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# Extinction of fear is facilitated by social presence: Synergism with prefrontal oxytocin

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ABSTRACT

This study addressed the question of whether extinction in pairs would have a beneficial effect on extinction of fear conditioning. To that end, we established an experimental setting for extinction in which we trained animals to extinguish contextual fear memory in pairs.

Taking advantage of the role of oxytocin (OT) in the medial prefrontal cortex (mPFC) in the mediation of memory extinction and social interaction, we also sought to study its role in social interaction-induced effects on extinction. Our results clearly show that the social presence of another animal in the extinction context facilitates extinction, and that this facilitation is mediated through mPFC–OT. Our results suggest that social interaction may be a positive regulator of fear inhibition, implying that social interaction may be an easy, accessible therapeutic tool for the treatment of fear-associated disorders.



## 1. Introduction

Fear conditioning involves association between a neural conditioned stimulus (CS) and an aversive unconditioned stimulus (US), resulting in robust fear memory that can be weakened through extinction (Berman and Dudai, 2001; Bouton and Nelson, 1994; Myers and Davis, 2002; Rescorla, 1996). Fear responses can be modulated by social presence. For example, they are attenuated by the presence of a calm companion in a variety of species, including human subjects. The postulated mechanism underlying socially-mediated fear reduction is that companions distract attention from the threatening environment and thereby reduce emotionality (Epley, 1974). This distraction hypothesis assumes that the more attention a companion attracts, the greater the magnitude of the fear reduction response. In humans, social support serves as a reliable protective factor against stress, and it was found that social support can reduce the hypothalamus–pituitary–adrenal axis responsiveness to social stress (Bruchey et al., 2010,b; Ditzen et al., 2008a,b; Heinrichs et al., 2003). Fear responses can be also socially transmitted, as rats that were exposed to an unfamiliar tone in the presence of a cage-mate, which had been previously fear-conditioned to the same tone, showed increased freezing levels in response to the stimulus the next day (Bruchey et al., 2010).

Similarly, Knapska et al. (2006) and Knapska and Maren (2009) designed an experimental rat model of between-subject transfer of emotional information, and showed that a conditioned rat that interacted with a naïve cage mate transferred fear memory to the naïve rat. This memory transfer resulted in a rapid increase in exploratory behavior of the observer, as well as a pattern of c-Fos activation in the observer's amygdala similar to the pattern observed in the shocked demonstrators. Together, these observations suggest that social animals obtain information via interaction with conspecifics, and use it to adapt to their environment.

Social interaction is mediated, at least in part, by the mammalian hormone and neuropeptide oxytocin (OT). Further, a link between OT, social interaction and regulation of fear was reported in a series of studies by Guzmán et al. (2013, 2014). The authors reported that OT in the lateral septum (LS), a brain region which subserves stress and fear (Sheehan TP, Chambers RA, Russell DS; 2004), can strengthen social memory regardless of its valence, but can bidirectionally affect fear. Specifically, OT in the LS strengthened negative social memory induced by social defeat, and enhanced subsequent contextual fear conditioning (Guzmán et al., 2013). Similarly, pharmacological antagonism as well as genetic down-regulation of the OT receptor (OTR) in the LS demonstrated that the OTR mediates the reduction of fear conditioning by preceding positive social interactions, an effect which was further facilitated by local oxytocin infusion (Guzmán et al., 2014). Furthermore, a recent study showed that oxytocin infusion into the dorsal LS before extinction training enhanced the extinction of social fear memory in mice (Zoicas I, Slattery DA, Neumann ID, 2014).

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Pavlovian extinction is orchestrated by the infralimbic subregion of the medial prefrontal cortex (IL–mPFC) (e.g., (Morgan et al., 1993; Quirk et al., 2000), which sends glutamatergic projections to a number of regions within the amygdaloid complex. The oxytocin receptors, which are implicated in anxiety and stress reduction (e.g., Dumont et al., 2009; Uvnäs-Moberg, 1997; Uvnas-Moberg, 1998 however, see King et al., 1985), are abundantly expressed in the mPFC (Gould and Zingg, 2003; Liu et al., 2005; Ninan, 2011), suggesting that they may play a role in fear extinction (reviewed in (Maroun and Wagner, 2015)). We have recently shown that when an OT agonist is microinfused into the IL, extinction of fear is indeed facilitated (Lahoud and Maroun, 2013).

Given the role of the mPFC in controlling emotional and social processes (e.g., (Kolb, 1974; Pellis et al., 2006; Uylings et al., 1991), the aim of this study was to address how mPFC OT modulates extinction of fear when social presence is within the context of extinction. Specifically, we sought to (1) develop a behavioral paradigm in which we can examine the extinction of a pair of animals simultaneously and to examine whether extinction in pairs would facilitate extinction of fear and (2) evaluate the role of mPFC OT in mediating the dyad effects on extinction of fear.

