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A dendritic organization of lateral amygdala neurons in fear susceptible and resistant mice

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ABSTRACT

Subtle differences in neuronal microanatomy may be coded in individuals with genetic susceptibility for neuropsychiatric disorders. Genetic susceptibility is a significant risk factor in the development of anxiety disorders, including post-traumatic stress disorder (PTSD). Pavlovian fear conditioning has been proposed to model key aspects of PTSD. According to this theory, PTSD begins with the formation of a traumatic memory which connects relevant environmental stimuli to significant threats to life. The lateral amygdala (LA) is considered to be a key network hub for the establishment of Pavlovian fear conditioning. Substantial research has also linked the LA to PTSD. Here we used a genetic mouse model of fear susceptibility (F-S) and resistance (F-R) to investigate the dendritic and spine structure of principal neurons located in the LA. F-S and F-R lines were bi-directionally selected based on divergent levels of contextual and cued conditioned freezing in response to fear-evoking footshocks. We examined LA principal neuron dendritic and spine morphology in the offspring of experimentally naive F-S and F-R mice. We found differences in the spatial distribution of dendritic branch points across the length of the dendrite tree, with a significant increase in branch points at more distal locations in the F-S compared with F-R line. These results suggest a genetic predisposition toward differences in fear memory strength associated with a dendritic branch point organization of principal neurons in the LA. These micro-anatomical differences in neuron structure in a genetic mouse model of fear susceptibility and resistance provide important insights into the cellular mechanisms of pathophysiology underlying genetic predispositions to anxiety and PTSD.

1. Introduction

Genetics is a determining risk factor for the development of anxiety disorders (Broekman, Olff, & Boer, 2007; Johnson, Mcguire, Lazarus, & Palmer, 2011). Family and twin studies have found that more than 30% of the variance associated with the development of emotional disorders such as Post Traumatic Stress Disorder (PTSD) is heritable (Kremen, Koenen, Afari, & Lyons, 2012; Skelton, Ressler, Norrholm, Jovanovic, & Bradley-davino, 2012). Pavlovian fear conditioning has been proposed to model key aspects of PTSD (Johnson et al., 2011; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). According to this theory, PTSD begins with a Pavlovian conditioned fear memory linking stimuli from the environment to significant threats to life. The development of PTSD is thus associated to an initial fear memory whereby PTSD contains elements of inappropriate stimuli and threat association and/or a memory of exaggerated magnitude, which could interact with its ability to be appropriately extinguished (Johnson et al., 2011). Previous studies have shown that a predisposition for Pavlovian fear is a highly heritable trait in mice, rats and humans (Balogh & Wehner,

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2003; de Castro Gomes & Landeira-Fernandez, 2008; Hettema, Annas, Neale, Kendler, & Fredrikson, 2003; Johnson et al., 2011). For modeling a genetic predisposition of excessive fear, several behaviorally selected lines of rats and mice have been developed using Pavlovian fear conditioning acquisition and extinction (Corda, Piras, Piludu, & Giorgi, 2014; de Castro Gomes & Landeira-Fernandez, 2008; Ponder et al., 2007; Shumake, Furgeson-Moreira, & Monfils, 2014), as well as several other anxiety-related phenotypes (for a review see Gomes et al., 2013). Reports consistently indicate that mouse and rat lines selected for phenotypic divergence in anxiety-like behaviors exhibit differential activation patterns in limbic circuitry that includes the amygdala complex (Mormède et al., 2002; Muigg et al., 2009; Singewald, 2007). Two mouse lines that have recently been developed using Pavlovian fear conditioning are the Fear Resistant (F-R) and Susceptible (F-S) mouse lines (McGuire et al., 2013; Parker, Sokoloff, Cheng, & Palmer, 2012; Ponder et al., 2007). F-S and F-R lines were derived from an F_8 advanced intercross line (AIL) of C57BL/6J and DBA/2J strains initially developed by Abraham A. Palmer at the University of Chicago (Parker et al., 2012). Previous investigations into the F-R and F-S lines revealed that intrinsic differences in limbic circuit activity were associated with phenotypic fear memory differences. F-S mice also exhibited higher levels of serum corticosterone before fear conditioning, as well increased hypothalamic corticotrophin-releasing hormone (CRH) mRNA expression compared with F-R animals (McGuire et al., 2013). F-S mice also exhibited a greater density of neurons expressing the phosphorylated form of mitogen-activated protein kinase (p44/42 ERK/MAPK) after Pavlovian fear conditioning (Coyner et al., 2014). ERK/MAPK is required for the consolidation of Pavlovian fear conditioning in the lateral amygdala (LA). Other recent studies have indicated additional anxiety-related phenotypic and neurophysiologic differences in the F-S and F-R mouse lines (Choi et al., 2012).

