Neurobiology of Learning and Memory 145 (2017) 7–17

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Investigating the role of dopamine receptor- and parvalbumin-expressing cells in extinction of conditioned fear

Heather B. Madsen [1], Alexandre A. Guerin [1], Jee Hyun Kim *

Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, Parkville, VIC 3052, Australia
Florey Department of Neuroscience and Mental Health, University of Melbourne, Parkville, VIC 3052, Australia

ARTICLE INFO

Article history:
Received 10 March 2017
Revised 9 August 2017
Accepted 21 August 2017

Keywords:
Dopamine
Parvalbumin
Fear extinction
Fear retrieval
Mice
Infralimbic cortex

ABSTRACT

The present study examined the pattern of activation of neurons that express dopamine receptors 1 and 2 (D1R and D2R), and parvalbumin (PV) in mice that underwent extinction of a fear memory. Adult male transgenic mice expressing D1R or D2R tagged with green fluorescent protein (GFP) were conditioned with 6 tone-shock pairings. The following day they were randomly divided into one of four experimental groups: extinction, retrieval, context or handled. Extinction groups were exposed to 45 tone presentations, retrieval groups were exposed to 5 tone presentations and the context groups were exposed to the chamber without any tones. Ninety minutes following their assigned treatment, mice were perfused and brain tissue processed for Fos/GFP/PV immunohistochemistry. Quantification of immunoreactivity revealed that extinction resulted in changes in the infralimbic cortex including increased Fos expression and a decrease in the number of D2R+ cells compared to all other groups. Conversely, fear memory retrieval resulted in increased activation of D2R+ cells in the prelimbic cortex compared to all other groups. Additional changes were observed in the extinction and retrieval groups that were different to the handled group, but not to the context group, which highlights that there is overlapping neurocircuitry between extinction and retrieval of fear memory, as well as with context exposure. These results provide novel insights into the roles of specific dopamine receptor subtypes, which will be valuable for informing future research that aims to strengthen extinction learning via dopaminergic mechanisms.

1. Introduction

Pavlovian fear conditioning and extinction have been studied extensively in the laboratory to understand learning and memory processes. While fear conditioning to a discrete conditioned stimulus (CS) forms a robust and context-independent memory, CS extinction memory is tied to the context where the memory is formed, hence the return of extinguished fear is a common phenomenon (Bouton, 2002). There is significant interest in trying to find ways to reduce the return of fear by enhancing the strength of extinction learning, as it forms the basis for exposure therapy used to treat many anxiety disorders (Hofmann, 2008; Kim & Ganella, 2015). A better understanding of the neurobiological basis of extinction is a critical step in achieving this goal.

Both the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) form the neural circuitry that underlies the acquisition and consolidation of extinction memory (Lin, Yeh, Lu, & Gean, 2003; Santini, Ge, Ren, Pena de Ortiz, & Quirk, 2004). These regions receive dopaminergic innervation from the ventral midbrain (Pinard, Muller, Mascagni, & McDonald, 2008; Pinto & Sesack, 2008) and dopamine signalling plays an important role in extinction learning (Abraham, Neve, & Lattal, 2014). For example, systemic administration of the dopamine precursor L-DOPA following extinction training has been shown to enhance extinction memory in both mice and humans (Haaker et al., 2013). Pre-extinction infusion of a dopamine 1 receptor (D1R) antagonist into the BLA impairs the acquisition of extinction memory (Hikind & Maroun, 2008), whereas infusion of a D1R antagonist or dopamine receptor 2 (D2R) antagonist into the infralimbic (IL) subregion of the mPFC impairs extinction consolidation (Hikind & Maroun, 2008; Mueller, Bravo-Rivera, & Quirk, 2010).

Importantly, D1R and D2R signalling can profoundly modulate parvalbumin (PV)-containing interneurons in the BLA and the mPFC (McDonald & Mascagni, 2001; Wilson et al., 2015), which may have a critical role in extinction (Brown et al., 2015). PV-interneurons

* Corresponding author at: Behavioural Neuroscience Division, The Florey Institute of Neuroscience and Mental Health, Kenneth Myer Building, 30 Royal Pde, Parkville, VIC 3052, Australia.
E-mail address: drjeehyunkim@gmail.com (J.H. Kim).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.nlm.2017.08.009
1074-7427/

make up approximately 50% of the interneuronal population in the neocortex (Markram et al., 2004) and are the largest interneuron population in the BLA (McDonald & Mascagni, 2001). PV-interneurons have mostly been studied in the context of fear learning; in the BLA they are important for acquisition of conditioned fear (Wolff et al., 2014) and in the mPFC they are involved in fear expression (Courtin et al., 2014). More recently however, evidence has emerged that PV-interneurons in the mPFC are important for extinction of reward-based learning (Sparta et al., 2014) and loss of PV interneurons in the IL has been associated with impaired fear extinction retention (Baker & Reichelt, 2016). While these results support a role for PV-interneurons in extinction learning, research in this area has so far been relatively limited. Also, very little is known on the interaction between PV-interneurons and dopamine that may subserve extinction processes.

