stressful experience can have numerous effects on learning processes, enhancements, impairments, or no effect at all. In previous studies, we found that exposure to an acute stressor of either inescapable tailshocks or swim stress enhances classical conditioning of an eyeblink response in male rats (Shors et al., 1990; Shors 2001). Here we evaluated the effects of acute stress on this task as well as trace fear conditioning, contextual fear conditioning and sensory preconditioning. Exposure to the acute stressor of tailshocks increased trace eyeblink conditioning but appeared to reduce fear conditioning as measured by an increase in movement during trace fear conditioning and sensory preconditioning compared to unstressed controls. However, since the stressor and the unconditioned stimulus (US) both consisted of shocks, these effects are likely attributable to US preexposure or extinction. This conclusion is supported by data indicating that acute swim stress did not alter performance in the fear conditioning tasks. Together, these data suggest that exposure to an acute stressful experience does not necessarily alter learning, especially that which involves fear conditioning procedures.

INTRODUCTION:

Exposure to stressful and fear-provoking events can impact an animal's subsequent ability to learn. There exists a sizable literature substantiating this phenomenon in laboratory animals, especially in the male rat. A search of stress and animal learning yields nearly 10,000 reports in the last twenty years. The degrees of stress invoked by such events are numerous and varied; extending from slight environmental changes (Shors, 2002; Shors, 1998) to catastrophic physiological insults such as global cerebral ischemia (Nelson, Lebessi, Sowubski, P, & Hodges, 1997). A variety of stressors have likewise been employed including handling, warm and cold water swim stress, shallow and deep water swim stress, restraint, restraint and tail shock, footshock, noise, immunological insults, chemical interventions, exposure to familiar and unfamiliar conspecifics, and prenatal manipulations (Nishimura, Endo, & Kimura, 1999; Kaneto, 1997; Paris, Lorens, Van de Kar, & Urban, 1989; Luine, Martinez, Villegas, McEwen & Magarinos,1996; Beylin & Shors, 2002; Beylin & Shors, 1998a; Servatius & Shors, 1996; Nelson et al., 1997; Windle et al., 1997; Shors, 2001; Kusnecov & Rabin, 1993; Bauer, Perks, Lightman, & Shanks, 2001; Tonkiss J. 2001; Shors & Wood, 1995; Rimondini, Agren, Borjessin, Sommer, & Heileg, 2003). Additionally, the types of behaviors and conditioning procedures used to assess the effects of stressor exposure are numerous, including heart rate conditioning, passive avoidance learning, fear-enhanced startle responses, escape during operant conditioning, spatial navigation in a water maze task, social conflict, fear conditioning, radial arm maze performance, immunological responses, gastric responses, FMRI responses, and conditioned motor responses such as the eyeblink (Wilson, Wilson, & Dicara, 1975; Holscher, 1999; Kaneto, 1997; Kusnecov et al., 1993; Maier, 1990; Overmier & Murison,2000; Servatius et al.,1994; Buchel, Dolan, Armory, & Frestong, 1999; LeDoux, 1997; Beylin et al., 2002; Shors, 2002; Wood, Young, Reagan, & McEwen, 2003). Given the variety of stressors and behavioral measurements, it is understandable that this literature has numerous inconsistencies and minimal consensus.

In our laboratory we have demonstrated that exposure to an acute stressful event enhances association formation during classical conditioning. This enhancement occurs during training on hippocampal-independent or dependent versions of an eyeblink conditioning task (Shors, Foy, Levine, & Thompson, 1990; Beylin & Shors, 1998a; Beylin & Shors, 1998b). Exposure to the stressor does not enhance responding in animals that have already learned this task or alter the magnitude of the unconditioned response (Shors, 2001). Thus, the data to date suggest that the effects of stress on eyeblink conditioning involve learning and are not result of alterations in

performance or sensitivity to the unconditioned stimulus (US) (Shors et al., 2000). One question that arises from these findings is whether the enhancing effect of stressful experience on learning is unique to classical eyeblink conditioning or whether it would emerge during other training situations and learning opportunities. In a set of experiments presented here, we evaluated the effects of exposure to an acute stressful event of either brief intermittent tailshocks or swim stress delivered either before or after conditioning, on four common animal learning tasks. The tasks we used included trace eyeblink conditioning, trace fear conditioning, contextual fear conditioning and sensory preconditioning. The common denominator among these tasks is their dependence on the hippocampus (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; McEwen, 1994; Beylin et al., 2002; Talk, Gandhi, & Matzel, 2003). Thus, an additional objective was to determine whether there is a common effect of acute stressful experience on learning across tasks that engage the hippocampus.

