

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.

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1. Introduction

Genetics is a determining risk factor for the development of anxiety disorders (Broekman, Olf, & 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 F₈ 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 (Coynier 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, Anastakis, & 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 S₄ 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 S₃ generation that created S₄ generation of F-S and F-R lines.

2. Methods

2.1. Animals

Mouse lines were derived from an F₈ 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, F₈ AIL S₁ 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 (S₂–S₄). 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 S₄ naïve F-S ($n = 5$) and F-R ($n = 5$) adult (8–12 weeks) males. Therefore behavioral data is presented for parental S₃ generation. The S₃ 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

S₃ 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 S₄ 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

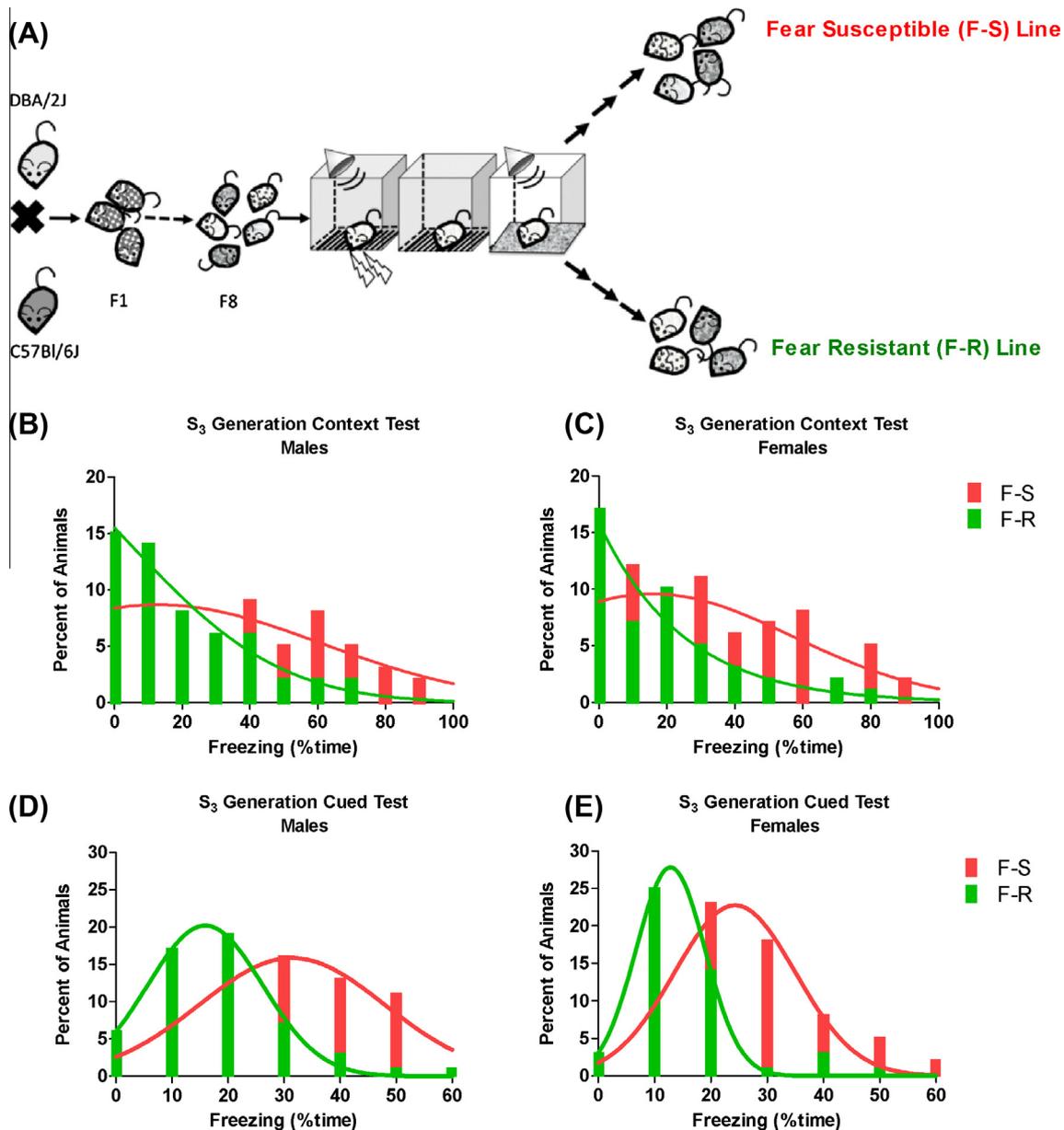


Fig. 1. Selective breeding procedure employed to produce the Fear Susceptible (F-S) and Fear Resistant (F-R) animals and behavioral evaluation of S₃ generation mice lines. Behavioral results are displayed as frequency distribution of mean percent (\pm SEM) of conditioned freezing in relation to mean percent of animal population. A: Mouse lines were initially derived from an F₈ advanced intercross line (AIL) between C57Bl/6J and DBA/2J strains. F₈ AIL S₁ animals were trained for contextual fear conditioning at University of Chicago and then shipped to the Uniformed Services University of the Health Sciences (USUHS), where selection for both contextual and cued fear conditioning was conducted for an additional 3 generations (S₂–S₄). For contextual fear, F-S line showed more conditioned freezing than F-R animals. ANOVA showed main effects for phenotype but not for sex. Independent *t*-test comparisons showed that F-S lines differed from F-R lines for both males (B) and females (C) ($p < 0.001$). For cued fear, analysis showed main effects for phenotype and sex (all $p < 0.05$). Post-hoc comparisons also showed that F-S lines presented more conditioned freezing to the tone CS than F-R lines for both males (D) and females (E) (all $p < 0.001$).

new environment, mice were then presented with two tones CS's which were identical to the training day, with a 30-s interval. Conditioned freezing response was registered and scored with ANY-MAZE analysis software (Stoelting, Wood Dale, IL, USA).

2.3. Tissue preparation

S₄ generation adult subjects were anesthetized with a 0.1 mg/kg ketamine/0.1 mg/kg xylazine cocktail and perfused intracardially with 0.9% saline. Brains were removed and stored in a Golgi–Cox solution (Sigma, St Louis, MO) for 14 days and then transferred to 30% sucrose solution until sectioning. All Golgi–Cox staining procedures were developed for this study using a standard protocol that has been previously described (Bergstrom, Donald,

