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Susceptibility and resilience to PTSD-like symptoms in mice is associated with opposite dendritic changes in the prelimbic and infralimbic cortices following trauma

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## **Abstract**

Post-traumatic stress disorder (PTSD) is triggered by exposure to traumatic events, but not everyone who experiences trauma develops this disorder. Like humans, PTSD-like symptoms develop in some laboratory rodents (susceptible individuals), while others express less or no symptoms (resilient individuals). Here, considering (i) the putative causal role of fear conditioning in PTSD development and (ii) the involvement of the medial prefrontal cortex (mPFC) in the regulation of conditioned fear response, we tested whether trauma-associated changes in the mPFC may discriminate stress-resilient from stress-susceptible mice. From data on avoidance behavior (as a major symptom), we found that trauma-exposed mice displayed a bimodal distribution in their step-through latency, with low avoider (stress-resilient) individuals and high avoider (stress-susceptible) individuals. Dendrites of Golgi–Cox-stained neurons were analyzed in two parts of the mPFC: the prelimbic (PrL) and infralimbic (IL) areas. In the resilient phenotype, the total number of dendrites decreased in the PrL and increased in the IL; however, it decreased only in the IL in the susceptible phenotype compared to controls. These findings demonstrate that the type of post-trauma morphological changes in the mPFC is associated with susceptibility or resilience to trauma-related symptoms.

## Introduction

Post-traumatic stress disorder (PTSD) is a debilitating disorder that occurs after being exposed to one or multiple traumatic events. Clinical studies have highlighted that only a minority of trauma-exposed individuals develop PTSD (Kessler et al., 1995; Chilcoat and Breslau, 1998; Breslau and Kessler, 2001). Like humans, laboratory mice (Lebow et al., 2012; Sullivan et al., 2017) and rats (Elharrar et al., 2013; Toledano and Gisquet-Verrier, 2014) also display a great heterogeneity in their response to trauma exposure, with some animals developing PTSD-like (or stress) behaviors, while others displaying less symptoms or remaining symptom-free.

The factors that make certain individuals susceptible to develop PTSD and others resilient are still unknown. It has been hypothesized that the natural ability to adapt to stress may be damaged in those developing PTSD (Lebow et al., 2012; Elharrar et al., 2013). This idea has been so far supported by rodent studies showing that changes in corticotropin-releasing factor receptor type 2, in the bed nucleus of the stria terminalis, characterized susceptibility to PTSD-like state (Lebow et al., 2012; Elharrar et al., 2013).

Studies on fear conditioning, as a key component of PTSD, have expanded our knowledge of brain circuits required for coping with stress (Maier and Watkins, 2010; Ago et al., 2017; Fucich et al., 2018). By investigating, in rodents, changes in brain activity in these circuits, which include the medial prefrontal cortex (mPFC), other neurobiological features of susceptibility to stress may be revealed. Indeed, the mPFC controls subcortical regions, mainly the amygdala and hippocampus, to regulate appropriate conditioned fear (Giustino and Maren, 2015). Findings on the mPFC also suggest that activity in its prelimbic area (PrL) is necessary to drive the expression of conditioned fear, while activity in its infralimbic area (IL) is important for the inhibition of conditioned fear (Milad and Quirk, 2002; Burgos-robles et al., 2009). We therefore hypothesized that, following trauma exposure, stress-susceptible and

stress-resilient subpopulations would manifest opposite changes in the PrL and IL.

The aim of the current study was to test this hypothesis in mice. Based on studies indicating that changes in neuronal excitability are associated with changes in dendritic morphology (Muller et al., 2000; Monfils et al., 2004), we hypothesized that examination of post-trauma alterations in the mPFC dendritic morphology would reveal opposite changes in the PrL and IL. Several animal models of PTSD have been developed to model different aspects of PTSD symptomatology. Among these models, there are restraint stress, forced swim stress, predator based psychosocial stress, social defeat stress, single prolonged stress and footshock stress. Here, we used the footshock stress, based on our previous studies (Bentefour et al., 2015, 2016, 2018), which were based on passive avoidance training. Such training induces persistent avoidance, persistent fear sensitization and increased anxiety-like behavior.

## **Materials and methods**

### **Animals**

The experiments were performed on forty-seven young male Swiss mice obtained from the animal care facility of the faculty of Science Semailia, Marrakech, Morocco. Animals were group housed (3-6 per cage) and maintained under constant conditions of ambient temperature ( $22 \pm 2^\circ\text{C}$ ), under a 12h light/12h dark cycle with food and water available *Ad libitum*. The behavioral experiments were conducted between 8AM and 3PM in conformity with approved institutional protocols. All animal procedures were in strict accordance with the guidelines of European Council Directive (EU2010/63). All efforts were made to minimize animal suffering.