Experiment 1: *Effects of stress on trace fear versus trace eyeblink conditioning:*

During classical eyeblink conditioning, a white noise conditional stimulus (CS) foreruns and predicts the occurrence of an aversive unconditional stimulus (US) which is stimulation to the eyelid. The stimulation elicits an eyeblink that serves as the unconditional response (UR). After repeated pairings of the CS followed by the US, the white noise itself comes to elicit an eyeblink, which is the conditioned response (CR). Fear conditioning is similar in that a white noise predicts the occurrence of a shock although the shock is delivered to the paws through a grid floor. The CR is measured as a decrease in movement in response to the CS and during the trace interval, which is an approximation of the fear response (Domjan, 1998; Anagnostaras, Maren, & Fanselow, 1999a). For both eyeblink and fear conditioning, the CS and US were separated by a trace interval (500 ms for the eyeblink task and 30 sec for the fear task). Acquisition of these responses using a trace paradigm is dependent on an intact hippocampus (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Beylin et al., 2001, Solomon et al., 1986; Shors, Townsend, Zhou, Kozorovitskiy, & Gould, 2002). During fear conditioning with a discrete CS such as the white noise, the animal also acquired fear about the context. This type of conditioning is known as contextual fear conditioning and some reports find it hippocampal-dependent (Maren & Fanselow, 1997); Anagnostaras et al., 1999a).

In the first experiment, we evaluated the effects of acute stress on classical eyeblink conditioning and fear conditioning using trace and contextual fear training paradigms. Groups were

exposed to brief intermittent tailshocks (30, 1 mA, 1/min) or left in their home cage. 24 hrs later, they were trained with either pairings of a white noise CS followed by an eyelid stimulation US or a white noise CS followed by a footshock US. In an attempt to achieve differing levels of fear conditioning, rats were exposed to either one or ten trials of training; the amount of fear acquired during training was assessed 24 hrs later as a decrease in movement in response to the white noise CS and during the trace interval. As the final part of this experiment, we examined the effects of stressor exposure *after* training with the fear conditioning paradigm.

Experiment 1 Methods:

Subjects.

For all experiments, Sprague-Dawley rats (220-250g) were individually housed in hanging wire cages and left undisturbed for one week following arrival at the animal facility. They had unlimited access to water and chow (Ralston-Purina, St. Louis, MO.) and were maintained on a 12:12 light: dark cycle with light onset at 0700. Testing and training occurred between 0900 and 1800.

Stressor exposure

Immediately before or after training, stressed rats were restrained in a Plexiglas cylinder and exposed to 30, 1 sec, 1 mA, 60 Hz shocks to the tail. They were then returned to their home cages or transported to the conditioning environment.

Eyeblink Conditioning

Rats were anesthetized with pentobarbital (40 mg/kg). Four electrodes (insulated silver wire, 0.05 in diameter) were implanted through the ridge of the eyelid, two to detect eyeblinks by transmitting electromyographic activity from obicularis oculi and two to deliver a periorbital shock of .07 mV. Electrodes were attached to a headstage implanted to the rats' skull. Postoperatively, rats were injected with 0.3 ml of penicillin (250,000 units/ml; Apothecon) intramuscularly and provided access to acetaminophen (IDE Interstate, Amityville, NY) diluted 1:100 in drinking water. After 5 days of recovery, rats were taken from their home cages and exposed to the conditioning environment. The conditioning environment consists of ten conditioning boxes each surrounded by a soundproof chamber. Within each chamber is a lighted box with gray metallic walls and floor grids (not connected to shock). Rats were acclimated to the conditioning chambers for a time equaling 100 trials (to be described) and returned to their home cage.

The next day, rats were returned to the conditioning chamber where spontaneous blink rate and eyeblink responses to 10 white noise alone trials were measured. Then they were exposed to 300 trials of trace eyeblink conditioning each day for two days. A 250-ms white noise burst (CS) of 82-dB preceded a 0.7- mV, 100-ms periorbital shock (US) separated by a 500-ms trace interval. Ten trials consisted of one CS alone trial, four paired CS/US trials, one US alone trial, followed by four paired CS/US trials. The CS had a 5ms rise-fall time and intertrial interval was randomized between 10-20s.

