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An organization of visual and auditory fear conditioning in the lateral amygdala

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ABSTRACT

Pavlovian fear conditioning is an evolutionary conserved and extensively studied form of associative learning and memory. In mammals, the lateral amygdala (LA) is an essential locus for Pavlovian fear learning and memory. Despite significant progress unraveling the cellular mechanisms responsible for fear conditioning, very little is known about the anatomical organization of neurons encoding fear conditioning in the LA. One key question is how fear conditioning to different sensory stimuli is organized in LA neuronal ensembles. Here we show that Pavlovian fear conditioning, formed through either the auditory or visual sensory modality, activates a similar density of LA neurons expressing a learning-induced phosphorylated extracellular signal-regulated kinase (p-ERK1/2). While the size of the neuron population specific to either memory was similar, the anatomical distribution differed. Several discrete sites in the LA contained a small but significant number of p-ERK1/2-expressing neurons specific to either sensory modality. The sites were anatomically localized to different levels of the longitudinal plane and were independent of both memory strength and the relative size of the activated neuronal population, suggesting some portion of the memory trace for auditory and visually cued fear conditioning is allocated differently in the LA. Presenting the visual stimulus by itself did not activate the same p-ERK1/2 neuron density or pattern, confirming the novelty of light alone cannot account for the specific pattern of activated neurons after visual fear conditioning. Together, these findings reveal an anatomical distribution of visual and auditory fear conditioning at the level of neuronal ensembles in the LA.

1. Introduction

It is widely thought that only a subset of neurons in a whole population encodes any given memory (Rumpel, LeDoux, Zador, & Malinow, 2005; Han et al., 2007; Bergstrom, McDonald, & Johnson, 2011; Chapeton, Fares, Lasota, & Stepanyants, 2012; Liu et al., 2012). What is not known is precisely which neurons in a population are allocated for memory encoding, and which neurons are not (Johnson, Ledoux, & Doyere, 2009). Localizing memory in neuronal subsets is a formidable research challenge (Krupa, Thompson, & Thompson, 1993) and is of clinical relevance for understanding disorders of learning and memory, such as post-traumatic stress disorder (PTSD) (Johnson, McGuire, Lazarus, & Palmer, 2011) and addictions (Hyman, 2005).

Pavlovian fear conditioning is an extensively used behavioral paradigm for studying learning and memory in the brain (Davis, 1992; LeDoux, 2000; Johnson et al., 2011; McGuire, Coyner, & Johnson, 2012). In Pavlovian fear conditioning, a previously innocuous sensory stimulus, such as a tone or light, quickly acquires negative valence (conditioned stimulus, CS) after being paired with a naturally fearful stimulus (unconditioned stimulus, US). As a result of CS and US pairing, a stable and lasting fear-evoking memory about the CS is formed. The molecular, physiological

Abbreviations: LA, lateral amygdala; LAd, dorsolateral amygdala; LAvl, ventrolateral amygdala; LAvm, ventromedial amygdala; LP, lateral posterior nucleus; TE2, secondary auditory cortical area 2; p-ERK1/2, phosphorylated extracellular signal-regulated kinase 1/2; LV, lateral ventricle; CS, conditioned stimulus; US, unconditioned stimulus; CV, coefficient of variance; SEM, standard error of the mean; aFC, auditory fear conditioning; vFC, visual fear conditioning; SD, standard deviation; PTSD, post-traumatic stress disorder.

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and anatomical framework of Pavlovian fear conditioning is well-characterized, with the lateral nucleus of the amygdala (LA) a key hub for the establishment of long lasting fear memory (Sah, Faber, Lopez De Armentia, & Power, 2003; Johnson & Ledoux, 2004; Lamprecht & LeDoux, 2004; Rodrigues, Schafe, & LeDoux, 2004; Pape & Pare, 2010). This makes the study of Pavlovian fear conditioning at the level of neuronal ensembles in the LA particularly advantageous because a significant portion of the engram has been localized (Schafe, Doyere, & LeDoux, 2005; Kwon & Choi, 2009).

We previously demonstrated that neurons activated after auditory fear conditioning are topographically organized in the LA (Bergstrom et al., 2011, 2013; Bergstrom, McDonald, Dey, Fernandez, & Johnson, 2013). The objective of the present study was to determine whether a redundant or new distribution of neurons is activated in the LA after visual fear conditioning. We selected the LA for mapping visual and auditory fear conditioning because it receives both visual and auditory sensory input (Doron & Ledoux, 1999; Pitkänen, 2000) and has been directly linked with the generation of new visually cued fear memories (Ledoux, Romanski, & Xagoraris, 1989; Campeau & Davis, 1995; Shi & Davis, 2001), although see (Tazumi & Okaichi, 2002). Precise topographic measures of an activated neuronal population following visual fear conditioning have never been conducted or compared with the topography of neurons activated after auditory fear conditioning. Detailed mapping of an activated neuron population in the LA following auditory or visual fear conditioning is an important preliminary step towards decoding the anatomical organization of more complex, multimodal associative fear memories in the brain.