### **Behavioral protocols**

Experimental timeline is shown in Figure 1. All behavioral apparatus and procedures are described in detail in our previous papers (Bentefour et al., 2015, 2016). Briefly, on day 1, different aspects of stress behaviors (including avoidance behavior, fear sensitization and anxiety-like behavior) were induced using a passive avoidance apparatus consisting of two equally sized compartments with two independent stainless-steel grid floors (59×19×24-cm height, each), which were separated by a guillotine door (5×5 cm). Each grid floor was connected to an independent shocker unit delivering scrambled shock (Bioseb, LE10026). The light compartment was illuminated by a 50-W bulb. The apparatus was cleaned with a 70 % ethanol solution before each mouse was introduced. Mice were placed in the illuminated compartment to freely enter the dark compartment, where they received two 2-second foot-shocks (1.5 mA, separated by 6 seconds). The control group was similarly placed in the apparatus but did not receive any footshock. After a 14-day period, animals were submitted to classical fear conditioning (with three 1-second foot-shocks, 0.4mA each, separated by 70 seconds) in a different context: a chamber (20×20×25-cm height) with a grid floor made of stainless steel connected to a link box for shock delivery (Bioseb, LE10026). The apparatus was cleaned with a lemon-scented liquid soap before each mouse was introduced. On day 15, avoidance behavior was scored in the morning (after the re-exposure to the passive avoidance apparatus), while fear sensitization was tested in the afternoon (after the re-exposure to the classical conditioning chamber for 60 seconds). For the avoidance testing, each mouse was introduced into the light compartment of the apparatus. Sixteen seconds later, the guillotine door, separated the light from the dark compartment, was opened and the time for the mouse to enter the dark compartment was determined. The guillotine door was closed for 60 seconds, then the mouse was taken from the apparatus and put back in its home cage. For fear sensitization, each mouse was placed in the classical fear conditioning context for 60 seconds to determine the time spent freezing. On day 16, anxiety-like behavior was assessed with an

elevated plus maze (EPM). From days 17-21, mice were daily exposed to the avoidance apparatus, where they were left to freely enter the dark compartment, and then they were locked for 10 minutes/day to induce extinction.

### **Behavioral scoring and analysis**

For avoidance behavior, step-through latency (to the dark compartment) was scored for each exposure to the avoidance apparatus. For fear sensitization, the amount of time spent freezing (Fanselow et al., 1994) in the conditioning chamber was measured using a 1-s time sampling technique. For anxiety-like behavior, the behavior of each animal was continuously recorded and rated later using Ethovision XT Noldus 8.5 video-tracking program. The anxiety index was calculated as elsewhere (Rao and Sadananda, 2016):  $\text{index} = 1 - [(\text{open-arm time}/\text{total time}) + (\text{open-arm entries}/\text{total entries})]/2$ .

After the completion of the behavioral study, the susceptible and resilient mice were distinguished following a method adapted from a rat study (Dopfel et al., 2019). Briefly, when we examined the step-through latency time expressed by each mouse, we found a distinct distribution between the Control group and the trauma-exposed mice. Indeed, the Control group showed a unimodal distribution while the trauma-exposed mice displayed a bimodal distribution. Within the trauma-exposed group, animals in the top and bottom tertiles of step-through latency time were separated into high-avoiders (stress-susceptible) and low-avoiders (stress-resilient) subgroups (n = 14 and n=15 for each subgroup, respectively). Five mice who expressed step-through latency time between the two tertiles were excluded. Behavioral data were retrospectively analyzed and shown accordingly.

### **Golgi-Cox staining**

At the end of the experiment, 5 mice from the Control group and 11 mice from the trauma-exposed group (including 5 stress-resilient individuals and 6 stress-susceptible individuals; individuals being randomly selected) were deeply anesthetized with an

intraperitoneal injection of a lethal dose of urethane (>40 mg/kg) and transcardially perfused with 0.9% saline followed by 4% of paraformaldehyde. The brains were extracted, post-fixed overnight in 4% paraformaldehyde. For Golgi-Cox staining, the brains were immersed in a solution composed of 3% solution of potassium dichromate for 15 days. The solution was changed using a glass micropipette in the dark each 4 days. The brains were transferred in a 2% silver nitrate solution for 3 days. During all the staining phases, the brains were kept in an opaque container in room temperature. The tissue was serially cut into 100 $\mu$ m sections, which were mounted into gelatin coated slides and dehydrated in graded ethanol starting from 50%, then xylene, before coverslipping the slide.

### **Image acquisition and morphometric analysis**

The regions of interest, the PrL and IL, were identified using the atlas of Paxinos and Watson (2008). The complete impregnation of several neurons was found in these regions. Pyramidal neurons were recognized according to their triangular soma shape and apical and basilar dendrites. To reduce bias in neuron selection, we only took neurons that (i) were fully impregnated, (ii) had no morphological changes due to incomplete dendritic impregnation of Golgi-Cox staining, and (iii) had no truncated branches. In addition, each selected neuron was traceable and entirely visible. The mean of 5 to 8 whole neurons/region/animal were considered for analysis. Images of neurons were acquired using a 40-x objective. These images were taken along a z-axis while modifying the microscope focus manually to capture the whole detail of the dendritic arborization. Using image j software, images were stitched together in a 3-D stack allowing visualizing all the images in one window using a scrollbar. This method enabled us to visualize the full dendritic detail for each neuron (Ago et al., 2017). We manually quantify, for each neuron, the number of apical dendrites from each visible order (primary, secondary, ...). We summed up the number of dendrites to obtain the



total number. The same procedure was followed to quantify the total number of basilar dendrites. All quantification was performed blindly.

## **Statistical analysis**

Data analysis was performed using GraphPad Prism v7 software. The Student's t-test was used for two-sample comparisons. One-way ANOVA was used to assess the effect of a single parameter, while the effect of independent variables was assessed using two-way ANOVAs followed by Holm-Sidak *post-hoc* for multiple comparisons. The significance was set at  $p < 0.05$ . Pearson's correlations were used to assess the association between avoidance behavior and dendritic morphology changes.

Difference in AIC ( $\Delta AIC$ ) was calculated using GraphPad Prism v7 software. To obtain the Akaike weight for the model we used the following formula:  $\exp(-0.5 * \Delta AIC)$  divided by the sum of these values across all models (Burnham and Anderson, 2003). The 3D scatter plot for data visualization was run using Python.