Eyelid EMG was bandpass filtered at 0.3-1.o kHz, amplified (10K) with a differential amplifier, and passed to a 16-bit A/D card (Keithley-Metrabyte, Tautin, MA). On paired trials, eyeblinks were considered CRs if they occurred 250 ms after white noise onset but prior to US administration (250-750 ms after CS onset). During CS alone trials, an eyeblink between 250 and 1,000 ms after white noise onset is considered a conditioned response. Eyelid EMG responses were counted as eyeblinks when they exceeded the pre-CS baseline responses' maximum EMG value plus four times the standard deviation.

Fear Conditioning

On the first day of training rats were transported to the conditioning room which consisted of four conditioning boxes each surrounded by a soundproof chamber. Within each chamber is a 12 in by 9 freeze monitor box (SD Instruments, San Diego, CA.) similar to a Skinner box. The freeze monitor boxes have a metallic grid floor (connected to shock) and clear Plexiglas sides and removable lid. Vertical and horizontal movements of animals were recorded by a photo beam activity system (SD Instruments, San Diego, CA.) and stored in 15s bins. On the walls of each enclosing soundproof chamber are detachable panels with 3 inch alternating black and white lines. Detachable panel lines were configured in a horizontal pattern or a vertical pattern. Light was provided to the chambers by 4 overhead florescent lamps. The door to each chamber remained open 3 inches for ambient light to enter (40 Lux). Rats were not presented with any stimuli during the first 6 minutes in the apparatus. Rats that were stressed before fear conditioning were acclimated to the conditioning environment 24h prior to conditioning whereas those stressed after fear conditioning were not acclimated. The apparatus was cleaned between trials.

Rats were exposed to ten or one conditioning trials with an intertrial interval of 240 s ($\pm$ 30s). A white noise CS (82 dB, 15 sec, 5msec rise/fall time) was followed by a 30 second trace interval prior to the US footshock (500msec, 1 mV). After training, rats were returned to their home

cages or exposed to stressor depending on group. The next day (24h later), rats were placed in the same conditioning chamber for a 300 second period, during which no stimuli were presented. Movement during this period was measured to assess fear to the context. Rats were returned to their home cages for at least 60 min and placed in a novel testing chamber. If a subject was trained in a box with horizontal lines, it was tested in a box with vertical lines. Other changes to the context included: grid floors were replaced with black Plexiglas floors (tactile), different scents for cleaning, luminosity in the conditioning chamber either matched the brightness during training and acclimation or was dimmed(visual), animals were removed from home cages and placed in conditioning chambers in a different order than training, the experimenter wore different type and color gloves and lab coat, and animals were transported to the conditioning room by a different route than during training and disoriented by turning the transport cage three times .

Rats were placed in the novel chambers and allowed a three minute acclimation period. Locomotion and rearing activity were measured to detect movements. A baseline response was measured for three min. Ten CS alone trials were delivered and movement during the CS and trace interval was measured.

Experiment 1 Results

Stress before training:

As shown previously, exposure to the stressor 24 hrs before eyeblink conditioning increased the number of CRs emitted during the trace interval during eyeblink conditioning [$F(1,8) = 11.62$; $p < .05$] (see figure 2B). For fear conditioning, stressor exposure was associated with increased movements during trace interval relative to the unstressed controls after either one or ten trials of training [$F(1, 28) = 9.82$; $p < .05$]. Movements during the trace interval were enhanced in animals stressed before one trial [$F(1,22) = 4.83$; $p < .05$] (see figure 1B) and before 10 trials [$F(1,20) = 7.03$; $p < .05$]. Stress exposure prior to trace fear conditioning did not alter movements in the training context and thus did not affect contextual fear conditioning.

Stress after training:

Movement was not affected by exposure to the stress of 30 intermittent tailshocks after 10 trials of trace fear conditioning [$F(1,21) = 5..24$; $p < .05$] but enhanced movement after only one trial of trace fear conditioning. Exposure to the stressor after fear conditioning did not alter movements in the training context ($p>0.05$).

