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Activation of nociceptin/orphanin FQ receptors inhibits contextual fear memory reconsolidation

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ABSTRACT

Several neuropeptidergic systems act as modulators of cognitive performances. Among them, nociceptin, an opioid-like peptide also known as orphanin FQ (N/OFQ), has recently gained attention. Stimulation of its receptor, the N/OFQ opioid receptor (NOP), which is expressed in brain regions involved in emotion, memory and stress response, has inhibitory effects on the acquisition and/or consolidation of spatial and emotional memory in rodents. Recently, N/OFQ was also proposed to be linked to the pathogenesis of Post-Traumatic Stress Disorder in humans. However, until now the effect of the activation of the N/OFQ-NOP system on already consolidated memory, such as during retrieval and reconsolidation phases, has never been explored. In the present study, we investigated the consequences of systemic injection of NOP agonists or i.c.v. injection of the N/OFQ peptide on the retrieval and the reconsolidation of contextual fear memory in mice. We demonstrate that the activation of the N/OFQ system impairs the reconsolidation of context-dependent but not cue-dependent aversive memories. We also show that this amnestic effect is associated with decreased c-Fos expression in the hippocampus and amygdala. Our data thus provide the first evidence that the NOP receptor could be targeted during the reconsolidation process to weaken maladaptive memories. The N/OFQ-NOP system might constitute in the future an interesting pharmacological target for interfering with so-called "pathological memories", in particular those involving maladaptive contextual memories.

1. Introduction

Long-term memory is a dynamic process. During memory retrieval the memory trace returns to a labile state and has then to be re-stabilized or reconsolidated to ensure long-term storage (Alberini and Ledoux, 2013; Nader, 2015; Nader et al., 2000). Interfering with the reconsolidation process is thus a way to alter the original memory. In humans, inhibiting reconsolidation is a promising strategy to treat disorders that involve maladaptive memories such as post-traumatic stress disorder (PTSD), phobia or addiction (Parsons and Ressler, 2013; Schwabe et al., 2014). Targeting reconsolidation using pharmacological agents, such as adrenergic antagonists, has been successful in attenuating memories in animal models of aversive or appetitive conditioning (Reichelt and Lee, 2013). This approach has also shown promising results in humans suffering from PTSD or phobia. However its effectiveness has also been questioned in some clinical populations (Kroes et al., 2016), highlighting the necessity to better understand the mechanism of memory reconsolidation and to identify new pharmacological targets to attenuate maladaptive memories in humans.

Nociceptin/orphanin FQ (N/OFQ) is a 17-amino acid neuropeptide related to the opioid family (Meunier et al., 1995; Reinscheid et al., 1995). However it does not interact with classical mu, delta and kappa opioid receptors but with another opioid-like G-protein-coupled receptor called ORL1 or NOP (Meunier et al., 2000).

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Consistent with its widespread distribution in the central nervous system N/OFQ has been shown to modulate many physiological functions in rodents such as pain, feeding, cardiovascular control, reward, stress/anxiety and depression-like behavior (Calo et al., 2000; Lambert, 2008; Zaveri, 2016). In addition, it has been suggested that endogenous N/OFQ could negatively modulate learning and memory processes (Manabe et al., 1998). Supporting this idea, NOP agonists have been shown to interfere with memory acquisition and consolidation in many learning tasks in pre-clinical animal models (Ouagazzal, 2015; Andero, 2015). More specifically, the injection of N/OFQ i.c.v. or of small molecule NOP agonists i.p. inhibits memory acquisition and/or consolidation in spatial, contextual and recognition memory tasks such as the Morris water maze (Higgins et al., 2002; Kuzmin et al., 2009), fear conditioning (Andero et al., 2013; Fornari et al., 2008; Goeldner et al., 2009; Mamiya et al., 2003), passive avoidance (Hiramatsu and Inoue, 1999; Liu et al., 2007; Mamiya et al., 1999) and novel object recognition (Goeldner et al., 2008). N/OFQ was also found to impair learning when injected directly in the dorsal hippocampus (Goeldner et al., 2008; Redrobe et al., 2000; Sandin et al., 1997, 2004) or the amygdala (Andero et al., 2013; Roozendaal et al., 2007). Moreover, alterations in the endogenous N/OFQ system have been described in mouse and rat models of PTSD (Andero et al., 2013; Zhang et al., 2012, 2015). Finally, a recent study identified a single-nucleotide polymorphism of the NOP receptor associated with increased PTSD symptoms in individuals exposed to child abuse (Andero et al., 2013). Altogether, these data suggest that the N/OFQ system could be targeted to interfere with maladaptive memories associated with PTSD. However, to further validate this hypothesis, the efficacy of NOP agonists to inhibit memory reconsolidation remains to be determined. While consolidation and reconsolidation processes show many similarities, they also involve specific molecular mechanisms, which take place in distinct brain regions according to different kinetics (Alberini, 2005; Li et al., 2013; von Hertzen and Giese, 2005). Therefore, in the present study we tested whether, similarly to their well-characterized effect on learning and memory consolidation, NOP agonists are able to interfere with the reconsolidation of fear memory.