French, & Smith, 2008). Brains were cut in 200 μ m coronal sections using a vibratome (Vibratome, USA) from bregma -0.58 to -3.16 mm and mounted in sequence.

2.4. Dendritic reconstruction and morphometry

Golgi–Cox stained sections containing the LA were visualized using light-microscopy and principal neurons were manually digitally reconstructed in 3D using NeuroLucida Software (MBF Biosciences, Williston, VT USA). Reconstruction of dendritic morphology and spine quantification were restricted to LA principal neurons under a 63X air objective. LA borders and subdivisions were demarcated based on a mouse brain atlas (K.B.G. Franklin 2012). Digital reconstructions were performed by an experimenter

(VCG) blind to treatment conditions. Only neurons isolated from each other, with untruncated dendrites and clear impregnation along the entire extent of the dendritic tree were chosen for digital reconstruction. Principal neurons were morphologically identified by an “apical-like” dendritic tree, biconical dendritic radiation and the presence of spines (Mancuso, Chen, Li, Xue, & Wong, 2012). 20 neurons for each group (F-S and F-R lines) and 2–6 neurons per mouse (5 mice/group) satisfied these criteria and were included for morphometric analysis. Quantitative morphometric parameters were extracted using NeuroExplorer software (MBF Bioscience, Williston, VT USA). Sholl analysis of concentric spheres, starting at 10 μm radius from the center of the soma with a fixed ratio of 20 μm , was applied to characterize the complexity of the dendritic tree (Milosević & Ristanović, 2007). Sholl analysis was also conducted on dendritic spines to determine their distribution. Morphometric parameters of the Sholl analysis included (1) number of nodes (or branch points) defined as the point where the dendrite sub-divides; (2) dendritic length, defined as the extension (in μm) of each dendritic branch; (3) trees endings, defined as the point where the dendritic tree terminates and (4) spine number and morphology. Spines were defined as small protrusions from the dendritic tree. The morphological subdivisions of spines (Thin, Mushroom, Stubby, Filopodia, Branched and Detached) were defined based on previous characterization (Knott & Holtmaat, 2008). Spines were manually counted and morphologically characterized across the extent of the dendritic tree analyzed. To ensure a comparable distribution of dendrite material across the length of the dendritic tree and across groups, we restricted morphometric analysis of dendritic structure and spine density to concentric circles $\leq 90 \mu\text{m}$ from the soma. Any missing values were replaced by the mean of the remaining distribution. Missing values accounted for 1.875% of the total observations.

