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Relief learning requires a coincident activation of dopamine D1 and NMDA receptors within the nucleus accumbens

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ABSTRACT

Relief learning is the association of a stimulus with the offset of an aversive event. Later, the now conditioned relief stimulus induces appetitive-like behavioral changes. We previously demonstrated that the NMDA receptors within the nucleus accumbens (NAC) are involved in relief learning. The NAC is also important for reward learning and it has been shown that reward learning is mediated by an interaction of accumbal dopamine and NMDA glutamate receptors. Since conditioned relief has reward-like properties, we hypothesized that (a) acquisition of relief learning requires the activation of dopamine D1 receptors in the NAC, and (b) if D1 receptors are involved in this process as expected, a concurrent dopamine D1 and NMDA receptor activation may mediate this learning. The present study tested these hypotheses. Therefore, rats received intra-NAC injections of the dopamine D1 receptor antagonist SCH23390 and the NMDA antagonist AP5, either separately or together, at different time points of a relief conditioning procedure. First, we showed that SCH23390 dose-dependently blocked acquisition and the expression of conditioned relief. Next, we demonstrated that co-injections of SCH23390 and AP5 into the NAC, at doses that were ineffective when applied separately, blocked acquisition but not consolidation or expression of relief learning. Notably, neither of the injections affected the locomotor response of the animals to the aversive stimuli suggesting that their perception is not changed. This data indicates that a co-activation of dopamine D1 and NMDA receptors in the NAC is required for acquisition of relief learning.

1. Introduction

During an aversive experience, animals can learn that particular stimuli predict the occurrence of an aversive event. This memory helps animals to adapt their behavior in order to prepare them for similar future events (Kamin, 1969; Kandel, 1999). For example, the learned stimuli are able to induce defensive behaviors later which help the animals to cope with the aversive event. This learning is called fear learning and is observed in all vertebrates studied so far, as well as in many invertebrates (Fendt and Fanselow, 1999; Kandel, 2001; LeDoux, 2000, 2012). Conversely, aversive events can also induce a positive emotional state in animals. Solomon (1980) argues that the initial fear before and during an aversive event is replaced by a relief on its termination, which has been described as a reward (Leknes et al., 2011). It has been shown that when animals learn the association of a neutral stimulus with the cessation of an aversive event, such a stimulus later induces appetitive-like behavioral changes, e.g. approach behavior (Andreatta et al., 2012; Tanimoto et al., 2004). Since the stimulus is presented at the moment of relief, this type of memory is called relief learning (Gerber et al., 2014; Lohr et al., 2007).

Fear, relief and also safety learning (i.e., the learning that a stimulus predicts the absence of an aversive event) are believed to be important for normal and pathological human behavior. Patients with post-traumatic stress disorder (PTSD) show increased fear learning and a deficit in fear extinction (Dunsmoor et al., 2015; Fani et al., 2012; Norrholm et al., 2011), as well as impaired safety

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learning (Jovanovic et al., 2010, 2012). Relief learning was not explored in these patients yet but may play a role in the development of PTSD and other psychiatric diseases (Gerber et al., 2014; Lohr et al., 2007), since PTSD patients show reduced accumbal activity and thus reward processing (Sailer et al., 2008).

We have previously found that the nucleus accumbens (NAC) has a critical role in relief learning: (a) in humans, the NAC is activated during a retention test on conditioned relief (Andreatta et al., 2012); in rats, (b) temporary inactivation of the NAC blocks acquisition and expression of conditioned relief (Andreatta et al., 2012; Mohammadi et al., 2014), (c) antagonizing accumbal NMDA receptors blocks acquisition but not expression of conditioned relief (Mohammadi and Fendt, 2015) and (d) inhibition of protein synthesis within the NAC blocks the consolidation of conditioned relief (Bruning et al., 2016). In these experiments, we induced relief learning by presenting a to-be-learned cue shortly after the offset of a mild electric stimulus and later measured an attenuation of the startle response during presentations of this cue, which then acts as a conditioned relief stimulus (Gerber et al., 2014). Even if different stimuli and behavioral paradigms are used for the investigation of relief learning, previous findings in the literature strongly support the crucial role of the NAC in relief learning (Becerra et al., 2013; Leknes et al., 2011; Navratilova et al., 2012).

The relief from an aversive event is rewarding (Navratilova et al., 2015; Seymour et al., 2005) and for instrumental reward-like learning, a coincident activation of accumbal NMDA and dopamine D1 receptors is necessary (Di Ciano et al., 2001; Smith-Roe and Kelley, 2000). Given the crucial role of the NAC in the reward system, the present study tested the hypothesis that a coincident activation of NMDA and dopamine D1 receptors within the NAC is required for relief learning.