2. Materials and methods

2.1. Animals

A total of 176 C57BL/6 male mice (10-12 week-old, Janvier Labs, Le Genest-Saint-Isle, France) were used. They were housed in collective cages (5 per cage) in a room with controlled temperature (21–23 °C), and a 12-h light/dark cycle (8.00 a.m.-8.00 p.m.). Food and water were provided *ad libitum*. All experiments were carried out in strict accordance with the European guidelines for the care of laboratory animals (European Communities Council Directive 86/609/ECC) and approved by the local ethical committee and the French Ministry of Education and Research (#1992–2015092911113438). All efforts were made to minimize animal discomfort and to reduce the number of animals used.

2.2. Drugs

Ro 65–6570 and N/OFQ were synthesized and purified in house (University of Ferrara) using previously described protocols (Guerrini et al., 1997; Wichmann et al., 1999). AT-403 was synthesized at Astraea Therapeutics. MK-801was purchased from Sigma (St. Quentin Fallavier, France). All small molecules were first prepared as stock solutions in 100% DMSO then diluted in saline (0.9% NaCl, maximal final DMSO concentration was 2%). For each experiment, vehicle refers to the control saline solution with an equivalent percentage of DMSO. The volume for intra-peritoneal (i.p.) injections was 10 ml/kg. N/OFQ peptide was prepared fresh in saline for intra-cerebro-ventricular (i.c.v.) injections. The volume for i.c.v. injections was 2 μl.

2.3. Surgery

The mice were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotactic apparatus (David Kopf Instruments, Tujunga, CA, USA). Guide cannulae were implanted into the lateral ventricle according to the following coordinates: AP 0 mm, Lat ±1 mm from bregma, and DV−2 mm from skull as previously described (Mouledous et al., 2010). After surgery, animals were allowed to recover for 7–9 days in their home cage before the start of the behavioral experiments. At the end of the behavioral experiments, all mice were deeply anaesthetized with an overdose of pentobarbital (Ceva Santé Animale, Libourne, France) and their brains removed for histological verification of cannulae placement as previously described (Massaly et al., 2013).

2.4. Fear conditioning

Conditioning was done in a rectangular conditioning chamber (length 35 cm, width 20 cm, height 25 cm) with a stainless steel rod floor as previously described (Daumas et al., 2004). The experimental device was lit by a 60-W white bulb. Two black-and-white patterns faced the conditioning chamber. The conditioning chamber was cleaned with 70% aqueous ethanol before each training session. For the consolidation experiment, conditioning consisted in a single session with two trials. After a 120 s exploration period, the sound (85 dB) was emitted for 30 s and a foot shock (0.7 mA) was superposed during the last 2 s of the tone. This sequence was repeated twice. Thirty seconds after the last foot shock, the mice were removed from the chamber and returned to their home cage. Twenty-four hours after, the mice were individually tested for freezing to the context in the conditioning chamber for 4 min. Two hours later, they were tested for freezing to the tone in a modified context consisting in a triangular chamber, with white Plexiglas walls and floor. The new chamber was washed with 1% acetic acid and lit by a 40 W red bulb. Two minutes after their placement in the modified context, the tone was presented for 2 min. All experiments were videotaped. Freezing, defined as the lack of all movement other than respiration and heart-beat, was scored manually in 5 s bins by an experimenter blind to the treatment groups.

For the contextual fear memory reconsolidation experiments, conditioning was similar except that no sound was emitted. Twenty-four hours after learning, the mice were subjected to a memory retrieval session by exposing them to the conditioning context for 2 min. This retrieval session was immediately followed by drug treatment. Twenty-four hours after memory retrieval, mice were individually checked for freezing to the context in the conditioning chamber for 4 min. Freezing was scored during both the 2 min retrieval and the 4 min test sessions.

For the tone fear memory reconsolidation experiment, another set-up was used that allowed the use of 3 different contexts. Conditioning was done inside a white chamber in a squared box (25 × 25 cm, height 34.5 cm) with a stainless steel rod floor and cleaned with 70% ethanol between each mouse. It consisted in a single session with two trials as described above.

Twenty-four hours after, the recall was performed inside a grey box in a cylinder (diameter 25 cm, height 34.5 cm) with a smooth floor and cleaned with 1% acetic acid between each mouse. The mice were subjected to this memory retrieval session by letting them explore the context for 1 min then exposing them to the sound for 30 s. This retrieval session was immediately followed by