2.5. Statistics

Fear conditioning was analyzed using two-way Analysis of Variance (ANOVA). “Phenotype” was the first factor with two levels (F-S and F-R) and “sex” the second factor with two levels (male and female). Post-hoc comparisons were performed using independent samples Student *t*-tests. Prior to analysis, we verified the data was normally distributed (Kolmogorov and Smirnov test). Outliers were detected using the extreme studentized deviate (ESD) method and subsequently excluded from the analysis and then replaced by the average of the remaining distribution. A mean value based on 2–6 neurons for each morphometric parameter was calculated for each subject. The mean value for each subject was used for all statistical comparisons. Statistical comparisons between F-S and F-R mice were performed through two-way repeated measures ANOVA. Between subject comparison was “phenotype” (F-S and F-R) and the within subject comparisons were “Sholl Radius”. In case of a significant interaction, multiple post hoc comparisons were performed with Student *t*-test. The level of confidence of all statistical comparisons was 95%. Analyses were conducted with SPSS (IBM SPSS, Armonk, New York, USA) and Graph Pad Prism (GraphPad Software, La Jolla, CA).

3. Results

3.1. *S*₃ generation of F-S and F-R lines differed in contextual and cued fear responses

In order to study baseline phenotypic differences we used experimentally naïve animals from the *S*₄ generation. In this sense, behavioral data is presented only for the whole parental generation (*S*₃) animals that created *S*₄ mice. A two-way ANOVA failed to

identify a sex \times phenotype interaction and failed to identify a main effect of sex on contextual fear (all $p > 0.05$). However, there was a main effect of phenotype ($F_{1,229} = 24.947$; $p < 0.001$) on contextual fear. Post-hoc analyses showed that F-S lines displayed increased conditioned freezing as compared to F-R lines for both males ($t_{117} = 3.529$; $p < 0.001$, Fig. 1B) and females ($t_{112} = 3.534$; $p < 0.001$, Fig. 1C). For cued fear, a two-way ANOVA failed to identify a two-way interaction, but showed main effects for phenotype ($F_{1,224} = 59.662$; $p < 0.001$) and sex ($F_{1,224} = 5.32$; $p = 0.22$). In the same manner, post hoc comparisons with independent *t*-test's also showed that the F-S line exhibited more conditioned freezing to the tone CS than the F-R line for both males ($t_{114} = 5.456$; $p < 0.001$, Fig. 1D) and females ($t_{110} = 5.507$; $p < 0.001$, Fig. 1E).

3.2. F-R and F-S lines do not differ on dendritic length, intersections, endings and spine morphology or distribution

All dendrites were reconstructed in 3D and spines characterized by morphological subtype (thin, stubby, mushroom, filopodia, branched and detached). Morphological parameters included branch point distribution, dendritic length, dendritic intersections and tree endings at concentric circles (Sholl analysis). Dendritic length, intersections, endings, spine distribution and morphology were not altered between F-S and F-R *S*₄ naïve mice. ANOVA on dendritic length revealed a non significant interaction and no phenotype main effects. Analysis of dendritic intersections on concentric circles also showed no interaction and no phenotype main effects (Fig. 2B); the same pattern of results was observed for endings points (all $p > 0.05$). In the present study, we reconstructed and segregated spines based on their morphology. ANOVA results showed no two-way interaction in the distribution along dendritic shafts of thin, stubby, mushroom, branched, filopodia and detached spines (all $p > 0.05$) (Fig. 2D).

