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Research Report

Periaqueductal gray c-Fos expression varies relative to the method of conditioned taste aversion extinction employed

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ABSTRACT

A conditioned taste aversion (CTA) is acquired when an animal consumes a novel taste (CS) and then experiences the symptoms of poisoning (US). Following CTA training, animals will avoid the taste that was previously associated with malaise. This defensive reaction to a learned fear can be extinguished by repeated exposure to the CS alone (CS-only; CSO-EXT). However, following a latency period in which the CS is not presented, the CTA will spontaneously recover (SR). Through the use of an explicitly unpaired extinction procedure (EU-EXT) we have shown that we can speed up extinction and attenuate SR of the CTA. Here we compared and contrasted the ability of CSO and EU extinction procedures to affect *c-Fos* expression in the periaqueductal gray (PAG). Fluid-deprived Sprague–Dawley rats acquired a strong CTA [via 3 pairings of 0.3% oral saccharin (SAC; the CS) and 81 mg/kg i.p. lithium chloride (LiCl; the US)] followed by extinction trials consisting of multiple exposures to either, (a) the CS every-other day (CSO-EXT), or (b) CS and US on alternate days (EU-EXT). A different group of rats did not receive multiple CS exposures and served as a "no extinction" (NE) control. Both extinction procedures resulted in ≥90% reacceptance of SAC (achieving asymptotic extinction). Some of the animals were sacrificed for c-Fos immunohistochemical analysis following asymptotic extinction. Other rats entered a 30-day latency period where they drank water only. These remaining animals were then tested for SR with a final exposure to SAC before being sacrificed for c-Fos immunohistochemistry. As reported previously, rats in the CS-only group exhibited a significant SR of the CTA. However, animals in the EU extinction group reached asymptotic extinction more rapidly than did CSO rats and they did not show SR of the CTA. As compared to rats that retained their CTA, both groups of extinguished rats showed suppression in the number of c-Fos-labeled neurons in all 4 longitudinal columns of the PAG. The number of c-Fos-labeled cells in the PAG was generally low but there was a reliable increase in c-Fos expression in dorsolateral PAG (dlPAG) following the SR test in the brains of rats that went through the EU-EXT procedure as compared with those that either went through the more-traditional CSO extinction procedure or experienced no extinction at all. The number of c-Fos-labeled neurons in the dlPAG was significantly correlated with the amount of SAC consumed at the SR test. Surprisingly, the brains of EU-extinguished rats and CSO extinguished rats did not differ in

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the number of c-Fos-labeled neurons in gustatory neocortex, medial prefrontal cortex, basolateral amygdala, or the central nucleus of the amygdala. Thus, behavioral differences in SR between the EU and CSO extinction animals were not represented by corresponding changes in the neural activity of several brain nuclei classically associated with extinction learning. However a detailed analysis of PAG *c-Fos* expression provided hints about some of the physiological changes evoked by these 2 extinction paradigms that produce very different behavioral outcomes. The findings are clinically relevant as we seek the development of treatments for deficits in fear extinction (e.g. PTSD, phobias).

1. Introduction

Many fears are learned when a previously neutral stimulus (CS) becomes associated with a naturally aversive one (US) through the process of Pavlovian conditioning (Pavlov, 1927). Therapy for anxiety disorders such as phobias and PTSD often utilize techniques based on learning theory that are aimed at breaking or weakening the CS+US bond (Norrholm et al., 2011). These exposure therapies are essentially a type of extinction that typically attempt to reduce fears by presenting the now-aversive CS without the US (CS-only extinction, CSO; Graham et al., 2011). Unfortunately, fears can re-emerge following the passage of time (i.e., spontaneous recovery; SR) or through other forms of relapse (Bouton, 1993; Rescorla and Heth, 1975).

