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Extinction of relapsed fear does not require the basolateral amygdala

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ABSTRACT

It is well established that extinguished fears are restored with the passage of time or a change in physical context. These fear restoration phenomena are believed to mimic the conditions under which relapse occurs in patients that have been treated for anxiety disorders by means of cue-exposure therapy. Here, we used a rodent model to extinguish relapsed fear and assess whether this new extinction prevents further relapse. We found that activity in the basolateral amygdala (BLA) is required to initially extinguish conditioned fear, but this activity was not necessary to subsequently extinguish relapsed fear. That is, extinction of spontaneously recovered or renewed fear was spared by BLA inactivation. Yet, this BLA-independent learning of extinction did not protect against further relapse: extinction of relapsed fear conducted without BLA activity was still likely to return after the passage of time or a shift in physical context. These findings have important clinical implications. They indicate that pharmacological agents with anxiolytic properties may disrupt initial cue-exposure therapy but may be useful when therapy is again needed due to relapse. However, they also suggest that these agents will not protect against further relapse, implying the need for developing drugs that target other brain regions involved in fear inhibition.

1. Introduction

Cue-exposure therapy is relatively effective at inhibiting irrational and excessive fears in patients suffering from anxiety disorders (Bisson et al., 2007; Harvey, Bryant, & Tarrier, 2003). Nevertheless, its long-term efficacy is challenged by the tendency of fear to return after treatment (Bradley, Greene, Russ, Dutra, & Westen, 2005). Animal models of fear conditioning and extinction are useful tools in studying the neuronal and psychological processes underlying fear inhibition and relapse (Myers & Davis, 2007). In the extinction of conditioned fear, repeated presentations of a cue (conditioned stimulus; CS) previously paired with a dangerous event (e.g., an aversive footshock; unconditioned stimulus; US) reduces the expression of conditioned fear responses (e.g., freezing). However, changes to the extinction context (e.g., physical, temporal, internal state) cause fear to be restored (Bouton, Westbrook, Corcoran, & Maren, 2006). For example, animals tested with the CS outside the physical context where extinction occurred show a renewal of fear responses, such as freezing (Harris, Jones, Bailey, & Westbrook, 2000). Extinguished fear responses also spontaneously recover with the passage of time (Rescorla, 2004). These fear restoration phenomena observed in animal models are likely those that characterize fear relapse after cue-exposure therapy in people.

The basolateral amygdala (BLA) has repeatedly been shown to be critical for the acquisition and extinction of conditioned fear (Duvarci & Pare, 2014; Myers & Davis, 2007; Quirk & Mueller, 2008). During acquisition, information about the CS and US converge in the BLA, activating a specific population of neurons that provides feedforward excitation of the central amygdala output neurons responsible for fear responses (Herry et al., 2008). During extinction, non-reinforced presentations of the CS activate a distinct population of neurons within the BLA that inhibits central amygdala output, leading to a decrease in fear responses (Herry et al., 2008). Manipulations targeting neuronal activity in the BLA before extinction have routinely demonstrated its importance in extinction learning (Davis & Bauer, 2012; Laurent & Westbrook, 2008; Laurent & Westbrook, 2009a; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Sotres-Bayon, Bush, & LeDoux, 2007; Zimmerman & Maren, 2010), identifying it as a target region for therapies that aid in the reduction of fear and anxiety. In line with this, pharmacological interventions that produce anxiolysis by acting on this amygdala-based fear circuit are often used in conjunction with behavioral therapies to facilitate fear inhibition (Otto, Pollack, & Sabatino, 1996; Wilhelm & Roth, 1997). In fact, some pharmacotherapies have been shown to protect against fear renewal (Haaker et al., 2013; Morris, Westbrook, & Killcross,

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2005). The anxiolytic effects produced by altered amygdala activity may be critical in reducing the incidence of relapse and increasing compliance among patient populations.

However, despite considerable research into the neuronal structures of fear extinction and how these regions respond to pharmacotherapies, surprisingly little is known of the mechanisms involved in the extinction of restored fear or how altering amygdala activity when relearning extinction may influence the incidence of further relapse. Yet, the restoration phenomena that develop after therapies make it likely that additional extinction learning will be required for long-term fear inhibition. Therefore, we aimed to examine the role of the BLA in the extinction of restored fear. To this end, we inactivated the BLA using the $GABA_A$ agonist, muscimol, when animals were learning, or relearning extinction. In Experiment 1, BLA inactivation occurred before initial fear extinction or before extinction of spontaneously recovered fear. In Experiment 2, BLA inactivation occurred before initial fear extinction or before extinction of fear that had been restored due to a context shift (renewal). We also asked whether inactivation of the BLA while relearning extinction protected against further fear restoration. To do so, we inactivated the BLA before the extinction of renewed fear and rats were tested back in their conditioning context to assess any effects on renewal (Experiment 3). In Experiment 4, the BLA was inactivated before extinction of reconditioned fear and rats were tested either one day or seven days later to assess any effects on spontaneous recovery.

