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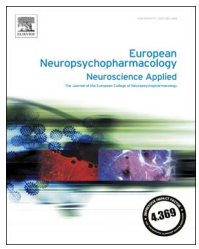

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Alpha$_1$-adrenergic receptor blockade in the VTA modulates fear memories and stress responses

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Abstract
Activity of the ventral tegmental area (VTA) and its terminals has been implicated in the Pavlovian associative learning of both stressful and rewarding stimuli. However, the role of the VTA noradrenergic signaling in fear responses remains unclear. We aimed to examine how alpha$_1$-adrenergic receptor (α_1-AR) signaling in the VTA affects conditioned fear. The role of α_1-AR was assessed using the micro-infusions into the VTA of the selective antagonists (0.1–1 μg/0.5 μl prazosin and 1 μg/0.5 μl terazosin) in acquisition and expression of fear memory. In addition, we performed control experiments with α_1-AR blockade in the mammillary bodies (MB) – a brain region with α_1-AR expression adjacent to the VTA. Intra-VTA but not intra-MB α_1-AR blockade prevented formation and retrieval of fear memories. Importantly, local administration of α_1-AR antagonists did not influence footshock sensitivity, locomotion or anxiety-like behaviors. Similarly, α_1-AR blockade in the VTA had no effects on negative affect measured as number of 22 kHz ultrasonic vocalizations during fear conditioning training. We propose that noradrenergic signaling in the VTA via α_1-AR regulates formation and retrieval of fear memories but not other behavioral responses to stressful environmental stimuli. It enhances the encoding of environmental stimuli by the VTA to form and retrieve conditioned fear memories and to predict future behavioral outcomes. Our results provide novel insight into the role of the VTA α_1-AR signaling in the regulation of stress responsiveness and fear memory.

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1. Introduction

Impaired fear regulation and fear conditioning are the core symptoms of stress-related disorders, and they might lead to the persistent flashbacks, nightmares, and intrusions of fear memories in posttraumatic stress disorder (PTSD) (Arnsten et al., 2015). Fear conditioning provides a useful model of psychopathology observed in the aftermath of stressful experience (Mahan and Ressler, 2012; Maren and Holmes, 2016). However, the neurobiological underpinnings of the formation and consolidation of fear memories are still not fully elucidated.

Dopamine (DA) signaling within the mesocorticolimbic brain structures is known to be involved in behavioral responses to both stressful and rewarding stimuli (Bromberg-Martin et al., 2010). Firing of some DA and GABAergic neurons which are typically located in the caudal ventral tegmental area (cVTA), briefly increases in response to footshock, conditioned aversive stimuli, restraint stress or social defeat stress (Anstrom et al., 2009; Anstrom and Woodward, 2005; Brischoux et al., 2009; Guarraci and Kapp, 1999), indicating that stress potently modulates VTA activity as well as DA signaling. In addition, exposure to stressful stimuli increases VTA GABAergic and decreases DAergic neuronal activity during the encoding of negative prediction error (Cohen et al., 2012). However, the underlying mechanisms that regulate VTA activity, its impact on behavioral stress responses and the formation of fear memories remains elusive.

The noradrenaline (NA) system encompassing NA neurons in the locus coeruleus (LC) and the area A1 and A2 in the medulla oblongata (for detailed neuroanatomy of the NA system see: Robertson et al. (2013) has been demonstrated to control the activity of VTA DA and non-DA neurons (Geisler and Zahm, 2005; Masana et al., 2011; Mejías-Aponte et al., 2009). Importantly, salient stimuli upregulate NA system activity similarly to the DA system, inducing burst firing of the LC NA neurons and subsequent phasic NA release at terminals (Bouret and Richmond, 2009; Park et al., 2012) suggesting a role of NAergic signaling in learning and memory. Accordingly, NA signaling has a facilitating role in acquisition and/or reconsolidation of emotional memories (Bernardi et al., 2009; Cahill et al., 1994; Dębiec et al., 2011; Ferry et al., 1999; Furini et al., 2010; Gelinas and Nguyen, 2005; McGaugh and Roozendaal, 2002; Milton et al., 2008; Sara et al., 1999; Schutsky et al., 2011). This is consistent with clinical studies suggesting elevated NA responsiveness in PTSD (Geracioti et al., 2001; Southwick et al., 1993) and the crucial role of NA signaling within the mesocorticolimbic brain structures in encoding fear memory (Wingenfeld et al., 2015); for review see: (Holmes and Quirk, 2010; Mueller and Cahill, 2010). Consequently, α_1-AR blocker prazosin proved to be effective in attenuating some symptoms of PTSD (Birnbaum et al., 1999; Germain et al., 2012; Koola et al., 2014; Raskind et al., 2013, 2007, 2003; Taylor et al., 2008, 2006); for review see: (Arnsten et al., 2015).

