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Habituation and extinction of fear recruit overlapping forebrain structures

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ABSTRACT

Establishing the neurocircuitry involved in inhibiting fear is important for understanding and treating anxiety disorders. To date, extinction procedures have been predominately used to examine the inhibition of learned fear, where fear is reduced to a conditioned stimulus (CS) by presenting it in the absence of the unconditioned stimulus (US). However, learned fear can also be reduced by habituation procedures where the US is presented in the absence of the CS. Here we used expression of the activity marker c-Fos in rats to compare the recruitment of several forebrain structures following fear habituation and extinction. Following fear conditioning where a tone CS was paired with a loud noise US, fear was then reduced the following day by either presentation of the CS or US alone (i.e. CS extinction or US habituation, respectively). This extinction and habituation training recruited several common structures, including infralimbic cortex, basolateral amygdala, midline thalamus and medial hypothalamus (orexin neurons). Moreover, this overlap was shared when examining the neural correlates of the expression of habituation and extinction, with common recruitment of infralimbic cortex and midline thalamus. However, there were also important differences. Specifically, acquisition of habituation was associated with greater recruitment of prelimbic cortex whereas expression of habituation was associated with greater recruitment of paraventricular thalamus. There was also less recruitment of central amygdala for habituation compared to extinction in the retention phase. These findings indicate that largely overlapping neurocircuitries underlie habituation and fear extinction and imply common mechanisms for reducing fear across different inhibitory treatments.

1. Introduction

Fear can be elicited by stimuli that predict the occurrence of aversive events. In laboratory settings, fear to a once neutral stimulus, such as a tone, is commonly produced by pairing it with an innately aversive stimulus, like footshock (the unconditioned stimulus, US) (Davis, 1992; LeDoux, 2000). Importantly, learned fear of the tone (the conditioned stimulus, CS) can be inhibited. For example, in an extinction procedure the CS is presented in the absence of the US and the fear response is gradually reduced (Maren & Quirk, 2004; Myers & Davis, 2002; Quirk & Mueller, 2008). Procedures used to reduce fear are of particular interest because impaired fear inhibition is thought to contribute to a number of anxiety disorders, including phobias, post-traumatic stress disorder, and panic disorder (Herry et al., 2008; Hofmann, 2008; Milad, Rauch, Pitman, & Quirk, 2006).

Learning to inhibit fear through extinction is dependent on the acquisition, consolidation and retrieval of new associative memories (e.g., forming or recalling an extinction memory). These processes are believed by many to occur over two phases: a within-session phase, where the CS is initially presented in the absence of the US and extinction is acquired, and a between-session retention phase, where the CS is again presented in the absence of the US in a subsequent session and extinction is recalled (Milad et al., 2006; Myers & Davis, 2002). The medial prefrontal cortex (mPFC) and the amygdala complex are critically involved in both these processes (Herry et al., 2010; Milad et al., 2006; Myers & Davis, 2002; Quirk & Mueller, 2008). Specifically, the infralimbic cortex (IL) and basolateral amygdala (BLA) are important for acquiring and consolidating the extinction memory. For example, pharmacological inactivation of either of these structures prior to extinction training, as well as protein synthesis inhibition after extinction training, prevent the reduction in fear to the CS at a subsequent retention test (Davis & Bauer, 2012; Hugues, Deschaux, & Garcia, 2004; Santini, Ge, Ren, Pena de Ortiz, & Quirk, 2004; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Zimmerman & Maren, 2010). The IL also mediates the expression and recall of extinction, most likely via interactions with the BLA and central

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amygdala (Ce) (Milad et al., 2006; Quirk, Likhtik, Pelletier, & Pare, 2003). For example, when animals are tested for extinction recall, IL and BLA neurons show increased activity (Barrett, Shumake, Jones, & Gonzalez-Lima, 2003; Milad & Quirk, 2002; Muigg et al., 2008), and lesions of IL prevent extinction recall (Lebron, Milad, & Quirk, 2004; Quirk, Russo, Barron, & Lebron, 2000).

While extinction is by far the predominant model of fear inhibition, it is not the only procedure for reducing fear to a CS. For example, US habituation (or US devaluation) where the US is presented alone after conditioning, also reduces the level of fear elicited by a CS previously paired with the aversive US (Rescorla, 1973). Theoretical accounts of US habituation suggest that non-associative mechanisms (such as reduced salience of the US representation or US devaluation), mediate the decrement in learned fear (Rescorla, 1973; Solomon & Corbit, 1974). These non-associative mechanisms stand in contrast to the associative mechanisms requiring learning of a new association (e.g., excitatory CS–No US association or an inhibitory CS–US association) and typically invoked to explain the reduction in fear following extinction (Bouton, 2004; Myers & Davis, 2002). However, we have shown that the reduction in fear observed following US habituation exhibits many of the same 'signature' characteristics as the reduction in fear following extinction. For example, CS-elicited fear after US habituation is regulated by context, whereby freezing is low when the CS is presented in the habituation context but high when presented in the conditioning context (i.e., ABA renewal) and fear to the CS can be reinstated by reminder cues (Storsve, McNally, & Richardson, 2012). Likewise, fear habitation and fear extinction share a common pharmacological sensitivity, with both impaired by systemic injection of an NMDA receptor antagonist prior to training (Storsve, McNally, & Richardson, 2010).