## **Results**

### **Labeling of mice as stress-susceptible or stress-resilient**

All trauma-exposed mice acquired avoidance behavior, which was characterized by increases of step-through latency (Fig. 2A: Shocked group). Unpaired t-test revealed a significant difference between the Control and Shocked groups ( $t=2.70$ ,  $p=0.009$ ). After examining the frequency distribution of step-through latency time of mice (Fig. 2B), we found that the Control group showed a unimodal distribution while trauma-exposed mice showed a high variability with bimodal distribution (bimodal Gaussian function fit quality:  $\Delta AIC = 0$ , Akaike weight for this model  $w=1$ ). We later considered animals in the top and bottom tertiles

of step-through latency time and separated them into high avoiders (stress-susceptible mice) and low avoiders (stress-resilient mice).

## **PTSD-like behaviors**

### ***Anxiety-like behavior***

Among trauma exposed mice, only the High avoiders displayed a higher anxiety index (Fig. 3C). A one-way ANOVA performed on anxiety index (AI) revealed a significant difference between groups ( $F_{(2, 39)} = 3.906, p=0.028$ ). The High avoiders had higher AI in comparison with the Control group ( $t=2.763, p=0.025$ ) but they did not differ from the Low avoiders:  $t=1.682, p=0.1911$ ). The Low avoiders and the Control group did not differ from each other ( $t=1.095, p=0.280$ ).

### ***Fear sensitization***

During the sensitization test, the High avoiders froze significantly more than the two other groups (Control group and Low avoiders; Fig. 3D). A one-way ANOVA applied on these data revealed a significant difference between groups ( $F_{(2, 39)} = 4.012, p=0.026$ ). The High avoiders froze significantly more than the Control group ( $t=2.499, p=0.049$ ) and the Low avoiders ( $t=2.357, p=0.048$ ). The difference between the Low avoiders and the Control group was not significant ( $t=0.854, p=0.184$ ).

### ***Avoidance extinction***

All trauma-exposed mice exhibited decreases in step-through latency during repeated situational exposure in the absence of foot-shock application (Fig. 3E). A two-way repeated measures ANOVA on extinction data (groups x sessions) showed main effects of group ( $F_{(2,39)}=15.570, p<0.001$ ) and session ( $F_{(4,39)}=41.060, p<0.001$ ), and a significant group x session interaction ( $F_{(8,39)}=17.960, p<0.001$ ). During Day 17 (i.e. first extinction day), step-through latency time was significantly higher for both the Low and High avoiders compared to the Control group (Holm-Sidak post-hoc test:  $t=2.781, p=0.005$  and  $t=7.124, p<0.001$ ,

respectively). Step-through latency was also significantly higher for the High avoiders compared to the Low avoiders ( $t=4.425$ ,  $p<0.001$ ). However, during Day 18 (i.e. 2th extinction day), only the High avoiders continued to exhibit significant higher step-through latency than the Control group and the Low avoiders ( $t=3.924$ ,  $p<0.001$  and  $t=2.319$ ,  $p=0.042$ , respectively). From Day19 (i.e. 3th extinction day), the three groups showed no significant difference and displayed identical low levels of latency on Day 21 (5th extinction day), indicating full extinction in the two trauma-exposed groups.

### ***3D-scatter plot for data visualization***

The 3D visualization was based on the 3 measured variables in avoidance, EPM and sensitization tests. It enabled the representation of the position of each mouse according to the 3 measured variables (Fig. 2F), with x-axis representing avoidance test data, y axis representing EPM test data, and z-axis representing sensitization test data. The High avoiders emerged from a separate cluster, while clusters of the Control group and the Low avoiders overlapped.

### **Quantification of the Golgi-Cox material**

Branching complexity of apical and basilar dendrites, dendritic length and branch order (Fig. 4A) were quantified in 106 PrL neurons (28, 33 and 45 neurons from the Control group, the Low avoiders and the High avoiders, respectively) and 73 IL neurons (24, 20 and 29 neurons the Control group, the Low avoiders and the High avoiders, respectively). The quantitative analysis was based on the branching patterns indicated on the drawing in Figure 3B.

### ***Evaluation of the number of dendrites***

***Apical dendrites:*** A two-way ANOVA (groups x areas) performed on the total number of dendrites (Fig. 4A) revealed main effects of area ( $F_{(2,13)} = 6.472$ ,  $p=0.017$ ). The group

factor was not significant ( $F_{(2,13)} = 0.859$ ,  $p = 0.434$ ), but the group x area interaction was significant ( $F_{(4,13)} = 14.87$ ,  $p < 0.001$ ). The Holm-Sidak *post-hoc* test on the PrL area showed that the Low avoiders had a significant reduction in the number of dendrites compared to the Control group and the High avoiders ( $t = 2.655$ ,  $p = 0.026$  and  $t = 3.158$ ,  $p = 0.011$  respectively). The High avoiders did not differ from the Control group ( $t = 0.384$ ,  $p = 0.703$ ). The same test on the IL area showed that the High avoiders expressed a significant reduction of the number of apical dendrites compared to the Low avoiders ( $t = 4.481$ ,  $p < 0.001$ ). The latter group did not manifest a significant increase in the overall number of apical dendrites, compared with both the Control group and the High avoiders ( $t = 2.238$ ,  $p = 0.066$  and  $t = 2.144$ ,  $p = 0.066$  respectively).

**Basilar dendrites:** A two-way ANOVA analysis (groups x areas), performed on the total number of basilar dendrites (Fig. 4B) revealed a main effect of group ( $F_{(2,13)} = 4.737$ ,  $p = 0.017$ ), but no main effect of area ( $F_{(2,13)} = 0.943$ ,  $p = 0.340$ ). The interaction between the two factors was significant ( $F_{(4,13)} = 11.210$ ,  $p < 0.001$ ). The Holm-Sidak *post-hoc* test on PrL data did not reveal any significant difference between the groups.