Despite the established NA system projections to the VTA and the receptor mechanisms, behavioral consequences of NA signaling in the VTA are poorly understood. Recently, it has been shown that VTA NA signaling is involved in cocaine-related behaviors (Goertz et al., 2015). In contrast, there are no reports to date of the role of VTA NA signaling in stress-related behaviors. Our study aimed to examine the role of the NA signaling via α_1-AR in the VTA in the acquisition and retrieval of conditioned fear memories. Exploring the receptor mechanisms of individual stress responsiveness is crucial for understanding the processes that determine susceptibility or resilience to diseases related to a traumatic stress or challenging situation.

2. Experimental procedures

2.1. Subjects

Male Sprague Dawley rats (280–350 g) were acquired from Charles River (Sulzfeld, Germany) and an Institute of Pharmacology PAS (Krakow, Poland) breeding facility. Animals were housed five per cage in a temperature and humidity controlled room (20–22 °C, 40–50% humidity), on a 12 h light/dark cycle (lights on at 7 a.m.), with *ad libitum* access to food and water. Before any surgical procedures, rats were allowed to acclimate to the facility for one week. After surgery, all animals were housed singly. All behavioral tests were performed during the light phase of the cycle. All experimental procedures were conducted according to the EU Guide for the Care and Use of Laboratory Animals and were approved by the Committee on the Ethics of Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences (Krakow, Poland) as well as the Committee on the Ethics of Animal Experiments at the Jagiellonian University.

2.2. Drugs

Prazosin hydrochloride (Praz; 0.1–1 μg, Sigma-Aldrich, Germany) – a selective α_1-AR antagonist – was dissolved in PBS and sonicated before microinjections. Terazosin hydrochloride (Teraz; 1 μg, Sigma-Aldrich, Germany) – another selective α_1-AR antagonist with better solubility – was dissolved in PBS. All drugs were infused into the VTA in a volume of 0.5 μL (Praz: 0.24–2.38 nmol/side; Teraz: 2.36 nmol/side) at a rate of 0.5 μL/min, using a Hamilton 25 gauge syringe. After infusion, the internal cannula was left in place for one additional minute to allow adequate absorption of the drug. The doses for all experiments were calculated based on previous work from our laboratory and others' demonstrating the ability of prazosin administration to modulate behavior (Azami et al., 2010; Ecke et al., 2012; Goertz et al., 2015).

2.3. Surgery

All rats were habituated to handling by the experimenters for at least five consecutive days prior to surgery. Rats were anesthetized with ketamine HCl (100 mg/kg, i.m., Biowet-Puławy, Poland) and xylazine (10 mg/kg, i.m., Biowet-Puławy, Poland) and placed in a stereotaxic frame (Stoelting Europe, Ireland) for intracranial cannula implantation. All coordinates were obtained from the rat brain atlas (Paxinos and Watson, 2007) with anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) positions referenced from Bregma. Bilateral guide cannula (Plastics One, Roanoke, VA, USA) were placed dorsal to the VTA (AP −5.2 mm, ML ± 0.5 mm, DV −7 mm). In addition, for control experiments, additional group of animals had bilateral guide cannulas placed in the mammillary body (MB) region (mammillary nuclei and medial supramammillary (AP − 4.5 mm, ML ± 0.5 mm, DV − 8.2 mm). Next, four anchor screws (Agnthos, Sweden) were mounted in the skull and dental cement (Duracryl, SpofaDental, Czech Republic) was used to ensure stability of the cannula. Guide cannula patency was ensured by inserting a matching dummy infusion cannula and a dust cap. After the surgery, animals were given an anti-inflammatory

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