To test whether accumbal dopamine D1 receptors are involved in relief learning, we locally injected the dopamine D1 receptor antagonist SCH23390 during different phases of relief conditioning. Intra-accumbal D1 receptor blockade inhibited both acquisition and expression of conditioned relief. A further experiment aimed to find a low dose of the NMDA receptor antagonist AP5 that does not block acquisition of conditioned relief after local injections into the NAC. Additionally, we injected ineffective doses of AP5 and SCH23390 into the NAC, separately or together, at different time points in the relief condition procedure. Our results indicate that co-activation of accumbal dopamine D1 and NMDA receptors is necessary for acquisition of conditioned relief.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, 7–9 weeks old weighing 280–350 g at the time of surgery were used. Rats were bred in our animal facility (original breeding stock; Taconic, Denmark) and housed in groups of 4–6 in standard cages (Macrolon type IV; 1820 cm^2). All animals were kept in a temperature-controlled animal facility (22 ± 2 °C; 50 ± 10% humidity) under a light/dark cycle of 12/12 h (lights on at 6:00 a.m.) with food and water *ad libitum*. Behavioral tests were performed during the light phase. All studies were conducted in accordance with the European regulations for animal experiments (2010/63/EU) and approved by the local authorities (Az. 42505-2-1172, Uni MD).

2.2. Stereotaxic surgery

Animals' heads were fixed into a rat stereotaxic apparatus under anesthesia (2–3% isoflurane with pure oxygen). The skull was exposed and two custom-made stainless steel guide cannulas (length: 8.0 mm; diameter: 0.7 mm) aiming to the NAC were bilaterally implanted (1.2 mm anterior and ±1.5 mm lateral to bregma and 3 mm ventrally from the brain surface; Paxinos and Watson, 2014). The cannulas were fixed to the skull using dental cement and 3 stainless steel screws. The animals were single-caged until they recovered from anesthesia and behavioral testing commenced 5–7 days after operation.

2.3. Startle apparatus

For measurement of acoustic startle reflex, rats were put in the animal enclosures (16 cm long and 9 cm inner diameter) located in boxes (35 cm × 35 cm x 38 cm) of a startle response system (SR-LAB, San Diego Instruments, San Diego, USA). Each enclosure had a motion-sensitive transducer underneath. The output signal of the transducers was digitized at sampling rate of 1 kHz and stored on a computer. As the startle magnitude, the mean transducer output during the time window 10–30 ms after startle stimulus onset was taken. The conditional stimulus (CS) was a white light (ca. 1000 lux) that was presented to the animals with 10 W bulbs, mounted on the back of the box. Scrambled foot shocks were administered by a floor grid (six parallel bars, 10 mm apart and 5 mm diameter). White background noise (50 dB sound pressure level (SPL)) and the acoustic startle probe (40 ms, 96 dB SPL white noise) were generated by loudspeakers mounted in the center of the ceiling of the test chambers.

2.4. Behavioral procedure

To test treatment effects on the acquisition and expression of conditioned relief, as well as on the reactivity to the unconditioned stimulus (US), we used the behavioral procedure as described in previous studies (Andreatta et al., 2012; Bruning et al., 2016; Kahl and Fendt, 2016; Mohammadi et al., 2014; Mohammadi and Fendt, 2015).

2.4.1. Baseline startle response test

After 5 min acclimatization, 10 startle stimuli were delivered with an inter-trial interval (ITI) of 30 s. Based on the mean startle amplitude of this session, the animals were allocated into different treatment groups with balanced mean baseline startle amplitude.

2.4.2. Relief conditioning (acquisition)

After an acclimatization period of 5 min, 15 pairings of an electric unconditioned stimulus (duration: 0.5 s; intensity: 0.4 mA) were delivered with a mean ITI of 150 s (range 90–210 s) followed by the light CS (duration: 5 s). The inter-stimulus interval (ISI) from US onset to CS onset was 3 s. No startle probes were presented during the conditioning session.

2.4.3. Retention test (expression)

After 5 min of accommodation, 10 startle stimuli were administered to habituate the startle response. Then, 20 further startle stimuli were presented, half of them in the absence and half of them in the presence of the light CS (in a pseudorandomized order; ITI: 30 s). The startle stimulus was presented 4.5 s after the onset of the 5 s light stimulus (cf. Andreatta et al., 2012). Peak amplitudes within the 100 ms after the startle stimulus onset were evaluated.

2.4.4. Reactivity to US

For testing the behavioral response to the aversive stimuli, animals were exposed to increasing intensities of electric stimuli (0.0, 0.1, 0.2, 0.3 and 0.4 mA; 60 s ISI). The mean locomotor response during the 500 ms duration of the electric shocks was calculated for each animal.