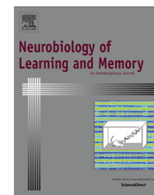
Neurobiology of Learning and Memory 144 (2017) 186–197

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme



Effects of chemogenetic excitation or inhibition of the ventrolateral periaqueductal gray on the acquisition and extinction of Pavlovian fear conditioning

Carolyn Arico [a], Elena E. Bagley [b], Pascal Carrive [c], Neda Assareh [a], Gavan P. McNally [a,*]

[a] School of Psychology, UNSW Sydney, Australia
[b] Discipline of Pharmacology, University of Sydney, Australia
[c] School of Medical Sciences, UNSW Sydney, Australia

ARTICLE INFO

Article history:
Received 29 May 2017
Revised 11 July 2017
Accepted 13 July 2017
Available online 14 July 2017

Keywords:
Periaqueductal gray
DREADD
Extinction
Fear
Conditioning
Conditioned suppression

ABSTRACT

The midbrain periaqueductal gray (PAG) has been implicated in the generation and transmission of a prediction error signal that instructs amygdala-based fear and extinction learning. However, the PAG also plays a key role in the expression of conditioned fear responses. The evidence for a role of the PAG in fear learning and extinction learning has been obtained almost exclusively using PAG-dependent fear responses. It is less clear whether the PAG regulates fear learning when other measures of learned fear are used. Here we combined a chemogenetic approach, permitting excitation or inhibition of neurons in the ventrolateral PAG (VLPAG), with conditioned suppression as the measure of learned fear to assess the role of VLPAG in the acquisition and extinction of fear learning. We show that chemogenetic excitation of VLPAG (with some encroachment on lateral PAG [LPAG]) impairs acquisition of fear and, conversely, chemogenetic inhibition impairs extinction of fear. These effects on fear and extinction learning were specific to the combination of DREADD expression and injection of CNO because they were observed relative to both eYFP controls injected with CNO as well as DREADD expressing controls injected with vehicle. Taken together, these results show that activity of L/VLPAG neurons regulates both the acquisition and extinction of Pavlovian fear learning.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Pavlovian fear conditioning enables learning about, and adaptive responding to, sources of danger in the world. The amygdala is critical for this learning as well as the responding (Davis, 1992; Lüthi & Luscher, 2014; Maren & Quirk, 2004; Pare, Quirk, & Ledoux, 2004; Schafe, Nader, Blair, & Ledoux, 2001). Principal cells of the basolateral amygdala (BLA) receive glutamatergic inputs from thalamus and cortex conveying information about the conditioned stimulus (CS) and aversive footshock unconditioned stimulus (US) (Farb & Ledoux, 1999; Lanuza, Moncho-Bogani, & Ledoux, 2008; Sah, Faber, Lopez De Armentia, & Power, 2003; Shi & Davis, 1999). The activity of these principal neurons is sufficient for fear learning (Johansen, Hamanaka et al., 2010). These neurons are subject to complex regulation by GABAergic interneurons (Ehrlich et al., 2009; Tovote, Fadok, & Lüthi, 2015; Wolff et al., 2014), show synaptic plasticity during fear conditioning, and form fear memories in an NMDA receptor-dependent manner (Marek, Strobel, Bredy, & Sah, 2013; Maren & Quirk, 2004; McKernan & Shinnick-Gallagher, 1997).

Several lines of evidence implicate the midbrain periaqueductal gray (PAG) and an ascending circuitry via midline thalamus and prefrontal cortex in instruction of this amygdala-based fear learning (McNally, Johansen, & Blair, 2011). Specifically, the PAG has been implicated in the generation and ascending transmission of a prediction error signal reporting the difference between the actual and expected outcomes of a fear conditioning trial (Johansen, Tarpley, Ledoux, & Blair, 2010; McNally, 2009; McNally & Westbrook, 2006; McNally et al., 2011; Ozawa et al., 2016). The PAG consists of four columns dorsomedial (DMPAG), dorsolateral (DLPAG), lateral (LPAG), and ventrolateral (VLPAG) to the cerebral aqueduct. It receives extensive projections from prefrontal cortex (including cingulate, prelimbic, infralimbic and orbital), extended amygdala (especially central nucleus and bed nucleus), and ascending projections from spinal and trigeminal dorsal horn (Carrive, 1993; Carrive & Morgan, 2003; Floyd, Price,