The same test on IL data indicated that the Low avoiders had a significant increase of the dendrites number as compared to the Control group ( $t = 2.980$ ,  $p = 0.012$ ). The High avoiders differed from both the Control and the Low avoiders ( $t = 2.363$ ,  $p = 0.025$  and  $t = 5.476$ ,  $p < 0.001$ , respectively).

### ***Evaluation of dendrite branch length***

**Apical dendrites:** A two-way ANOVA (groups x areas) on the total branch length (Fig. 4C) showed no significant effect of area ( $F_{(2,13)} = 2.278$ ,  $p = 0.143$ ), and no main effect of group ( $F_{(2,13)} = 1.204$ ,  $p = 0.143$ ). However, the interaction between the two factors was significant ( $F_{(4,13)} = 15.11$ ,  $p < 0.001$ ). The Holm-Sidak *post-hoc* test on PrL data showed that the Low avoiders expressed a significant decrease of the total branch length when compared

to the Control group and the High avoiders ( $t=2.466$ ,  $p=0.040$  and  $t=2.930$ ,  $p=0.020$ , respectively). However, the High avoiders did not differ from the Control group ( $t=0.353$ ,  $p=0.726$ ). The same test on IL data revealed that the Low avoiders had a significant increase of the total branch length compared with the Control group and the High avoiders ( $t=2.247$ ,  $p=0.044$  and  $t=4.779$ ,  $p<0.001$ , respectively). However, the High avoiders expressed a significant decrease compared with the Control group ( $t=2.247$ ,  $p=0.044$ ).

**Basilar dendrites:** A two-way ANOVA on the total length dendrites in the IL (Fig. 4D) did not reveal any significant group effect ( $F_{(2, 13)}=0.860$ ,  $p=0.434$ ), neither an area effect ( $F_{(2, 13)}=0.774$ ,  $p=0.386$ ). However, a significant group x area interaction was found ( $F_{(4, 13)}=12.71$ ,  $p<0.001$ ). The three groups differed from each other (Holm-Sidak *post-hoc* test). Regarding PrL data, the Low avoiders did not show any significant increase in the total length dendrites in comparison with the Control group ( $t=1.933$ ,  $p=0.124$ ). The difference was significant between the Low and High avoiders ( $t=2.591$ ,  $p=0.045$ ). However, the High avoiders did not differ from the Control one ( $t=0.571$ ;  $p=0.512$ ). The same trend was observed for IL data. The Low avoiders differed significantly from the Control group ( $t=2.759$ ,  $p=0.020$ ) and from the High avoiders ( $t=4.438$ ,  $p<0.001$ ). No significant difference was recorded between the High avoiders and the Control groups ( $t=1.557$ ;  $p=0.131$ ).

## **Correlation between behavior and dendritic morphology**

### ***PrL dendritic morphology***

Pearson correlation analysis performed on avoidance data and the total number of apical dendrites in the PrL revealed a slight positive correlation between the two parameters ( $r=0.610$ ,  $p=0.045$ ). This means that the High avoiders tend to have more dendrites in the PrL (Fig. 5A). The same analysis concerning the basilar dendrites in the PrL did not reveal any clear correlation between the two parameters ( $r=0.307$ ,  $p=0.359$ ). This means that the high or

low avoidance status does not depend on the changes in the total number of basilar dendrites in the same region (Fig. 5B).

### ***IL dendritic morphology***

Pearson correlation analysis performed on avoidance data and the total number of apical dendrites in the IL revealed a strong negative correlation between the two parameters ( $r = -0.874$ ,  $p < 0.001$ ). This means that the High avoiders tend to have less dendrites in the IL (Fig. 5C). Similarly, Pearson correlation on avoidance data and the total number of basilar dendrites in the IL showed a negative correlation ( $r = -0.816$ ,  $p = 0.002$ ), which confirms that the high avoidance status is characterized by lower total number of basilar dendrites in the IL (Fig. 5D).

## **Discussion**

In this study, we examined whether trauma exposure would cause opposite changes in the mPFC of mice that develop at least 3 PTSD-like symptoms (stress-susceptible group) and those developing less symptoms (stress-resilient group). To this end, a reliable evaluation method was used to allow the differentiation of trauma-exposed mice into susceptible and resilient groups. The Golgi-Cox method revealed significant decreases and increases in the total number of dendrites in the PrL and IL, respectively, in the stress-resilient group. As predicted, opposite changes were found in each mPFC area in the stress-susceptible group.

### **The differentiation of mice into susceptible and resilient groups**

Several scoring methods have been used to estimate the inter-individual differences regarding stress reactivity (Cohen et al., 2004; Kanarik et al., 2011; Nalloor et al., 2011; Toledano and Gisquet-Verrier, 2014; Febbraro et al., 2017). Some of these methods are prospective and others are retrospective. Regarding the prospective methods, reactivity to trauma are predicted (from pre-disposing factors), before trauma exposure. Examples of pre-

disposing factors include an exaggerated startle response, a high level of anxiety-like behavior (Nalloor et al., 2011) and locomotor reactivity to novelty (Cordero et al., 2003). With respect to retrospective methods, the susceptible and resilient groups are distinguished only following trauma exposure (Cohen et al., 2004; Toledano and Gisquet-Verrier, 2014), as in depression studies (Kanarik et al., 2011; Fabbraro et al., 2017). Here, we choose a retrospective method, especially because it does not require behavioral scoring before trauma exposure. This avoids applying some behavioral tests before and after trauma, such as EPM as it is well-known that repeating this test increases open arm avoidance (Carobrez and Bertoglio, 2005). We found that avoidance in trauma-exposed mice followed a bimodal distribution that allowed us to distinguish two groups: the high and low avoiders (based on a method used recently by Dopfel et al., 2019). We also found that both groups expressed different behaviors related to trauma (anxiety, fear awareness and fear extinction) and therefore they displayed two different phenotypes related to trauma.