Experiment 1 Discussion:

Data from the present experiments indicate that exposure to a stressful event of brief intermittent tailshock has different effects on conditioning of an eyeblink versus a fear response. As shown previously, exposure to the stressor increased the number of CRs emitted during trace eyeblink conditioning. In contrast, exposure to the same stressful stimulus either before or after training on a fear conditioning protocol impaired performance of CR. These data suggest that exposure to an acute stressful event does not necessarily induce enhancing effects on conditioning, even though they are both dependent on an intact hippocampal formation. What might be the reasons for differing effects of stress on eyeblink versus fear conditioning? One possibility is that fear conditioning is in and of itself stressful (and more stressful than eyeblink conditioning). Thus, stressor exposure may somehow interact with the stressful quality of the training experience to induce a negative impact on fear conditioning as opposed to the enhancing effect observed during eyeblink conditioning. More likely is the possibility that the effect of stressor exposure on fear conditioning is confounded by the use of shock during both stressor exposure and during fear training. Although shock is also used as the US during eyeblink conditioning, it is much less aversive and only sufficient for eyelid closure. The effects of exposure to the inescapable tailshocks prior to the footshock used for fear conditioning effectively pre-exposed the rat to the US, thus reducing the fear associated to the CS later when they are "trained." When the tailshocks were delivered after training, they may have acted to extinguish the fear acquired during conditioning. Therefore, in the next set of experiments, rats were exposed to a very different type of stress, 20 minutes of swim stress, which has been shown to enhance classical eyeblink conditioning in a manner indistinguishable from the intermittent tailshock stressor (Shors, 2001; Servatius and Shors, 1996).

Experiment 2 Methods:

Stressor exposure

Immediately before or after training on the fear conditioning paradigm, rats were placed in a container of room temperature water (22 degree C) for twenty minutes. Afterwards, rats were dried and returned to their home cages or transported to the conditioning environment. Unstressed rats remained in their home cages. All groups were exposed to 10 trace trials of the CS and US footshock either before or after stressor exposure and tested 24h later.

Experiment 2 Results:

Exposure to swim stressor did not alter fear conditioning as measured by a decrease in movement during the trace interval when stress was administered before [F (1,10) = .12;n.s.] or after [F(1,13) =2.75;n.s.] fear training (see figure 3B). Swim stress, before or after training, did not alter expression of fear associated with the training context ($p>0.05$). There was no effect on contextual fear associated with the training context using an examination of all fear conditioning stress paradigms ($p>0.05$).

Experiment 2 Discussion:

Here we examined the effects of swim stress on fear conditioning and there was none. These data are consistent with our hypothesis that the detrimental effect of tailshock stress on fear conditioning is due to a preexposure or extinction, depending on whether the stressor was delivered before or after training. Thus, unlike eyeblink responding, in which both intermittent tail shock and swim stress facilitate learning, stressor exposure itself does not enhance conditioning of a fear response. Although our data may be consistent with preexposure or extinction effects, it is also possible that these are simply performance deficits after tailshock stress. To address this issue, we next evaluated the effects of acute stressor exposure on sensory preconditioning. In this way, we could evaluate the effects of stressor exposure on forming an association that is not directly dependent on exposure to the shock stimulus.

Experiment 3 Sensory Preconditioning:

During sensory preconditioning, two neutral stimuli are paired and one stimulus is later paired with a separate, distinct stimulus (US). Usually, the initial pairing is between two relatively innocuous stimuli such as tones and the second pairing involves an aversive stimulus such as a footshock (Miller & Barnet,1993 ; Matzel, Schachtman, & Miller,1988 ; Matzel, Shuster, & Miller, 1987)(Domjan, 1998). Since the initial association does not directly involve shock, it was anticipated that the results from these studies would distinguish between the effects of stress on performance versus those on learning. Also, as with trace eyeblink and fear conditioning, learning the preconditioned association is dependent on an intact hippocampus (Talk, 2002). Rats were exposed to the stressor of intermittent tailshocks or swim stress prior to sensory preconditioning (Before) or after sensory preconditioning but prior to fear conditioning (After). Additional groups

were not stressed but exposed to sensory preconditioning, either as described or in an explicitly unpaired manner.

Experiment 3 Methods:

Sensory Preconditioning

The sensory preconditioning fear paradigm was a 3 day procedure. On the first day, rats are acclimated to the conditioning chamber for 6 min and then exposed to 10 pairings of a 15s white noise stimuli (82 dB, 15 sec, 5msec rise/fall time) followed immediately by a 15s tone (82 dB, 5msec rise/fall time) with an ITI of 240s ($\pm$ 30s). Additional groups were exposed to random presentations of the tone and white noise with the stipulation that no stimuli be presented more than twice in a row and the ITI was $\pm$ 105s. Rats exposed to paired and unpaired stimuli were trained for the same period of time (session length) and numbers of stimuli. After preconditioning with the S1 and S2 stimuli, rats were returned to their home cages. 24 hrs alter, they were trained with 10 trials of fear conditioning stimuli as before using a CS tone and footshock US. All rats were returned to their home cages.