Our results clearly show that social presence in the extinction context has a facilitatory effect on the extinction of fear memory, and demonstrate that this effect is mediated by the mPFC oxytocinergic system.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed on male Sprague Dawley rats (Harlan Laboratories, Jerusalem) weighing 200–280 g. The animals were housed in Plexiglas cages (five rats per cage) and were maintained on a free-feeding regimen with a 12-hr light/12-hr dark schedule (light phase 7:00 A.M–7:00 P.M.). In all experiments, excluding the first (Experiment 1), a week after arrival, the rats were anesthetized with ketamine (65 mg per kg, i.p.) and xylazine (7.5 mg per kg, i.p.) and placed in a stereotaxic frame, with body temperature maintained at 37 ± 0.5 °C. The animals were implanted bilaterally with stainless steel guide cannulae (23 gauge) aimed at the IL-mPFC [anteroposterior, +3.2 mm; lateral, ±0.5 mm; ventral, −4.6 mm relative to bregma]; (Paxinos and Watson, 1998). The cannulae were held in place with acrylic dental cement and secured with two skull screws. A stylus was placed in the guide cannula to prevent clogging. The animals were allowed one week to recuperate before being subjected to experimental procedures. The procedures were performed in strict accordance with the University of Haifa regulations and the US National Institutes of Health guidelines (NIH publication number 8023).

### 2.2. Housing

All animals were housed in groups of 4–5 rats per cage. Animals that were assigned into the "pairs" condition were always taken from the same home cage. This pairing assignment method, which is based on familiarity, is used to prevent aggressive behavior, as described in previous studies (Alberts and Galef, 1973; Wongwitdecha and Marsden, 1996; Brill- Maoz and Maroun, unpublished data).

### 2.3. Contextual fear conditioning

Based on our previous conditioning and extinction protocols (Kritman and Maroun, 2012; Motanis and Maroun, 2010), each rat was placed in a conditioning chamber with black methacrylate walls, a transparent front door, a top-view video camera, and a metal grid floor. To form the conditioning, each animal received three footshocks delivered through the grids (0.9 mA for 0.5 s) with 2 min intervals. A similar, weak conditioning protocol was used in some experiments, as indicated, in which shock intensity was 0.5 mA.

The rat was given 2 min for acclimatization to the context before delivery of the first shock and additional 2 min after the last shock. In total, the whole conditioning session lasted about 8 min (for details:(Kritman and Maroun, 2012; Lahoud and Maroun, 2013; Motanis and Maroun, 2010). The chamber was cleaned with 70% ethanol wipes and dried with paper towels after each trial. All animals underwent fear conditioning separately.

### 2.4. Retrieval session (T1)

The conditioned rats were placed in a conditioning chamber for 5 min during which no shock was delivered. Afterwards, based on freezing rate data (comparable freezing levels ± 10%), they were divided into two groups: paired (pairs) and individuals (single). This allocation served to ensure even distribution of basal freezing rates.

### 2.5. Extinction sessions (T2 and T3)

Rats underwent two consecutive extinction sessions separated by 24 h. The "pairs group" underwent extinction training together and rats in the "single group" underwent the same procedure but individually.

### 2.6. Reinstatement of fear

Immediately after the termination of the last session of extinction (T3), animals received an electric shock (0.9 mA for 0.5 s). Following the reinstatement, animals were divided into three groups to be tested for the success of reinstatement 24 h later (post reinstatement; PR). While the "single" group was tested as singles, the Pairs group was divided into two groups (1) animals that were tested at PR as pairs (pairs tested as pairs) and (2) pairs that were tested as singles (pairs tested as singles). The "pairs tested as singles" group underwent an additional session on PR2 and was tested as singles.

### 2.7. Freezing

The animal's movements were recorded by color video camera (Imaging Source) for offline analysis. Data were analyzed by high-sensitivity movement—transducer of Ethovision software (Noldus). Motion and exploration were recorded and analyzed using a designated software component of Ethovision (animals were marked with a Xylene free, instant drying, waterproof permanent marker to enable the differentiation between them). Freezing duration was defined as the overall time duration in which the animal ceases all types of movement except for respiration (Blanchard and Blanchard, 1972; Guthrie et al., 2000; Guzowski et al., 1999; Kim and Fanselow, 1992). Freezing duration was measured and averaged over all sessions. For the conditioning session, the first two minutes served for acclimatization to the chamber, during which the rat explored the cage. The last two minutes were used to estimate the success of the conditioning. For the extinction session, freezing was averaged over the whole session.

### 2.8. Drug microinjection

All microinjections were performed 30 min before the T2 extinction session. In order to allow microinjection, the stylus was removed from the guide cannula and a 28 mm gauge injection cannula was inserted. The injection cannula was connected via PE20