The study of two different types of fear conditioning in the LA required we first experimentally control for differences in the strength of the expressed memory so the underlying neuron population was of equivalent size, and thus comparable. Memory strength was modified by calibrating the intensity of auditory and visual CS salience to produce equivalent levels of a conditioned defensive "fear" response (freezing). Topographic measurements of the activated neuronal population were conducted by mapping the 3D coordinates of LA neurons expressing the phosphorylated form of extracellular signal-regulated kinase 1/2 (p-ERK1/2), a well-validated molecular marker of learning-induced synaptic plasticity following fear conditioning (Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Schafe et al., 2000; Radwanska, Nikolaev, Knapska, & Kaczmarek, 2002; Paul et al., 2007; Schafe, Swank, Rodrigues, Debiec, & Doyere, 2008; Kim, Hamlin, & Richardson, 2009; Kim, Li, Hamlin, McNally, & Richardson, 2012; Olausson et al., 2012; Besnard, Laroche, & Caboche, 2013; Coyner et al., 2013) and see (Sweatt, 2001; Thomas & Huganir, 2004; Cestari, Rossi-Arnaud, Saraulli, & Costanzi, 2013) for review.

We found both a common and distinct anatomical organization of p-ERK1/2-expressing neurons in the LA after auditory and visual fear conditioning. This organization was independent of both the size of the total activated neuron population and the relative strength of the memory, suggesting that some portion of the auditory and visual fear memory trace is allocated differently based on the anatomical distribution of p-ERK1/2-expressing neurons in the LA. These data provide the first insight into how Pavlovian fear conditioning, formed through different sensory modalities, is represented and organized at the level of neuronal ensembles in the LA.

2. Materials and methods

2.1. Subjects

All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Experimental Animals* and were approved by the Uniformed Services University Institutional Animal Care and Use Committee (IACUC). Subjects were experimentally naïve male Sprague–Dawley rats (Taconic Farms, Derwood, MD). Rats weighing 225–250 g on arrival to the vivarium were group housed (2/cage) on a 12 h light:dark cycle (lights on 0600; lux 15) with food and water provided without restriction. Bedding was changed 2/week. The vivarium humidity (55%) and temperature (20.5 °C) was constantly maintained. Rats were allowed at least seven days of acclimation to the vivarium and handled on three days prior to testing. All experiments were conducted during the light phase. Rats weighed 413 ± 6.7 g (342.8–512.8 g) at time of testing. Disclosure of housing and husbandry procedures was in accordance with recommendations for standard experimental reporting in behavioral neuroscience research (Prager, Bergstrom, Grunberg, & Johnson, 2011).

2.2. Pavlovian fear conditioning

Sprague–Dawley rats ($N = 44$) were randomized into two experimental conditions (auditory fear conditioning, aFC, $n = 14$; visual fear conditioning, vFC, $n = 17$) and two control conditions (shock alone, Shock, $n = 6$; box alone, Box, $n = 7$). All rats were allowed to explore (habituated) both the fear conditioning (Context A) and testing (Context B) chambers in counterbalanced order for 30 min each on three consecutive days prior to fear conditioning. Context A and B were distinguished by olfactory, visual, and tactile cues (background lux 1.0 and db < 50 for both chambers). On the training day, following three min of acclimation in Context A, rats were presented either two pairings of an auditory CS (2 kHz, 55 dB, 20 s) or visual CS (1 Hz: 0.5 s On/0.5 s Off for 20 s, 35 lux) that co-terminated with a mild foot shock US (0.6 mA, 500 ms). The mean random intertrial interval (ITI) duration was 120 s. Rats were removed from the chamber 60 s after the final stimulus presentation and returned to the vivarium. There were two control conditions. In the Shock alone condition (Shock), rats were presented the US without the auditory or visual CSs. Rats in the Box alone control condition (Box) were handled, habituated and exposed to Context A for the same duration of time as the experimental conditions but did not undergo fear conditioning.

Twenty-four hours later, a randomized subset of rats in the aCS ($n = 7$) and vCS ($n = 8$) conditions were placed into Context B for three minutes and then were replayed either the auditory CS or visual CS three times for 20 s each to test the expression of the auditory or visual cued fear memory. The mean ITI was 120 s. An experimenter blind to the experimental condition of the animals scored freezing behavior from digitized videos. Freezing is a behavioral index of conditioned fear (Blanchard & Blanchard, 1969). For the CS test, freezing was scored during the three min prior to the CS and during the CSs (20 s intervals). A mean freezing value was calculated during the presentation of the CS and transformed into a percentage freezing. Mean freezing percentage was the dependent variable for all behavioral analyses.

2.3. p-ERK1/2 immunohistochemistry

The presence of p-ERK1/2 in LA neurons served as a molecular marker of neuroplasticity associated with Pavlovian fear conditioning consolidation (Schafe et al., 2000) and see (Davis & Laroche, 2006) for review. The expression of p-ERK1/2 following auditory fear conditioning is predominantly localized to principal cell-type neurons in the LA (Bergstrom, McDonald, Dey, Tang, et al., 2013).

2.3.1. Tissue preparation

Rats were anesthetized for perfusion exactly 60 min after fear conditioning (Schafe et al., 2000). Rats were anesthetized with an intraperitoneal (i.p.) injection of a ketamine/xylazine (100 mg/kg,

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