24h later, animals were tested in novel contexts to fear associated with the white noise (S1) and tone (S2). After a 6 minute acclimation period in the conditioning boxes animals received 5 presentations of white noise alone with an ISI of 240s ($\pm$ 30s). After S1 testing, animals were returned to their home cages. Four hours later, animals were tested for fear associated with the tone. After a 6 minute acclimation period, rats received 5 presentations of tone alone with an ISI of 240s ($\pm$ 30s). The context used during S2 testing was varied from the S1 testing context with a stainless steel floor, different scented cleaning solution, differing experimenter clothing, and novel route to chambers. Vertical stripes in the conditioning chambers were replaced by 2 solid black and 2 solid white walls. Either immediately before or after sensory preconditioning, rats were exposed to the stressor of tailshocks or swim stress.

Experiment 3 Results:

Groups that received paired and unpaired sensory preconditioning did not differ in the fear associated with the tone that had been paired with shock; both groups reduced their movements in a novel context when exposed to tone (see figure 4B). However, the animals that received unpaired sensory preconditioning group differed significantly from those that received paired sensory preconditioning in movement reduction to the S1 white noise stimuli (the preconditioned

association) [$F(1,14) = 4.78$; $p < .05$]. Those exposed to paired stimuli during the first association expressed more fear to the first neutral stimulus than those exposed to unpaired stimuli. Thus, sensory preconditioning using a trace fear paradigm does support learning about the first association (S1-S2).

Stress before sensory preconditioning:

Tailshock stress before sensory preconditioning did not effect responding to either the fear or preconditioned conditioned stimuli. Furthermore, swim stress after SPC had no effect on conditioned responding (see figure 5B). During training, no rats developed conditioned responding to either conditioning stimulus (tone, white noise) during auditory stimulus onset throughout testing (data no shown).

Stress after sensory preconditioning:

Rats that were exposed to the stressor after sensory preconditioning (S1-S2 pairing) but prior to fear conditioning (S2-shock) did not reduce movements in response to the first stimulus (S1) as much as the unstressed controls [$F(1,16) = 5.07$; $p < .05$]. Their movements were also ~ 200% greater than the animals that received stress before SPC (see figure 4). Rats that were exposed to the tailshock stressor after sensory preconditioning were also impaired when tested to fear associated with the tone 24 h subsequent to $S2^+$ training. Rats stressed after SPC, increased their movements by >300% compared to the subjects stressed before sensory preconditioning and >400% compared to those that were unstressed.

Experiment 3 Discussion:

Data from the present experiment indicate that exposure to an acute stressor of intermittent tail shocks or swim stress prior to sensory preconditioning neither impaired nor facilitated learning of the relationship between white noise (S1) and tone (S2). That is, rats that were exposed to the stressors prior to sensory preconditioning performed in a similar manner to unstressed controls. These data suggest that exposure to an acute stressful event does not alter sensory preconditioning as stress before eyeblink conditioning does. The present data are relevant to those from the first experiment in which rats were exposed to intermittent tail shock and then fear conditioning 24 hrs later. Recall that they expressed a deficit in performing the fear conditioned response. In this experiment, sensory preconditioned animals stressed before preconditioning had a learning

opportunity after stressor exposure (the S1-S2 pairings) and before fear conditioning. It may be that exposure to these stimuli after stressor exposure prevented the performance deficit that occurred in experiment 1 when the animals were stressed and then immediately fear conditioned.

Animals that receive intermittent tail shocks after SPC but before fear conditioning showed reduced responding to S1 and S2. As before, it was hypothesized that exposure to the 30 tail shocks followed by tone/shock pairings impaired performance via US pre-exposure rather than by affecting association formation directly. Simply put, the tone was not a good predictor of aversive stimuli onset. This explanation also addresses why responding to the white noise preconditioned CS was reduced. Since no fear developed to the tone (S2) during fear conditioning, the CS was ineffective as an associative stimulus for the other neutral stimulus used during sensory preconditioning. This interpretation is supported by the fact the animals that received a swim stressor were not affected during sensory preconditioning at any stage of testing (figure 5).