## **Behavioral results of the stress-susceptible and stress-resilient groups**

### ***Avoidance behavior***

Avoidance is one of the core symptoms of PTSD (Sheynin et al., 2017). Our team has repeatedly shown that trauma exposure (using 1.5-mA foot-shocks) produces persistent avoidance behavior towards the trauma environment (Bentefour et al., 2015, 2016, 2018). However, in each of these studies, all trauma-exposed mice were included in a single trauma-exposed group, without distinguishing susceptible from resilient mice. Here, we distinguished stress-susceptible individuals, characterized by a more pronounced avoidance response, and stress-resilient mice, characterized by less avoidance behavior. A similar finding has been recently reported in a rat study (Le Dorze and Gisquet-Verrier, 2016b).

### ***Anxiety-like behavior***

People with PTSD, in addition to their core symptoms (re-experiencing, avoidance and hyperarousal), often struggle with frequent and intense symptoms of anxiety (Olatunji et al., 2018). Almost all studies on rodent PTSD models include assessment of anxiety-like behavior. Here, we found that only mice with the susceptible phenotype spent lower time in the open arms and had a greater anxiety index compared to the no trauma-exposed mice. Similar results have been reported by other authors (Toledano and Gisquet-Verrier, 2014; Le Dorze and Gisquet-Verrier, 2016a, b; Brodник et al., 2017).

### ***Fear sensitization***

Hyperarousal, implicating non-associative fear learning processes (Siegmund and Wotjak, 2007), can be assessed in laboratories with the acoustic startle response in both PTSD patients (Calhoun et al., 2011) and rodent models of PTSD (Levkovitz et al., 2015). In PTSD rodent models, hyperarousal can also be assessed as sensitized contextual fear established with a weak fear conditioning (Rau and Fanselow, 2009; Bentefour et al., 2015), as in the present study, where only susceptible mice exhibited greater fear sensitization.

### ***Avoidance extinction***

Post-trauma symptoms, including avoidance, are considered part of the normal reactions to trauma exposure. Resilient individuals show steadily decreasing of these symptoms over time (Rothbaum and Davis, 2003). Among vulnerable individuals, the most common trajectory is also progressive recovery (Kessler et al., 1995). In other words, complete recovery is often reached in less than 1-month post-trauma in resilient individuals and by 3 months post-trauma in vulnerable individuals (Yehuda and LeDoux, 2007). One can, therefore, hypothesize that, during extinction training, avoidance would disappear more rapidly in resilient than in susceptible mice. Our data showed that all trauma-exposed mice successfully extinguished their avoidance response during extinction training, similarly to our previous study (Bentefour et al. 2016). Nevertheless, the fact that extinction occurred earlier



in resilient mice than in susceptible individuals does not confirm the above hypothesis because all trauma-exposed mice did not start extinction training with similar avoidance behavior.

### **Quantification of the Golgi-Cox material**

The analysis of the total number and branch length of dendrites in the PrL showed that the susceptible phenotype was characterized by the elevation, of both parameters for apical and basilar dendrites mostly compared to the resilient mice. Similar morphological changes have previously been linked in rats with chronic stress (Cook and Wellman, 2003), mild and short-term stress (Brown et al., 2005) and repeated stress (Radley et al., 2006). However, there are also other studies in rodents that have shown that stress causes a decrease in length and number of branches of apical dendrites (Radley et al., 2004) or even a lack of change in the PrL (Izquierdo et al., 2006; Febbraro et al., 2017). Nevertheless, beyond this discrepancy, none of these studies show dendritic plasticity, in the PrL, specific to the susceptible phenotype. The PrL is known to send extensive excitatory projections to the basolateral amygdala (Cheriyian et al., 2016), a key region in expressing fear-related behavior. Increased activity in the PrL could, therefore, lead to stronger amygdala activation. Given the involvement of amygdala hyperactivation in the expression of PTSD symptoms, our data on the PrL may, at least in part, explain the higher levels of avoidance, the emergence of fear sensitization, and the higher levels of anxiety-like behavior observed with the susceptible mice. We can also conclude that a decreased PrL plasticity may lead, on the contrary, to a protection against the development of PTSD-like state. This corresponds to what we observed with the resilient phenotype.

Here, we also found that the susceptible phenotype was characterized by a reduced number and branch length of both apical and basilar dendrites in the IL, compared with both the Control and resilient groups. These data are consistent with previous reports indicating

that exposure to stress induces a significant retraction of terminal branches of apical dendrites in this area (Izquierdo et al., 2006; Moench et al., 2016). Stress-susceptible rodents have also been found to display low immunoreactivity to c-fos (Febbraro et al., 2017), low expression of proBDNF (the molecular precursor of BDNF) (Sun et al., 2018) and high expression of FKBP5 (a binding protein increasing the affinity of glucocorticoids with their cytoplasmic receptors) (Criado-Marrero et al., 2017). This further demonstrates that stress-related susceptibility is associated with dysfunctions in the IL. Moreover, studies based on synaptic plasticity have shown long-term depression in the IL of mice exposed to fear conditioning (Herry et al., 1999; Herry and Garcia, 2002). In fact, this region is known to play a major role in suppressing fear response (Milad and Quirk, 2002), via its projections to the amygdala (McDonald et al., 1996). More specifically, the IL exerts, via GABAergic intercalated cells, feed forward inhibition of central amygdala output neurons, which triggers the expression of fear-related behavior (Quirk et al., 2003). In addition, the IL sends direct excitatory projections to the basolateral amygdala, activating fear extinction neurons (Cho et al., 2013; Bloodgood et al., 2018). Related to this, it has been suggested that resilient humans are less likely to consolidate emotional memories and have a greater ability to extinguish traumatic memories (Charney, 2004). The decreased activity in the IL could have facilitated the development of PTSD-like state in mice with the susceptible phenotype, while increased activity in this area could have contribute to resilient responses.