In addition to neuroendocrine and behavioral traits, changes in dendrite morphology and spine patterning may also relate to a genetic predisposition in the strength of fear memory formation (Borrie et al., 2014; Camp et al., 2012; Dias et al., 2014; Mitra, Adamec, & Sapolsky, 2009; Nietzer et al., 2011; Pignataro & Ammassari-Teule, 2015; Pillai et al., 2012). Dendritic morphology shapes circuit signaling and modifications to dendrite and spine structure represent an important neuroanatomical correlate of memory formation and storage in the brain (Papoutsi, Kastellakis, Psarrou, Anastasakis, & Poirazi, 2014). Amygdala dendrites are especially sensitive to stress. Rats subjected to chronic or acute stress showed enhanced dendritic morphology and increased spine number in amygdala principal neurons, the main class of glutamatergic excitatory neuron involved in fear memory acquisition in the amygdala (Leuner & Shors, 2013; Padival, Blume, & Rosenkranz, 2013; Vyas, Bernal, & Chattarji, 2003; Vyas, Jadhav, & Chattarji, 2006; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002). In addition to stress, changes in dendrite and spine structure have been linked with fear conditioning and extinction (Heinrichs et al., 2013).

LA subnuclei incorporate afferent multimodal sensory information required for the establishment of mammalian associative fear memories, for both continuous and discrete conditioned stimuli (Bergstrom et al., 2012; Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001). Sensory information necessary for the generation of Pavlovian fear conditioning is subjected to a substantial level of processing before it leaves the LA. Excitability of LA neurons is closely linked with the development of Pavlovian fear (Gouty-Colomer et al., 2015) and blockade of intracellular signaling cascades essential for synaptic plasticity in the LA impairs Pavlovian fear conditioning (Blair et al., 2001; Nader, Schafe, & Ledoux, 2000; Schafe & LeDoux, 2000). Dendrites and spines contain the structural apparatus required for synaptic plasticity (Faber, Callister, & Sah, 2013; Papoutsi et al., 2014; Spruston, 2008). How dendrite morphology and spine density of LA principal neurons in the LA segregate with the F-R and F-S mouse lines is unknown. To address this question, we used a Golgi–Cox staining preparation and naïve S_4 generation F-S and F-R mouse lines to investigate dendritic morphology, spine morphology, spine density and spine distribution in LA principal neurons. Since we employed experimentally naïve animals to study baseline phenotypic differences in dendrite morphology, we also characterized behavioral differences in contextual and cued fear acquisition in the parental S_3 generation that created S_4 generation of F-S and F-R lines.

2. Methods

2.1. Animals

Mouse lines were derived from an F_8 advanced intercross line (AIL) of C57BL/6J and DBA/2J mouse strains originally developed in the laboratory of Abraham A. Palmer at the University of Chicago (Parker et al., 2012). In this study, the line was reestablished in the laboratory of Luke Johnson at the Uniformed Services University of the Health Sciences (USUHS) (McGuire et al., 2013). To create the new divergent F-R and F-S mouse lines, F_8 AIL S_1 animals were trained for contextual fear conditioning at University of Chicago and then sent to USUHS, where selection for both contextual and cued fear conditioning was maintained for 3 generations (S_2–S_4). Contextual freezing was employed as the main criterion of selection to generate the F-S and F-R lines (Fig. 1A). Auditory cued fear was used as secondary criterion, in order to differentiate animals with similar levels of contextual freezing during the selective breeding process (for details see McGuire et al., 2013). In order to study baseline phenotypic differences in dendrite morphology we used fear memory S_4 naïve F-S ($n = 5$) and F-R ($n = 5$) adult (8–12 weeks) males. Therefore behavioral data is presented for parental S_3 generation. The S_3 population consisted of 131 animals from the F-S line (64 males and 67 females), and 102 animals from the F-R line (55 males and 47 females). All animals were housed 2–5 per cage in a climate-controlled vivarium on a 12:12 light cycle (lights on 06:00) with *ad libitum* access to food and water. All experimental procedures were reviewed and approved by the appropriate (University of Chicago and USUHS) Institutional Animal Care and Use Committee (IACUC).

2.2. Fear conditioning procedure

S_3 generation F-R and F-S mice (8–12 weeks old) were tested for contextual and cued conditioned fear with a fear conditioning standard protocol previously described (McGuire et al., 2013; Ponder et al., 2007). This protocol was carried out prior the breeding procedures employed to create S_4 generation. Briefly, the fear conditioning procedure involved an acquisition session (day 1), a context fear test session (day 2) and a cued fear test session (day 3). During acquisition, each animal was placed in the observation chamber for 3 min, followed by two presentations of a pure tone conditioned stimulus (CS), which was co-terminated with three footshocks (2 s, 0.5 mA) delivered with a 30 s interval. The context fear test session occurred approximately 24 h after training and consisted of placing the animal for 10 min in the same chamber in which the three footshocks had been administered on the previous day. No footshock or other stimulation occurred during this period. Context-fear behavior was registered in the first 5 min. In order to avoid generalizations among experimental days, the conditioning chamber was altered for visual, olfactory and tactile cues for cued fear test on day 3. After 3 min of exploration in this