The data presented so far suggest that exposure to an acute stressful event does not necessarily enhance conditioning during tasks that engage the hippocampus. It should be noted that in many of the fear conditioning experiments, animals expressed considerable fear as measured by the decrease in movement during the trace interval. This was even the case after exposure to just one pairing of the CS and the US. Thus, we may be observing a ceiling effect which would preclude observing an enhancement after stressor exposure.

General Discussion:

As a whole, these findings indicate that exposure to an acute stressor of intermittent tailshocks enhances the acquisition of a classical conditioned eyeblink response in male rats but has no enhancing effect on learning during trace or contextual fear conditioning. Exposure to an inescapable tailshock stress did impair performance during trace fear conditioning, but the effects appear to be due to US preexposure in the case of stress before training and extinction in the case of stress after training. This conclusion was drawn since exposure to a stressor with no shock involved (swim stress) had no effect on fear conditioning. Previous studies had shown that swim stress does enhance eyeblink conditioning and certainly elicits very high corticosterone concentrations in the blood, higher even than exposure to the tailshock stressor (Paris, Lorens, Van de Kar, & Urban, 1989)(Shors, 2001a).

In the final experiment, we examined the effects of tailshock and swim stress on sensory preconditioning. Sensory preconditioning involved learning an association between two stimuli,

neither which was a shock stimulus. Exposure to the intermittent tail shock stressor before sensory preconditioning training did not alter learning of the first association although it did slightly impair the later fear conditioning. It seems likely that a US pre-exposure effect of intermittent tail shock in rats that received tail shock *after* sensory preconditioning but *before* fear conditioning again induced a deficit in conditioned responding to the tone (S2), just as US pre exposure did to the white noise CS in experiment 1. Together, these data suggesting that the decrease in conditioned responding or increase in movement is simply a result of poor learning during the fear conditioning procedures as a results of tailshock exposure. This is supported by the final experiment in which swim stress had no effect whatsoever on sensory preconditioning.

Data presented here suggest that exposure to an acute stress of intermittent tailshock does indeed alter fear conditioning. However, the effects are not ones involving stress, per se, but rather US pre-exposure, generalization, or extinction effects. These responses do of course involve learning but might not necessarily be interpreted as "stress effects on learning." One of the reasons for conducting these studies was to evaluate the generality of the stress effect on conditioning. As noted and demonstrated here, acute stress does enhance eyeblink conditioning in male rats. Also, there are numerous reports in the literature that exposure to stressors of various sorts enhancing fear conditioning. For example, restraint alone stress before training can facilitate both contextual and cued fear conditioning when administered chronically (Conrad, Magarinos, LeDoux, & McEwen, 1999.). Acute restraint stress has also facilitated contextual fear conditioning (Cordero, Venero, Kruyt, Sandi, 2003), although the stress was administered for 2 hours. Here stress did not enhance contextual fear, however, we acutely stressed rats for a ½ hour session and used restraint paired with tailshock or swim stress. Whether or not reported stress effects on conditioning represent "true" learning effects is debatable based on the data described here. These data would suggest some caution in making broad generalizations concerning how stress affects conditioning when differing behavioral paradigm and stressors are utilized.

Reference List

Anagnostaras,S.G., Maren,S., DeCola,J.P., Lane,N.I., Gale,G.D., Schlinger,B.A., & Fanselow,M.S. (1998). Testicular hormones do not regulate sexually dimorphic Pavlovian fear conditioning or perforant-path long-term potentiation in adult male rats. *Behavioral Brain Research,* 92(1), 1-9.

Anagnostaras,S.G., Gale,G.D., & Fanselow,M.S. (2001). Hippocampus and Contextual Fear Conditioning: Recent Controveries and Advance. *Hippocampus,* 11, 8-17.

Anagnostaras, S.G., Sheena, J.A., Frankland, P.W., & Silva, A.J. (2000) Computer Assisted Behavioral Assessment of Pavlovain Fear conditioned Mice. *Learning & Memory* 7, 58-72.

Anagnostaras,S.G., Maren,S., & Fanselow,M.S. (1999). Temporarily graded retrograde amnesia of contextual fear after hippocampal damage in rats: Within-subjects examination. *Journal of Neuroscience,* 19(3), 1106-1114.

Bauer,M.E., Perks,P., Lightman,S.L., & Shanks,N. (2001). Restraint stress is associated with changes in glucocorticoid immunoregulation. *Physiology & Behavior,* 73(4), 525-532.

Beylin,A.V., & Shors,T.J. (1998a). Stress enhances excitatory trace eyeblink conditioning and opposes acquisition of inhibitory conditioning. *Behavioral Neuroscience,* 112(6), 1327-1338.

Beylin,A.V., & Shors,T.J. (1998b). Stress-induced facilitation of trace eyeblink conditioning is dependent on the presence of glucocorticoids. *Behavioral Neuroscience,* 24, 173-180.

Beylin,A.V., & Shors,T.J. (2002). Glucocorticoids are necessary for enhancing memory

Buchel,C., Dolan,R.J., Armory,J.L., & Frestong,K.J. (1999). Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. *Journal of Neuroscience,* 19, 10869-10876.

Conrad, C. D. (1999) Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioral Neuroscience,* 113, 902-918.

Cordero MI, Venero C, Kruyt ND, Sandi C. (2003). Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Evidence for a role of corticosterone. *Hormones and Behavior* 44, 338-345.

Domjan,M. (1998). *The Principles Of Learning And Memory*. (4 ed.). Pacific Grove: Brooks/Cole Publishing.

Holscher,C. (1999). Stress impairs performance in spatial water maze learning tasks. *Behavioral Brain Research,* 100 (1), 225-235.

Kaneto,H. (1997). Learning/memory processes under stress conditions. *Behavioural Brain Research,* 83(1-2), 71-74.

Kusnecov,A.W., & Rabin,B.S. (1993). Inescapable footshock exposure differentially alters antigen- and mitogen-stimulated spleen cell proliferation in rats. *Journal of Neuroimmunology,* 44(1), 33-42.

Ledoux, J. (1997). The amygdala: contributions to fear and stress. *Seminars in the Neurosciences,* 6, 231-237.

Luine, V.N., Martinez,C., Villegas,M., Margarinos,A.M., McEwen,B.S.(1996). Restraint stress reversibly enhances spatial memory performance. *Physiology and Behavior*,59, 27-32

Maier,S.F. (1990). Role of fear in mediating shuttle escape learning deficit produced by inescapable shock. *Journal of Experimental Psychology: Animal Behavior Processes,* 16, 137-149.

Maren,S., & Fanselow,M.S. (1997). Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiology of Learning & Memory,* 67(2), 142-149.

Matzel, Louis D., Schachtman, Todd R., & Miller, Ralph R.(1988). Learned irrelevance exceeds the sum of CS-preexposure and US-preexposure deficits. *Animal Behavior Processes.* Vol 14(3), 311-319.

Matzel, Louis D., Shuster, Karl, & Miller, Ralph R.(1987). Covariation in conditioned response strength between stimuli trained in compound. *Animal Learning & Behavior*. Vol 15(4), 439-447.

McEchron,M.D., Bouwmeester,H., Tseng,W., Weiss,C., & Disterhoft,J.F. (1998). Hippocampectomy disrupts auditory trace fear conditioning and contextual fear conditioning in the rat. *Hippocampus,* 8(6), 638-646.

McEwen,B.S. (1994). The plasticity of the hippocampus is the reason for its vulnerability. *The Neurosciences,* 6, 239-246.

McEwen,B.S. (1999). Stress and hippocampal plasticity. *Annual Review of Neuroscience,* 22, 105-122.

Miller, R. & Barnet, R.(1993). The role of time in elementary associations. *Current Directions in Psychological Science*. Vol 2(4) , 106-111.

Nelson,A., Lebessi,L., Sowubski, P, & Hodges,H. (1997). Comparison of effects of global cerebral ischemia spatial learning in the standard and radial water maze. *Behavioral Brain Research,* 85, 93-115.

Nishimura,J.I., Endo,Y., & Kimura,F. (1999). A long-term stress exposure impairs maze learning performance in rats. *Neuroscience Letters,* 273(2), 125-128.

Overmier, J. B.& Murison, R.(2000). Anxiety and helplessness in the face of stress predisposes, precipitates, and sustains gastric ulceration. *Behavioural Brain Research* 110, 161-174.

Paris, J. M. , Lorens, Van de Kar, & Urban (1989) A comparison of acute stress paradigms: Hormonal responses and hypothalamic serotonin. *Physiology & Behavior*. Vol 39(1), 33-43.

Rimondini,R., Agren,G., Borjessin,S., Sommer,W., & Heileg,M. (2003). Persistent behavioral and autonomic supersensitivity to stress following prenatal stress exposure in rats. *Behavioural Brain Research.,* 140(1-2), 75-80.

Servatius,R.J., & Shors,T.J. (1996). Early acquisition but not retention of the classically conditioned eyeblink response is N-methyl-d-aspartate (NMDA) receptor dependent. *Behavioral Neuroscience*, 110, 1040-1048.

Servatius, R. J, Ottenweller, J., Bergen, M., & Soldan, S. (1994). Persistent stress-induced sensitization of adrenocortical and startle responses. *Physiological Behavior* 56(5), 945-954.

Shors,T.J., Seib,T.B., Levine,S., & Thompson,R.F. (1989). Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science*, 244.

Shors,T.J. (1998). Stress and sex effects on associative learning: For better or for worse. *The Neuroscientist*, 4, 353-364.

Shors,T.J. (1999). Acute stress and re-exposure to the stressful context suppress spontaneous unit activity in the basolateral amygdala via NMDA receptor activation. *Neuroreport*, 10, 2811-2815.

Shors,T.J. (2001a). Acute stress rapidly and persistently enhances memory formation in the male rat. *Neurobiology of Learning and Memory*, 75, 10-29.

Shors,T.J. (2001b). The modulation of memory formation by stressful experience and sex differences in the brain. In H. E. Steinmetz, M. A. Gluck, & P. R. Solomon (Eds.), *Model Systems and the Neurobiology of Associative Learning*. (pp. 331-360). Mahwah: Erlbaum Associates.

Shors,T.J. (2002). Opposite effects of stressful experience on memory formation in males versus females. *Dialogues in Clinical Neuroscience*, 4, 39-47.

Shors,T.J., Beylin,A.V., Wood,G.E., & Gould,E. (2000). The modulation of Pavlovian memory. *Behavioral Brain Research*, 110, 39-52.

Shors,T.J., & Dryver,E. (1992). Effects of stress and long-term potentiation (LTP) on subsequent LTP and the theta burst response in the dentate gyrus. *Brain Research*, 666, 232-238.

Shors,T.J., Foy,M.R., Levine,S., & Thompson,J.K. (1990). Unpredicatable and uncontrollable stress impairs neuronal plasticity in the rat hippocampus. *Brain Research Bulletin*, 24, 663-667.

Shors,T.J., & Wood,G.E. (1995). Contribution of stress and gender to exploratory preferences for familiar versus unfamiliar conspecifics. *Physiology and Behavior*, 58, 995-1002.

Shors, T.J. (2001). Acute stress rapidly and persistently enhances memory formation in the male rat. *Neurobiology of Learning & Memory*, 75(1),163-171.

Shors,T.J., Townsend,D.A., Zhou,M., Kozorovitskiy,Y., & Gould,E. (2002). Neurogenesis May Relate to Some but not All Types of Hippocampal-Dependent Learning. *Hippocampus*, 12, 578-584.

Solomon,P.R., van der Schaaf,E.R., Thompson,R.F., & Weisz,D. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience*, 100, 729-744.

Talk,A.C., Gandhi,C.C., & Matzel,L.D. (2003). Hippocampal Function during behaviorally silent associative learning: Dissociation of memory storage and expression. *Hippocampus*, 12, 648-656.

Tonkiss J., Almieda S. S. and Galler J. R (2001). Prenatally malnourished female but not male rats show increased sensitivty to MK-801 in a differential reinforcement of low rates task. *Behavioral Pharmocolog,y* 9, 49-60.

Wilson,L.M., Wilson,J.R., & Dicara,L.V. (1975). Facilitation of Pavlovian conditioned cardiodecelerations following preshock in immobilized rats. *Physiology and Behavior*, 15, 653-658.

Windle,R.J., Wood,S., Shanks,N., Perks,P., Conde,G.L., da Costa,A.P.C., Ingram,C.D., & Lightman,S.L. (1997). Endocrine and behavioral responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity. *Journal of Neuroendocrinology*, 9, 407-414.

Wolfer,DP., Stagljar-Bozicevic,M., Errington,M.L., & Lipp,HP. (1998). Spatial Memory and Learning: Fact or Artifact? News Physiology *Science,* 13, 118-123.

Wood,G.E., Young,T., Reagan,L., & McEwen,B. (2003). Acute and Chronic restraint stress alter the incidence of social conflict in male rats. *Hormones and Behavior,* 43, 205-213.