Our results also highlighted the fact that there is a strong negative correlation between avoidance and the number of dendrites in the IL. We did not find any strong correlation between these two parameters in the PrL. Using avoidance training, Jinkes et al (1997) have shown that the IL is implicated in the inhibition of behaviors associated with aversive outcomes. Brain stimulation studies have demonstrated that electrical stimulation of this area during passive avoidance conditioning impairs the retention of the behavioral response

(Santos-Anderson and Routtenberg, 1976). This indicates the importance of the IL in modulating stress-related responses. To our knowledge, our study, using morphological plasticity approach, is the first to show that higher number of dendrites in the IL is associated with less avoidance, and therefore with a state of resilience. Altogether, this suggests that the IL is more implicated in the process of stress resilience.

## **Conclusion**

The current study demonstrates that trauma-exposure induces not only prefrontal changes in susceptible but also in resilient individuals. The study shows that resilience does not correspond to the maintenance of prefrontal cortex dendritic morphology in its pre-traumatic state, nor to a rapid return to the pre-traumatic state of dendrites plasticity, but to the development of a protective prefrontal neuronal plasticity.

## **Authors' Contributions**

A.L., Y.B., M.B., S.B.M., and R.G. conceived and designed the study.

A.L. and Y.B. acquired the data.

A.L. analyzed the data.

A.L., S.B.M. and R.G. drafted the article.

All authors interpreted the data, discussed the results, and commented on the article.

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*Conflict of Interest:* None declared.

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## Figure Legends

Figure 1. Timeline of the behavioral experiments.

Figure 2. Behavioral data. A) Step-through latency (as avoidance behavior indicator) as expressed by the Control group and the trauma-exposed mice (Shocked group) during avoidance test at 15 days post-trauma exposure. B) The distributions of step-through latency time of all mice (Control and Shocked groups). The step-through latency time distribution in the Shocked group was fit to a Gaussian mixture model, which demonstrated a bimodal distribution. C) The Anxiety index recorded during the elevated plus maze test. D) Freezing (as fear sensitization indicator) recorded during contextual fear conditioning testing. E) Step-through latency recorded during extinction training (Day17-Day21). F) 3D scatter plot for data visualization of the three measured variables. Back symbols refer to mice used for Golgi-cox staining. In panels A, C, D, & E, bars represent mean  $\pm$  SEM. Holm-Sidak *post-hoc*, \*\*\* $p < 0.001$ , \*\* $p < 0.01$  and \* $p < 0.05$ : significant difference compared to the Control group;

### $p < 0.001$ , ## $p < 0.01$  and # $p < 0.05$ : significant differences in comparison with the Low avoiders.

Figure 3: Dendrite organization in the PrL and IL of the Low and High avoiders. A) Photomicrographs showing representative pyramidal neurons in the PrL and IL in the trauma-exposed group (Low and High avoiders). B) Schematic illustration of the dendritic branching pattern used for quantitative analysis.

Figure 4: Trauma exposure-induced dendritic morphological changes in the mPFC. A) Changes in the total number of apical dendrites in the PrL and IL. B) Changes in the total number of basilar dendrites in the PrL and IL. C) Changes in the total apical dendrites branch length in the PrL and IL. D) Changes in the total basilar branch length in the PrL and IL. Data in panels A-D are presented as mean  $\pm$  SEM. Holm-Sidak *post-hoc*, \*\*\* $p < 0.001$ , \*\* $p < 0.01$  and \* $p < 0.05$ : significant differences compared to the Control group; ### $p < 0.001$ , ## $p < 0.01$  and # $p < 0.05$ : significant differences in comparison with the Low avoiders.

Figure 5: The relationship between avoidance behavior and the total number of dendrites in the PrL and IL cortices. A) Correlation between step-through latency time and the total number of apical dendrites in the PrL. B) Correlation between step-through latency time and the total number of basilar dendrites in the PrL. C) Correlation between step-through latency time and the total number of apical dendrites in the IL. D) Correlation between step-through latency time and the total number of basilar dendrites in the IL.