The overall aim of the present study was to gain further insight into the role of dopamine signalling and PV-interneurons in extinction of conditioned fear. To achieve this we examined the pattern of activation of D1R-, D2R- and PV-expressing cells in the prelimbic cortex (PrL), IL and BLA as a result of extinction compared against retrieval, context exposure, and handling. Reporter mice expressing green fluorescent protein (GFP)-tagged D1R or D2R were used in combination with immunohistochemistry for GFP, PV and Fos, an immediate early gene used as a marker of neuronal activation (Dragunow & Faull, 1989). Information about the pattern of activation of these cells during extinction will be extremely valuable in the quest to identify pharmacotherapies that enhance the acquisition and retention of extinction memory.

2. Methods

2.1. Animals

A total of 56 male mice were used in this study (see Table 1 for n for each group). Separate breeding colonies of Drd1a-EGFP and Drd2-EGFP mice bred on a Swiss background were established at the Florey Institute of Neuroscience and Mental Health, Melbourne, Australia. Mice were originally generated by the Gene Expression Nervous System Atlas (GENSAT) program at the Rockefeller University, New York, USA (Gong et al., 2003). All mice were 10–14 weeks of age at the start of experimentation, and only mice hemizygous for the mutation were used in the study.

All mice were initially group housed in individual ventilated cages (IVC) with littermates, and were transferred to open top cages and maintained on a 12-h reversed light/dark cycle (lights off at 7 am) at least one week prior to the start of experimentation. Food and water were available *ad libitum*. All experiments were conducted according to *the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (8th Edition; Australian Government Publishing Service, 2013). All procedures were approved by the Animal Ethics Committee (AEC) of the Florey Institute of Neuroscience and Mental Health.

2.2. Genotyping

Mouse pups were genotyped using PCR. DNA was extracted from tail samples using the REDExtract-N-AMP™ Tissue PCR Kit (Sigma-Aldrich, Castle Hill, Australia) and PCR was performed using GoTaq® Green Master Mix (Promega, Alexandria, Australia) according to recommended protocols. The following forward and reverse primers were used:

D1-EGFP forward primer: 5′-ACC GGA AGT GCT TTC CTT CTG GA-3′
D1-EGFP reverse primer: 5′-TAG CGG CTG AAG CAC TGC A-3′

Table 1
Number of mice in each group for each genotype.

	Genotype	
Group	Drd1a-EGFP	Drd2-EGFP
Tone	3	4
Handled	7	7
Context	5	6
Retrieval	7	5
Extinction	6	6

D2-EGFP forward primer: 5′-GAG GAA GCA TGC CTT GAA AA-3′
D2-EGFP reverse primer: 5′-TGG TGC AGA TGA ACT TCA GG-3′

Primers were purchased from Geneworks, Hindmarsh, Australia.

2.3. Behaviour

2.3.1. Apparatus

Fear conditioning and extinction sessions took place in the Contextual NIR Video Fear Conditioning System for Mouse (Med Associates Inc., VT, USA) and freezing behaviour was recorded and automatically quantified using Video Freeze® software (Med Associates Inc.). The conditioned stimulus (CS) was a tone (volume: 80 dB, frequency: 5000 Hz) and the unconditioned stimulus (US) was a 1 s (1.0 mA). Fear chambers were designated as either 'context A' or 'context B'. Context A had curved white walls with green stripes, the tray beneath the grid was lined with paper towel and the house light remained on during the sessions. Context B had plain steel walls, the tray beneath the grid was lined with aspen bedding and the house light remained off.

2.3.2. Fear conditioning

On Day 1, all mice were fear conditioned as previously described (Handford, Tan, Lawrence, & Kim, 2014). The first 120 s in the chambers served as a baseline freezing recording period. This was followed by the mice receiving the first of six CS-US trials. For each trial, a 10 s tone CS was presented co-terminating with a 1 s footshock US. The inter-trial (ITI) was average 110 s ranging from 85 to 135 s. After the end of the last pairing, mice remained in the chamber for a further 120 s before being returned to their home cage. There was an additional group of mice (Tone) that were handled but not conditioned. These mice were run separately from all the other groups, therefore, it is not included in any of our analyses.

2.3.3. Extinction

Mice were randomly assigned to one of four experimental groups: Extinction, Retrieval, Context or Handled. On day 2, mice in the Extinction, Retrieval and Context groups were placed in a context different from where they were conditioned. A different context was used to examine CS-elicited freezing (Handford et al., 2014). After a 120 s baseline recording period, mice in the Extinction group received 45 10 s CS presentations with an ITI of 10 s. They remained in the chambers for an additional 120 s after the last CS presentation. Mice in the Retrieval group were placed in the extinction context for the same length of time except that they were only exposed to 5 CSs presented at the beginning of the session (separated by a 10 s ITI). Mice in the Context group were also exposed to the extinction context for the same period of time but without any CS presentations. Mice in the handled group were transported and handled in the same manner as mice in the other groups, except that they were returned to their home cage without being placed in the extinction chamber. There was an