Substantial recent efforts have been made to devise more effective therapies that reduce or eliminate the SR or relapse of fears (Kaplan et al., 2011). Moreover, there has been great interest in identifying the brain areas and neurochemical mechanisms involved in the acquisition, extinction, and SR of conditioned emotional responses (CERs) (for a recent review see Herry et al., 2010) so that more-effective behavioral (Urcelay et al., 2009) and pharmacological therapies (Debiec et al., 2011; Graham et al., 2011) may be developed. This work has been quite fruitful and we now know, for example, that the mPFC, amygdala, hippocampus, and the midbrain periaqueductal gray are involved in various aspects of the extinction of conditioned fears (Barad, 2005; Li et al., 2009; McNally et al., 2004; Peters et al., 2009; Quirk and Mueller, 2008; Sierra-Mercado et al., 2011; Sotres-Bayon and Quirk, 2010).

In order to take advantage of our knowledge about how learning, more generally, could influence development of therapies for fear and anxiety, Groblewski et al. (2009) have called for studies that go beyond the CER. Our recent research has been investigating the conditioned taste aversion (CTA) (Garcia, et al., 1955). Rats will avoid a taste that has been previously associated with malaise (Mickley et al., 2004, 2005). The CTA has been described as a defensive reaction to a learned fear (Parker, 2003) but the extent to which fear mediates the aversion is not settled (Akirav et al., 2009). Still, it is a form of aversive learning that is biologically meaningful and has distinct characteristics (e.g., rapid acquisition and resistance to extinction; Nolan et al., 1997) that may make it a useful model as we seek therapies for anxiety disorders such as phobias and PTSD. Further, CTA extinction employs some of the same neural circuits as does the extinction of CERs. Specifically, we have reported changes in neural activity (as measured through c-Fos immunohistochemistry) in the amygdala and mPFC that correlate with various stages of extinction and SR of a CTA (Mickley et al., 2004, 2005, 2007).

Additional behavioral studies from our laboratory indicate that there are techniques that may be employed to modulate the speed and stability of CTA extinction. For example, we have reported that, by explicitly unpairing the CS and US during extinction training (i.e., through alternate day presentations; EU-EXT), we could speed the rate of extinction and substantially reduce SR (Mickley et al., 2009) when compared to extinction procedures during which the CS-only is presented (CSO-EXT). These studies build on the reports of other labs indicating that the EU-extinction procedure can thwart renewal of conditioned fears (Rauhut et al., 2001; Thomas et al., 2005).

Given the distinctive behavioral outcomes produced by the EU-EXT and CSO-EXT paradigms, the current study was aimed at describing neural correlates that distinguish between these 2 methodologies. In particular, we focused on c-Fos protein expression in the PAG following CTA extinction and SR tests. It is well known that the PAG is involved in defensive reactions to natural and learned fears (Carrive, 1993) and stimulation of the PAG produces an increase in running, jumping, blood pressure, tachycardia, and blood flow redistribution (Morgan et al., 1998). We also selected the PAG for analysis since it has a close functional connection with the mPFC and amygdala — structures that contribute to modulation of emotional responses (Floyd et al., 2000; Pare et al., 2004; Peters et al., 2009; Price, 2005; Ulrich-Lai and Herman, 2009) and extinction of conditioned fears (McNally et al., 2004, 2005). The PAG is not part of the classically identified neural circuit that subserves CTA learning (Yamamoto, 1993; Yamamoto and Fujimoto, 1991; Yamamoto et al., 1994, 1997). However, the mPFC and amygdala that connect with PAG and mediate CER extinction and renewal (Bruchey et al., 2007; Quirk and Mueller, 2008) also play important roles in CTA extinction and SR (Mickley et al., 2004, 2005, 2007).

Here we report that CTA extinction, produced by either EU-EXT or CSO-EXT methods, produced suppression in the number c-Fos immunoreactive neurons in the PAG. However, following the SR test, there was a significant increase in c-Fos expression in the dlPAG only in the group of rats that experienced EU-EXT and did not exhibit SR of the CTA.

2. Results

2.1. *CTA acquisition*

Following three saccharin (SAC) and lithium chloride (LiCl) pairings all rats had acquired a strong CTA. SAC consumption