Given that US habituation and CS extinction show similarities in the way fear expression is regulated, it is possible that the same neural mechanisms mediate the decrement in fear observed using these two procedures. However, in contrast to the extensive literature examining the neural basis of extinction, little is known about the neurocircuitry underlying fear inhibition via US habituation.

Here, we used the immediate early gene (IEG) c-Fos to compare neuronal activity during the acquisition and expression of fear reduction caused by these two procedures. Adult rats first received pairings of a tone CS and a loud noise US. The following day, rats were given either CS extinction training or US habituation. In Experiment 1, neuronal activity was examined two hours later to determine which neural structures were activated. Then, in Experiment 2, neuronal activity was assessed the following day, for the remaining animals, after testing for fear to the CS to determine which neural structures were engaged when fear was being inhibited. The use of IEGs allows for high-resolution spatial mapping of multiple neural structures, and hence is a useful initial step to compare and contrast the regions and circuit(s) recruited by these procedures. Our focus was on structures within the mPFC and amygdala complex given the extensive evidence that these structures are critically involved in CS extinction. In addition, we examined thalamic and hypothalamic afferents to these structures, which have been implicated in regulation of fear and fear learning (Furlong, Cole, Hamlin, & McNally, 2010; McNally, Johansen, & Blair, 2011), to establish a wider network of relevant neurocircuitry following the two procedures.

2. Methods

2.1. Subjects

Experimentally naive Sprague-Dawley-derived rats (bred in the School of Psychology, The University of New South Wales) were housed in groups of eight (weighing 340–560 g, $N = 46$). Rats were maintained on a 12 h light/dark cycle (lights on at 07:00) with food and water available *ad libitum*. All procedures were approved by the Animal Care and Ethics Committee at The University of New South Wales and conducted in accordance with *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th Edition, National Health and Medical Research Council of Australia).

2.2. Apparatus

Two distinct experimental chambers were used, referred to as chambers A and B. The chambers were of the same size [30 cm (l) × 30 cm (w) × 23 cm (h)], but differed on a number of other features. Chamber A consisted of a Plexiglas floor, and Plexiglas walls that were lined with vertical black and white stripes (2.5 cm apart) and was illuminated by white light. Chamber B consisted of Plexiglas walls and a floor composed of 3 mm stainless steel rods set 1 cm apart. Each chamber was housed separately in a wood sound-attenuating cabinet where illumination and background noise were provided by infrared light and a ventilation fan (50 dB). All presentations of auditory stimuli were controlled by custom software and were presented through two high-frequency speakers mounted within each chamber. Infrared cameras were also mounted on the rear wall of each cabinet and connected to a DVD recorder for measuring freezing to the CS.

2.3. Behavioral procedures

2.3.1. Stage one: fear conditioning

On day 1, rats were fear conditioned to a tone by pairing it with a loud noise. Rats were placed in chamber A for 2 min before presentation of a 10 sec tone CS (3 kHz, 75 dB). During the last 0.1 sec of the CS a white noise US was presented (120 dB, with a 1 ms rise-fall time). Ten CS–US pairings were given with a mean inter trial interval (ITI) of 85 sec. Freezing was scored during each CS presentation. Freezing was manually scored, every 3 sec, and was defined as the absence of all movement except that required for respiration.

2.3.2. Stage two: fear inhibition

On day 2, rats were placed in chamber B for habituation (habituation group), extinction (extinction group), or no treatment (control group). Specifically, rats in the habituation group were exposed to 150 presentations of the white noise US alone (with 60 sec adaption period and 8 sec ITIs). For rats in the extinction group, 30 tone CS alone presentations were given (for 10 sec each, with 60 sec adaptation period and 20 sec ITIs). Rats in the control group were placed in the chamber for the equivalent length of time (22 min) but received neither the CS nor the US. Freezing was scored during each CS presentation, and reported as 10 blocks each consisting of 3 CS presentations for the extinction group only. All animals were then returned to their home cages. Half the rats from each condition were sacrificed 1 h 40 min after the end of their respective sessions and their brains extracted for immunohistochemical analysis (experiment 1, $N = 24$).

2.3.3. Stage three: expression of fear inhibition

Half the rats from each of the three conditions were tested for CS-elicited fear on day 3. All of these rats were placed in chamber B and exposed to 15 CSs (each 10 sec long, with 1 min adaptation period and 10 sec ITIs). Freezing was scored during each CS presentation, and reported as 5 blocks consisting of 3 CS presentations. Rats were then returned to their home-cages and sacrificed 1 h 40 min later for immunohistochemical analysis (experiment 2, $N = 22$).