* Corresponding author at: School of Psychology, UNSW Australia, Sydney, NSW 2052, Australia.
E-mail address: g.mcnally@unsw.edu.au (G.P. McNally).

http://dx.doi.org/10.1016/j.nlm.2017.07.006
1074-7427/© 2017 Elsevier Inc. All rights reserved.

Ferry, & Keay, 2006; Keay & Bandler, 2004; Rizvi, Ennis, Behbehani, & Shipley, 1991). The PAG, in turn, has significant ascending projections to hypothalamus, midline and intralaminar thalamus (Krout & Loewy, 2000), as well as descending projections to premotor and sensory regions in the brainstem and spinal cord (Carrive & Morgan, 2003; Keay & Bandler, 2004). We and others have shown that pharmacological manipulation of the PAG, and the VLPAG in particular, alters the acquisition and extinction of fear learning. For example, microinjections of opioid receptor antagonists into the VLPAG prevent the associative blocking (Cole & McNally, 2007; Cole & McNally, 2008; McNally & Cole, 2006), overexpectation (Cole & McNally, 2008), and extinction (McNally, Lee, Chiem, & Choi, 2005; McNally, Pigg, & Weidemann, 2004b; Parsons, Gafford, & Helmstetter, 2010) of Pavlovian fear conditioning whereas broad inhibition of PAG via infusions of the GABA agonist muscimol disrupts both fear learning and the transmission of shock US related information to basolateral amygdala (Johansen, Tarpley et al., 2010).

However, this evidence implicating PAG in fear learning suffers from two limitations. First, it relies almost exclusively on assessment of fear via the species-typical defense response of freezing. Freezing, as a measure of fear, is useful because it is rapidly established, easily measured, and part of the natural defensive repertoire of rodents (Blanchard & Blanchard, 1971; Bouton & Bolles, 1980; Fanselow & Lester, 1988). However, understanding the role of PAG in fear learning using freezing can be confounded by the role of PAG in fear expression (Assareh, Sarrami, Carrive, & McNally, 2016; Chen et al., 2015; Tovote et al., 2016) and its obligatory role in expression of freezing as a conditioned fear response (Amorapanth, Nader, & Ledoux, 1999; Carrive, 1993; Tovote et al., 2016; Vianna, Graeff, Brandao, & Landeira-Fernandez, 2001; Vianna, Graeff, Landeira-Fernandez, & Brandao, 2001; Walker & Carrive, 2003). Any claim regarding the role of PAG in the acquisition and extinction of fear requires evidence from measures of fear learning that do not rely on PAG for their expression. Second, this evidence rests heavily on pharmacological manipulation of PAG. The strongest evidence is derived from studies of opioid receptor manipulations in VLPAG that show selective antagonism of mu-opioid receptors affects the acquisition and extinction of fear learning (Cole & McNally, 2007; McNally & Cole, 2006). A difficulty here is that opioid receptors are expressed on both PAG cell bodies and the terminals of major inputs to PAG (Mansour, Fox, Akil, & Watson, 1995; Mansour, Fox, Burke, Akil, & Watson, 1995; Mansour, Khachaturian, Lewis, Akil, & Watson, 1998), making it difficult to relate the effects of these pharmacological manipulations to the activity of PAG neurons (Behbehani, 1995; da Costa Gomez & Behbehani, 1995; Ozawa et al., 2016).