Figure 1

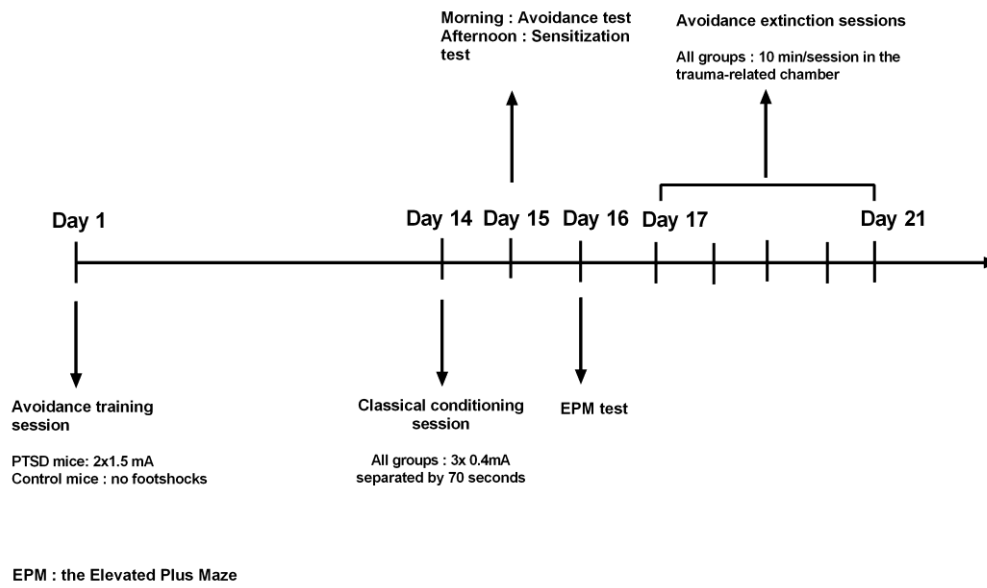


Figure 2

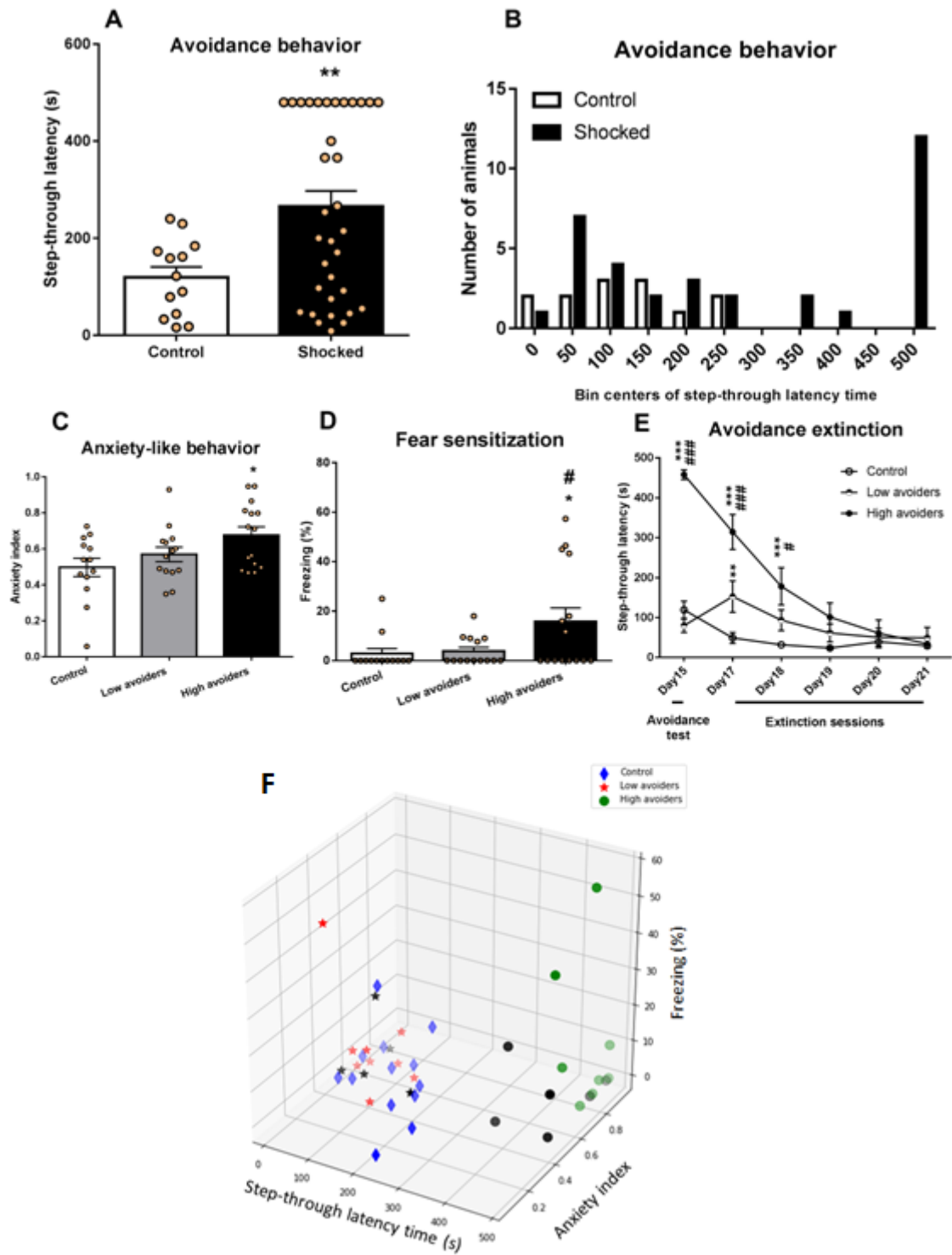


Figure 3:

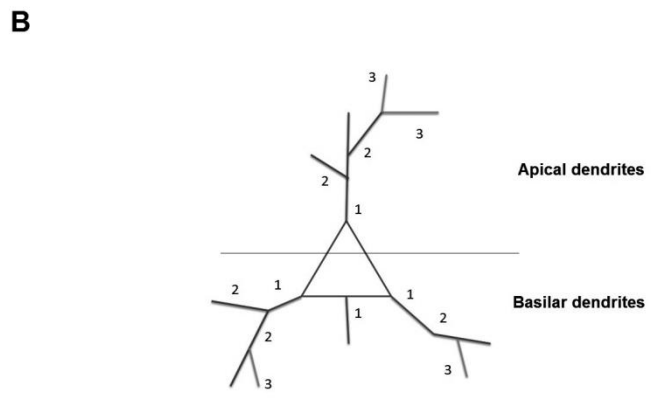
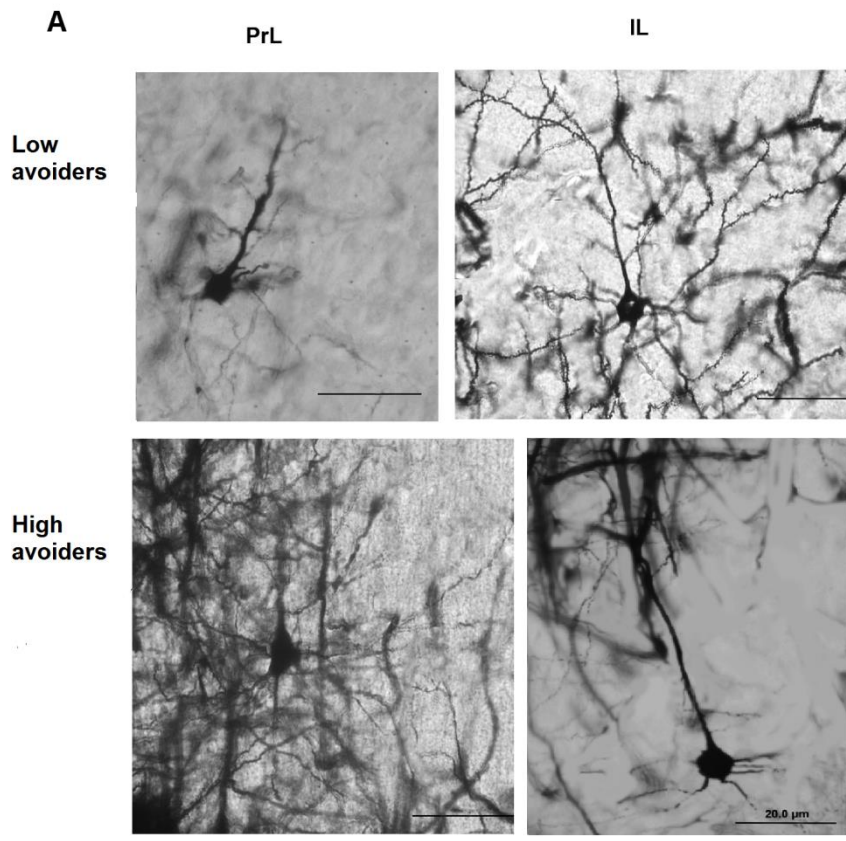


Figure 4:

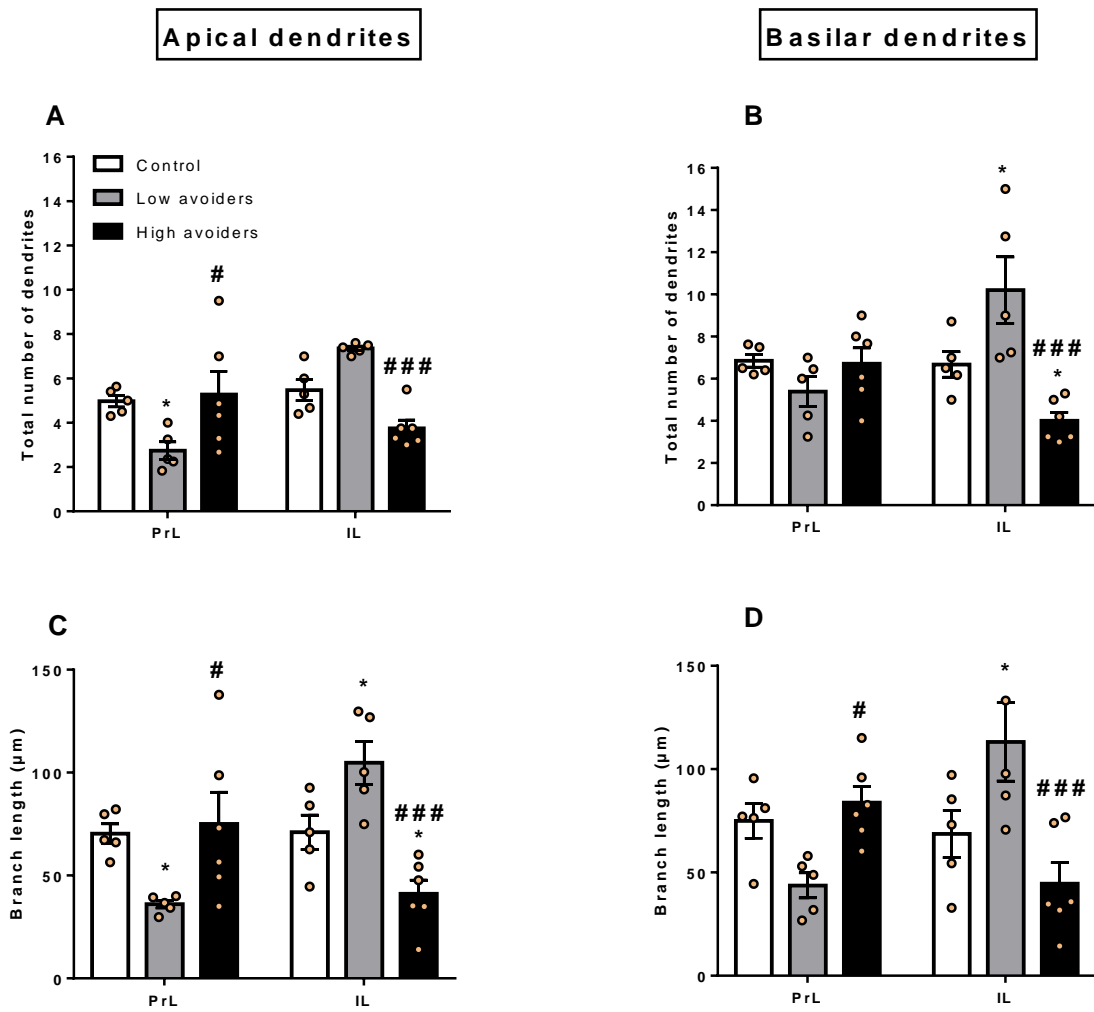




Figure 5:

