Physiology & Behavior xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Repeated stressor exposure enhances contextual fear memory in a beta-adrenergic receptor-dependent process and increases impulsivity in a non-beta receptor-dependent fashion

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HIGHLIGHTS

- Changes in associative learning following repeated stressor exposure
- Repeat stress increases contextual fear memory processing.
- Beta-receptor blockade prevents exaggerated freezing in contextual fear conditioning.
- Stress reduces latencies in passive avoidance test suggesting increased impulsivity.

ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 18 February 2015
Accepted 4 March 2015
Available online xxxx

Keywords:
Chronic mild stress
Norepinephrine
Propranolol
Associative learning
Contextual fear conditioning
Passive avoidance

ABSTRACT

Memory formation is promoted by stress via the release of norepinephrine and stimulation of beta-adrenergic receptors (β-ARs). Previous data demonstrate that repeated stressor exposure increases norepinephrine turnover and β-AR signaling within the amygdala, which led to the hypothesis that some stress-induced behavioral changes are likely due to facilitated associative learning. To test this, Fischer rats were exposed to chronic mild stress for four days. On day 5, subjects (including non-stressed controls) were injected with the beta-blocker propranolol or vehicle prior to conditioning in an operant box (animals receive two mild foot shocks) or passive avoidance apparatus (animals received a foot shock upon entry into the dark chamber). Twenty-four hours later, subjects were returned to the operant box for measurement of freezing or returned to the passive avoidance apparatus for measurement of latency to enter the dark chamber. Subjects were also tested in an open field to assess context-independent anxiety-like behavior. Animals exposed to chronic stress showed significantly more freezing behavior in the operant box than did controls, and this exaggerated freezing was blocked by propranolol during the conditioning trial. There was no effect of stress on behavior in the open field. Unexpectedly, retention latency was significantly reduced in subjects exposed to chronic stress. These results indicate that chronic exposure to stress results in complex behavioral changes. While repeated stress appears to enhance the formation of fearful memories, it also results in behavioral responses that resemble impulsive behaviors that result in poor decision-making.

1. Introduction

Exposure to life stressors is a risk factor for development of psychopathological disorders including post-traumatic stress disorder (PTSD). One of the major diagnostic criteria for PTSD is the presence of recurrent distressing memories. In fact, the aim of behavioral treatments such as exposure therapy is to reduce the emotional and physiological responses associated with aversive memories. Acute exposure to severe stressors or repeated exposure to chronic mild stressors (CMSs) can both result in enhanced anxiety-like behaviors; however, it has not been well documented to what extent behavior changes are a result of enhanced associative learning processes.

Exposure to acute stress prior to classical conditioning trials facilitates learning and the formation of the memory [1,2]. Classical learning is the process of pairing a neutral cue (conditioned stimulus) with an aversive, unconditioned stimulus such that the conditioned stimulus alone elicits a response [3]. For example, exposure to tail shock prior to the pairing of white noise with a brief periorbital shock results in significantly more conditioned eyeblink responses when the white noise is presented alone during testing [4]. Most evidence points to the release of norepinephrine (NE) and stimulation of central beta-noradrenergic receptors (β-ARs) within the amygdala as the mechanism by which

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http://dx.doi.org/10.1016/j.physbeh.2015.03.008
0031-9384/

stressor exposure facilitates memory formation. Evidence for this phenomenon is quite extensive and is summarized in multiple reviews [5–7]. It has also been shown that administration of β-AR agonists directly into the basolateral amygdala (BLA) during training trials increases memory retention while blockade of β-ARs in the BLA reduces memory formation [8–12]. Exposure to restraint stress enhances subsequent long-term potentiation (LTP) in the BLA pathway induced in vitro, which can be prevented by prior administration of a β-AR antagonist [13]. While glucocorticoids released by the adrenal cortex during times of stress contribute to enhanced memory formation, their effects largely appear to be mediated through augmenting adrenergic signaling [10,14–17]. Together, these data suggest that β-ARs are involved in a common final pathway in memory consolidation.

Repeated exposure to mild stressors enhances NE turnover in the amygdala [18]; however, the effect this has on behaviors dependent on associative learning has not been fully determined. We previously published that some behavioral changes (e.g. decreased exploratory behavior, social withdrawal) following exposure to CMS can also be attributed to distal environmental cues (lighting, ambient temperature, and olfactory cues in the procedural room). This is significant because others have published that chronic stress does not induce anxiety-like behaviors in an open field test or elevated-plus maze [19–21] but does enhance freezing behavior in a contextual fear-conditioning test [1].

Here, we tested the hypothesis that exposure to CMS would alter behavioral responses in tasks that depend on classical fear conditioning. Undisturbed home cage control (HCC) and CMS animals were tested in two behavioral tests that require associative learning (e.g. contextual fear conditioning in an operant box and retention latencies in a passive avoidance test) or in a third behavioral test that is not associative learning dependent (e.g. open field). We predicted that animals exposed to CMS would show increased freezing behavior in an operant box and increased retention latencies in a passive avoidance apparatus following conditioning, but no anxiety-like behaviors in the open field test. We also tested whether administration of propranolol (a β-AR antagonist) prior to conditioning trials in the operant box and passive avoidance chamber would prevent or attenuate chronic stress-induced behavioral changes.

2. Methods

2.1. Subjects

Thirty-two Fischer-344 rats were purchased from Harlan Laboratories (Indianapolis, IN), weighing approximately 200–262 g ($\bar{x}$ = 230.1 g; σ = 14.5 g) at baseline. Animals were housed individually in clear Plexiglas habitats (40 × 20 × 20 cm), with food and water available *ad libitum*, and kept on a 12:12 light/dark cycle, with lights on at 07:00 h. Upon delivery, rats spent the first seven days becoming acclimated to the environment and were then handled for five additional days prior to the commencement of the study. Studies were conducted in accordance with the guidelines of the PHS Guide to the Care and Use of Laboratory Animals, and approved by the Kent State University Institutional Animal Care and Use Committee.

2.2. Repeated stress protocol

Subjects were exposed to a series of stressors following an established protocol [23], or were left undisturbed as HCC animals. This four day repeated stress paradigm was chosen since we previously demonstrated that it results in increased NE turnover in the amygdala, sensitized β-AR mediated responses, and enhanced fear conditioning to distal contextual cues [23]. On the morning (08:00–10:00) of day 1, CMS rats were placed in DecapiCone rodent restrainers (Braintree Scientific, Inc., Braintree, MA) for 60 min, before being returned to their home cage. At 15:00 h, food was removed from CMS rat cages for 18 h. On day 2, CMS subjects were placed in novel habitats, each with a piece of filter paper containing 35 μL trimethylthiazoline (a component of fox feces) to simulate predator odor [22] (Contech Enterprises, Victoria, BC). Rats were then placed back in their home cages but were housed in constant light conditions overnight. On the morning of day 3, animals were exposed to restraint stress again for 60 min, then at 15:00 h their bedding material was dampened with approximately 1500 mL tap water. On day 4, subjects were exposed to forced swim for 5 min in glass cylinders measuring 49 × 18.7 cm (inner height and diameter, respectively) filled approximately to the 37.5 cm line with water at a temperature of 21 °C. Following this task, subjects were placed in cages containing dry bedding. At 15:00 h, water bottles were removed from CMS home cages for 18 h and returned on the following morning. Following CMS, and prior to behavioral and physiological observations, subjects weights were recorded.

2.3. Behavioral testing

Behavioral assays were performed to test associative learning (e.g. contextual fear conditioning and passive avoidance) and generalized anxiety (e.g. open field), with tests occurring 2 h apart. The order of tests was counterbalanced to protect against possible order effects. A second cohort of animals was tested in a passive avoidance task only.

2.3.1. Conditioned fear

One day following the stress protocol (day 5), subjects (n = 32) were placed in an 8.5× 8.5″ × 11″ (21.59 × 21.59 × 27.94 cm) operant box (Lafayette Instrument Company, Lafayette, IN) with a floor consisting of a series of electrically conductive steel bars; after 2 min, animals received two foot shocks (1.5 mA for 2 s), the second shock administered 2 min after the first. One minute following the second foot shock, animals were removed from the box and placed back into their home cages. Twenty-four hours later, subjects were placed back into the operant box and behavior was recorded for 15 min and freezing behavior was evaluated. Freezing behavior was defined as complete immobility, save for movements necessary for respiration. Scoring was performed by a trained researcher blind to group assignment. Scores were obtained by checking the video every 10 s for 15 min, and one point was assigned for each instance of freezing behavior, with a maximum possible score of 90 pts. Points were not assigned if at any time point within the ten second interval a subject showed any additional voluntary movement beyond what was required for respiration.

2.3.2. Open field

Subjects were placed in a 60 × 60 × 60 cm Plexiglas box and spontaneous motor activity was recorded using a ceiling mounted video camera for 10 min. Movements within the open field were evaluated using EthoVision XT tracking software (v8.0, Noldus Information Technology, Wageningen, NL). Total time spent in the center section of the open field was measured with lower values indicating an increase in anxiety-like behavior; measurements of locomotion – distance traveled and time spent in motion – were also recorded. The parameters of the open field were defined by creating a 10 × 10 grid (100 subdivisions) on the space within the viewport in the tracking software; the inner 64% of these subdivisions (8 × 8) was designated as the center section.

2.3.3. Passive avoidance

A second group of animals (n = 32) was tested in a passive avoidance apparatus. The apparatus consisted of two chambers: an illuminated, transparent “safe” compartment and a darkened, opaque “shock” compartment. The compartments were divided by a wall with a door that closed upon entry into the opposing chamber. Both chambers sat atop a floor constructed of electrically conductive steel bars; the floor was tilted in the center, which triggered the closing of the door between the chambers when the subject had fully crossed the threshold. On day 5, subjects were placed in the “safe” compartment of the apparatus.

Please cite this article as: R.M. Camp, J.D. Johnson, Repeated stressor exposure enhances contextual fear memory in a beta-adrenergic receptor-dependent process and increases impulsivi..., Physiol Behav (2015), http://dx.doi.org/10.1016/j.physbeh.2015.03.008