Here we addressed these limitations using a chemogenetic approach to manipulate PAG neuronal activity during the acquisition and extinction of fear learning as measured via conditioned suppression. The chemogenetic approach allowed us to express the excitatory (hM3Dq) or inhibitory (hM4Di) designer receptors exclusively activated by designer drugs (DREADDs) (Armbruster, Li, Pausch, Herlitze, & Roth, 2007; Urban & Roth, 2015) in VLPAG neurons and to remotely control the activity of these neurons via systemic injection of clozapine-*N*-oxide (CNO). Conditioned suppression of lever pressing for reward (Estes & Skinner, 1941) is a robust and well-established measure of fear. The expression of conditioned suppression is highly correlated with freezing (Bouton & Bolles, 1980) but conditioned suppression is not due to behavioural interference from, or response competition with, freezing (Amorapanth et al., 1999; Ayres & Vigorito, 1984; Bevins & Ayres, 1992). It provides a measure of fear independent from PAG because animals with virtually complete VLPAG lesions express fear to an auditory CS as measured via conditioned suppression but do not express freezing to this CS (Amorapanth et al., 1999).

2. Materials and methods

2.1. Subjects

Subjects were 130 experimentally naive adult male Sprague-Dawley rats (280–400 g), obtained from Animal Resource Centre (Murdoch, Western Australia). There were 8 animals for *in vitro* slice experiments. 64 rats in Experiment 1, 47 rats in Experiment 2, and 15 rats in Experiment 3. Rats were housed in groups of four in a colony room maintained on a 12:12 hr light–dark cycle (lights on at 7 am); all procedures were conducted during the light phase. Food and water were available *ad libitum* prior to behavioural training in all experiments. Rats in Experiments 1 and 2 were food restricted three days prior to behavioural procedures and maintained on 90% of their free feeding weight, water access remained unrestricted. The procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales and the University of Sydney, and conducted in accordance with the *Australian Code for the Care and Use of Animals for Scientific Purposes* (eight edition).

2.2. Apparatus

For Experiments 1 and 2, all behavioural procedures were conducted in a set of eight identical Med Associates chambers that measured 24 cm (length) × 30 cm (width) × 21 cm (height). The floor consisted of steel rods 4 mm in diameter, spaced 15 mm apart. The top and rear walls, and front hinged door of these chambers were made of clear Perspex. The end walls consisted of stainless steel panels. The chambers were illuminated during testing with a house light located in the rear wall. A magazine (5 cm × 5 cm in diameter) with a dish was located in the middle panel on the front wall of the chamber and attached to a pellet delivery system. A retractable lever was situated on the front wall, 4 cm to the right of the magazine. Depression of the lever resulted in delivery of a 45 mg grain pellet (Able Scientific Biotechnology). These chambers were situated in sound-attenuating cubicles (83 cm length × 59 cm width × 59 cm height) where ventilation fans produced a constant background noise. Two CSs were used. The visual CS (CSA) was a 60 s, flashing LED (8 cm length × 5 cm width × 3 cm height) mounted to the ceiling of the sound attenuating chamber. The auditory CS (CSB) was a 60 s, 80 dB clicker delivered through a speaker mounted to right side wall of the chamber. The US was a 0.8 mA scrambled footshock delivered through the grid floor; it was 0.5 s in duration.

For Experiment 3, two transparent plastic circular bowls (35 cm deep, 40 cm diameter) with corncob bedding served as the test chambers. The bowls were situated in sound attenuating chambers (83 cm length × 59 cm width × 59 cm height) with video cameras located above and to the rear of the bowls allowing an unobstructed view of the chambers. An LED (8 cm length × 5 cm width × 3 cm height) situated at the rear right wall of the chambers produced constant illumination during habituation and test sessions.

2.3. Viral vectors

Adenoassociated viral (AAV) vectors encoding eYFP, hM4Di DREADD, or hM3Dq DREADD were obtained from the University of North Carolina Vector Core (Chapel Hill NC). The vectors used in these experiments were AAV5-hSyn-eYFP (2×10^{12}vp/ml titer),

Download English Version:

https://daneshyari.com/en/article/5043122

Download Persian Version:

https://daneshyari.com/article/5043122

Daneshyari.com