3.3. F-R and F-S lines differ on branch point distribution

ANOVA on branch point distribution along the extent of the dendritic tree showed a significant two-way interaction ($F_{4,32} = 4.126$; $p < 0.05$). These results suggested that phenotypic differences are specific to sub-regions of the dendritic tree. A follow up Pairwise post hoc student *t*-test comparisons indicated more branch points for F-R mice at a distance of 30 μm from the soma. In contrast, F-S animals exhibited more branch points at a 50 μm radial distance from the soma (all $p < 0.05$) (Fig. 2F).

4. Discussion

We investigated the anatomical structure (dendrites and spines) of lateral amygdala (LA) principal neurons in two mouse lines behaviorally selected for Pavlovian fear conditioning susceptibility (F-S) or resistance (F-R) (Bergstrom, McDonald, Dey, Fernandez, & Johnson, 2013; Gouty-Colomer et al., 2015; Radley et al., 2004; Spruston, 2008). We hypothesized that F-S and F-R naïve mice would exhibit differences in LA principal neuron structure. In the first experiment, behavioral results from the *S*₃ generation indicated stronger contextual and cued fear responses in F-S compared with F-R mice, for both males and females. In the second experiment, 3-D morphometric analysis of LA principal structure from the naïve *S*₄ generation revealed no gross differences in dendritic length, intersections or endings across phenotype. There were also no differences in the density or morphology of dendritic spines. A spatial analysis of the distribution of branch points across the length of the dendritic tree revealed a significant increase in branch points at more distal locations in the F-S compared with