2. Materials and methods

2.1. Subjects

144 experimentally naïve male Sprague-Dawley rats (Rattus Norvegicus) were obtained from the Animal Resources Centre (Perth, Western Australia). They were housed in groups of 4 in a temperature- and humidity-controlled colony room with *ad libitum* access to food and water. The room was maintained on a 12 h light/dark cycle (7 am–7 pm) and all experimental procedures took place within the light cycle. Rats were treated in accordance with the Australian National Health and Medical Research Council guidelines, and the Animal Care and Ethics Committee of the University of New South Wales approved all procedures.

2.2. Apparatus

Behavioral training and testing took place in three distinct sets of 4 chambers, labeled Context A, B and C. In experiments were multiple contexts were used, contexts were counterbalanced across subjects. Context A measured 30 cm length × 26 cm depth × 30 cm height. The floor was composed of stainless rods, 2 mm in diameter and spaced 10 mm apart, center to center. The sidewalls and ceiling were made of stainless steel and painted white; the front and back walls were made of clear Plexiglass. Context B measured 30 cm length, × 26 cm depth × 30 cm height. The floor was composed of stainless rods, 7 mm in diameter and spaced 18 mm apart, center to center. The sidewalls and ceiling were made of stainless steel and the front and back walls were made of clear Plexiglass. The third set of 4 chambers measured 24 cm length, × 21 cm depth × 20 cm width. The floor was composed of stainless rods, 2 mm in diameter and spaced 13 mm apart, center to center. The sidewalls were made of stainless steel and the ceiling and front and back walls were made of clear Plexiglass. All chambers were enclosed in sound- and light-attenuating shells and equipped with a shock generator, which delivered an unscrambled 0.5 mA, 0.5 s shock to the floor. A speaker mounted to the back wall of each chamber delivered a 70 dB white noise and a 620-Hz square wave tone. A white fluorescent light mounted to the back of each chamber allowed for the presentation of a light CS, flashing at a rate of 2/s in an otherwise dark session. All chambers were connected to computers located outside the testing rooms that controlled stimulus presentations via Matlab software (MathWorks). Cameras in each chamber were connected to DVD recorders that recorded the activity within the chamber. Immediately before the commencement of the behavioral procedures of each experiment, all rats were habituated to the context(s) for 20 min twice per day for two days, and then habituated to the CSs the following day by presenting them twice in each context. All CS durations were 30 s and intertrial intervals across experiments were fixed 3 min.

2.3. Drugs

The $GABA_A$ agonist, muscimol (Sigma), was dissolved in 0.9% (wt/vol) non-pyrogenic saline for a final concentration was 1 μg/μL (Laurent & Westbrook, 2008). Non-pyrogenic saline was used as a control vehicle.

2.4. Surgery and drug infusion

Rats were anesthetized with isoflurane gas and placed in a stereotaxic frame (Kopf, Tujunga, CA, USA). The incisor bar was adjusted to align bregma and lambda on the same horizontal plane. Holes were drilled into the skull above the basolateral amygdala (BLA) using an electric hand drill. 26-gauge guide cannula (Plastics One, Roanoke, VA) were implanted and aimed at the following coordinates, relative to bregma: A/P: −2.3; M/L: ±4.9; D/V: −7.9. Guide cannulas were fixed in position with dental cement and dummy cannulas were kept in each guide at all times, except during infusions. At the end of surgery, rats received a prophylactic intraperitoneal injection of 0.4 mL solution of procaine penicillin (Ilium Benicillin, Australia). Rats were allowed 5 days of recovery before the commencement of behavioral procedures.

At the time of microinfusions, dummy cannulas were removed and 33-gauge injector cannulas that projected 1 mm beyond the end of the guide cannula were inserted. Injector cannulas were connected to a 25 μL Hamilton syringe attached to an infusion pump (Harvard Apparatus). A total volume of 0.3 μL muscimol or vehicle was delivered at a rate of 0.1 μL/min. Injector cannulas were left in place for an additional 1 min after the infusion to allow for the drug to diffuse. Immediately after the infusion, dummy cannulas were replaced, and rats were returned to their home cages for 20 min before being placed into the behavioral chambers. Six hours later, rats received control infusions to control for the effect of the drugs, per se, on subsequent test performance. Rats in the muscimol groups received saline infusions, whereas rats in the saline groups received muscimol.

2.5. Histology

Rats were euthanized with a lethal dose of sodium pentobarbital and decapitated. Their brains were extracted, and 40 μm coronal cryostat sections were taken through the amygdala, mounted onto slides, and stained with cresyl violet. Locations of the cannula tips were determined by a trained observer unaware of the subjects' group assignment using boundaries defined by Paxinos and Watson (2006). Subjects with misplaced cannula were excluded from statistical analysis.