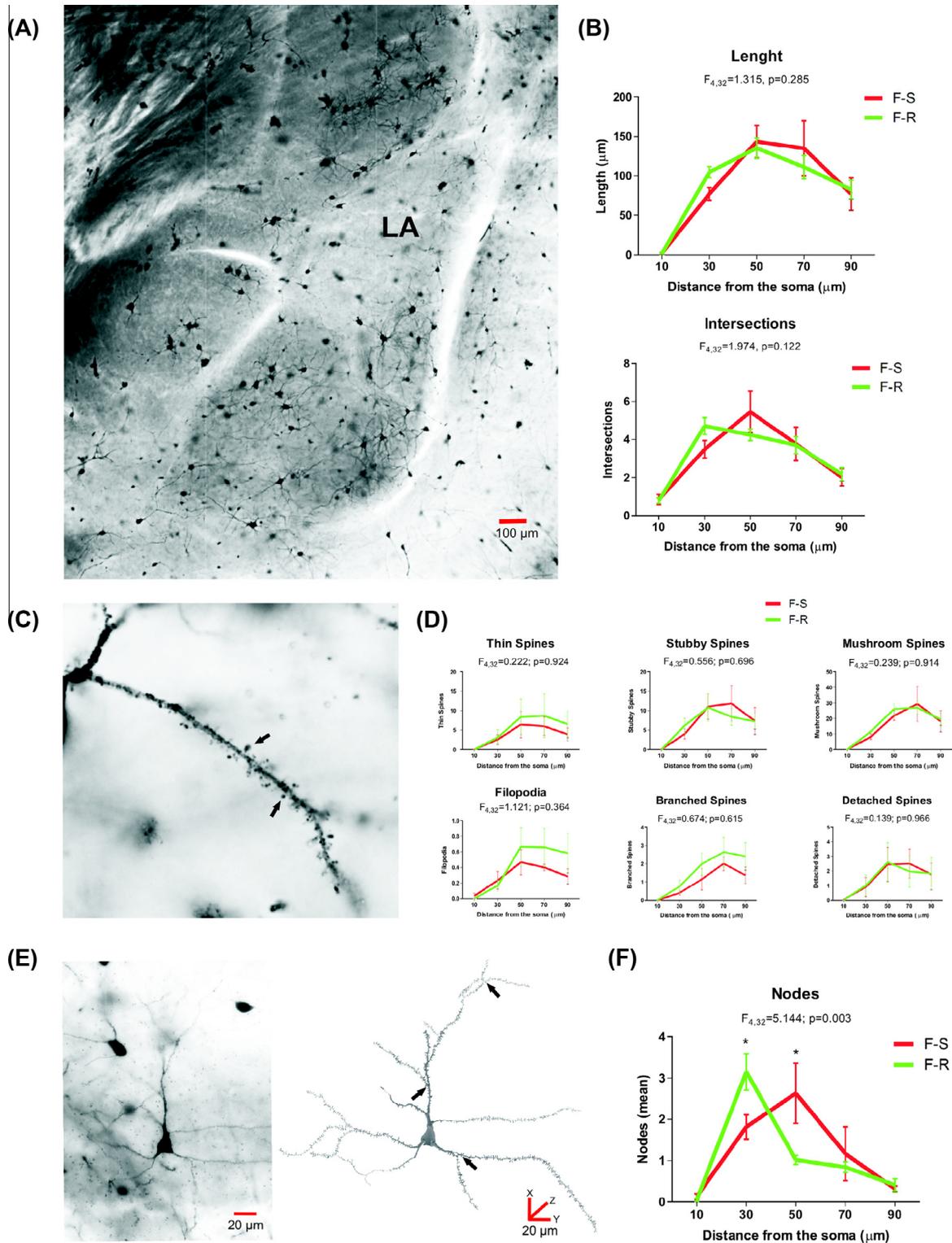


Fig. 2. Dendrite structural measures and spine density of F-S and F-R lines. Results indicated a difference between lines in the spatial distribution of branch points across the length of dendritic trees. A: Frontal photomicrograph showing lateral amygdala (LA) sub-region from a Golgi-Cox stained brain section; B: data displayed as length (top) and intersections (bottom) mean (\pm SEM) for 20 μm of increasing distance from the center of the cell body. Repeated measures ANOVA showed no significant interaction between lines and dendritic length and dendritic intersections (all $p > 0.05$). C: Photomicrograph of a dendritic branch from a LA pyramidal neuron; arrows indicate dendritic spines; D: data displayed as spines (thin, stubby, mushroom, filopodia, branched and detached) mean (\pm SEM) for 20 μm of increasing distance from the center of the cell body; repeated measures ANOVA showed no significant interaction between lines and all dendritic spines categories distribution (all $p > 0.05$); E: representative photomicrograph of a LA pyramidal neuron with the respective digital reconstruction; arrows indicate bifurcating nodes; F: data displayed as mean (\pm SEM) of branch points distribution at increasing distance from the center of the cell body; repeated measures ANOVA showed a significant interaction between branch points and mouse lines ($p < 0.05$). Post-hoc comparisons showed significant differences between F-S and F-R lines at 30 μm and 50 μm of radial distance from the cell body (all $p < 0.05$).

F-R line (see representative Dendrogram – Fig. 3). To our knowledge, this is the first report showing an association between inherited Pavlovian fear conditioning resistance and susceptibility with LA principal neuron dendritic morphology in experimentally naïve mice.

Behavioral evaluation the S_3 generation indicates F-S mice consistently exhibit more conditioned freezing than F-R mice in response to contextual and auditory environmental cues. These findings extend our previous reports, where S_3 behavioral data were reported for the breeders employed to create S_4 generation (McGuire et al., 2013). Considerable evidence from human and non-human studies indicate that fear conditioning in response to contextual cues or to a discrete CS are mediated by distinct but interacting neural circuitry (Ferreira, Moreira, Ikeda, Bueno, & Oliveira, 2003; Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011; Kim & Fanselow, 1992; Ledoux, 2000; Sylvers, Lilienfeld, & LaPrairie, 2011). These results support the hypothesis that Pavlovian fear conditioning models at least two distinct dimensions of anxiety-related disorders. PTSD, which is characterized by intense phasic fear or cue-specific fear reactivity, can be modeled by aversive learning in response to a discrete CS (Davis, Walker, Miles, & Grillon, 2010; Grillon, 2002). Generalized anxiety disorder (GAD) is characterized by a persistent, diffuse and non-cue-specific anxiety, and may be better modeled by contextual fear conditioning. Indeed, considerable evidence indicates isomorphism between freezing in response to contextual stimuli paired with electrical shocks and GAD (Brandão, Zanoveli, Ruiz-Martinez, Oliveira, & Landeira-Fernandez, 2008; Davis et al., 2010). The fact that F-S lines exhibit more freezing than F-R mice in response to both continuous and discrete environmental cues suggests the neural circuitry responsible for defensive responsiveness was conserved across the selective breeding process used to generate the F-S and F-R lines. Accordingly, the tendency to associate environmental stimuli to a broad spectrum of stressful conditions is a feature consistently observed in anxious patients (Newport & Nemeroff, 2000).

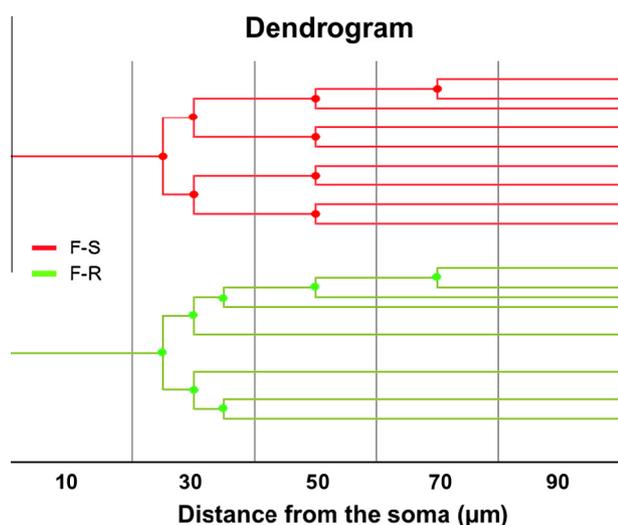


Fig. 3. Dendrogram scaled to dendritic length of LA pyramidal cells. Pyramidal cells play a key role in the neural processing involved in the acquisition of pavlovian fear. Red and Green lines represent, respectively, dendritic trees of F-S and F-R lines; colored dots within each dendritic tree represent bifurcating nodes. In order to facilitate schematic visualization, number of bifurcating nodes depicted in the Dendrogram was increased, by multiplying the average results of the present study by ~ 1.5 . Analysis of digitally reconstructed dendrites indicated a difference in the spatial distribution of branch points across dendritic trees. Whereas F-R mice have more branch points ($\sim 5:3$) at closer distances (range of 30 μm) to the cell body, F-S animals presents more nodes ($\sim 4:1$) farther from the soma (range of 50 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The LA is a key network hub for the establishment of Pavlovian fear conditioning. LA subnuclei incorporate afferent multimodal sensory information required for the establishment of associative fear memories, for both continuous and discrete conditioned stimuli. However, sensory information involved in the generation of Pavlovian fear conditioning is subjected to a high degree of processing before leaving the LA. Excitability of LA neurons is closely linked with the development of Pavlovian fear conditioning. Alterations in dendrite morphology and spine patterning after Pavlovian fear conditioning have consistently been observed in threat learning circuits, including the hippocampus (Conrad, Magariños, LeDoux, & McEwen, 1999), medial prefrontal cortex (mPFC) (Vetere et al., 2011) and amygdala (Heinrichs et al., 2013; Radley et al., 2006). The pattern of structural remodeling most frequently observed includes increases in dendritic branch points, dendritic length and spine density on both basal and apical trees (Ostroff, Cain, Jindal, Dar, & Ledoux, 2013). Contextual fear conditioning has also been associated with increases in dendritic arborization and spine density on principal neurons from both the CA1 area of hippocampus and LA (Trabalza, Colazingari, Sgobio, & Bevilacqua, 2011). Supporting the relationship between dendritic spine structure and fear conditioning are findings indicating that fear conditioning and fear extinction bi-directionally remodel dendritic spines (Lai, Franke, & Gan, 2012).

Relatively little is known about the cellular and molecular mechanisms involved in individual differences in response to trauma (i.e., before the stressful stimulus is presented). Describing the neural mechanisms involved in different strengths of fear responses could add vital data to our understanding of several anxiety disorders, including PTSD. Rodent genetic models are of high relevance for understanding the functional neuronal networks involved in aberrant fear memory formation and expression (Gomes et al., 2013). The aim of the present study was to investigate baseline differences in dendrite and spine structure in the F-S and F-R selected lines. Evidence from genetic studies has consistently correlated individual differences in fear memory responsiveness with morphological alterations in neural structures from brain regions involved in fear learning (Camp et al., 2012; Dias et al., 2014; Holmes & Singewald, 2013; Izquierdo, 2006). Morphological changes in neuronal structure were also observed in response to innate fear eliciting situations like predator-stress exposure (Mitra et al., 2009), in pre-clinical models of behavioral therapy like fear extinction (Camp et al., 2012) and in rodent genetic models of divergent HPA axis activation (Pillai et al., 2012).

Accordingly, F-S lines express higher levels of corticosterone compared to F-R lines (McGuire et al., 2013). Therefore, circulating corticosterone may represent an important mechanistic variable in the present study. The influence of elevated corticosterone on dendrite morphology and/or spine density in rodent genetic models, and how these variables interact with memory strength, is a matter of investigation (Schwabe, Wolf, & Oitzl, 2010). For example, elevated glucocorticoid levels have consistently been related with reduced dendritic arborization in the hippocampus, which in turn may compromise the efficacy of synaptic transmission (Magariños, McEwen, Flügge, & Fuchs, 1996). Amygdala neurons are particularly sensitive to glucocorticoids (Vyas et al., 2006). Stress was found to increase the dendritic arborization of BLA principal neurons and induce anxiogenic behavior. Camp et al. (2012) found enlarged dendritic arbors in basolateral amygdala neurons in the extinction-impaired 129S1/SvImJ (S1) compared with the C57B6/J inbred mouse strain following fear extinction training. Importantly, naïve S1 animals showed increased corticosterone levels in response to either acute restraint stress or extinction training (Camp et al., 2012). Moreover, the authors reported increased apical dendritic material at distal locations from the soma. This finding is consistent with the branch point results in the present

study (Fig. 2F) and is consistent with other reports (Adamec, Hebert, Blundell, & Mervis, 2012; Mitra et al., 2009; Padival, Blume, Vantrease, & Amiel Rosenkranz, 2015). Importantly, axonal inputs from GABA inter-neurons were found to be more perisomatic (closer to the soma) in comparison with excitatory inputs from pyramidal neurons. Muller et al. (2006) showed that the perisomatic region of BLA principal neurons is densely innervated by symmetrical synapses (presumably inhibitory), whereas the density of asymmetrical synapses (presumably excitatory) increases as the distance to the soma increases. These results suggest the density of LA principal neuron dendritic branch points at varying distances from the soma may play a role in synaptic plasticity underlying Pavlovian fear conditioning. In the present study, we observed that alterations in branch point distribution at distal points from the soma are a consistent phenotype associated with genetic differences in fear memory formation. One open question is how the dendritic branch point distribution in the LA might interact with a distributed associative threat learning circuit. Future investigation will be required to determine the relative contribution of dendrite structure to the behavioral expression of Pavlovian fear conditioning in the F-S and F-R mouse lines.

5. Conclusions

In summary, F-S and F-R animals from the S₃ generation displayed marked differences in contextual and cued fear conditioning. F-S lines exhibited a greater conditioned freezing response than F-R mice, for both males and females. These results further bolster the use of F-S and F-R mouse lines as a genetic model for the study of associative fear learning. The F-S and F-R lines are also a suitable mouse model for understanding fear learning variance and have the potential to expand our understanding of GAD and PTSD. F-S mice exhibit more dendritic branch points at distal locations (50 μm) from the cell body than F-R mice, suggesting that dendritic branching in the LA is a phenotype associated with excessive fear memory formation. Considering the small effects of a large set of genes influencing abnormal emotional responses and the complexity of neuronal function we are just beginning to identify the underpinnings of anxiety disorders.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense, nor the U.S. Government.

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