Long-term Hippocampal and Amydalar Dendritic Spine Alterations after Adolescence Psychosocial Stress

behnam vafadari (behnam.vafadari@gmail.com)
Helmholtz Center Munich German Research Center for Environmental Health: Helmholtz Zentrum Munchen Deutsches Forschungszentrum fur Gesundheit und Umwelt https://orcid.org/0000-0002-9384-0388

shiladitya mitra
Max-Planck-Institute of Psychiatry: Max-Planck-Institut fur Psychiatrie

Leszek Kaczmarek
Nencki Institute of Experimental Biology: Instytut Biologii Doswiadczalnej im M Nenckiego Polskiej Akademii Nauk

Short Report

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Long-term hippocampal and amydalar dendritic spine alterations after adolescence psychosocial stress

Behnam Vafadari[1, 3], Shiladitya Mitra[2, 3], Leszek Kaczmarek[3]

1-Chair and Institute of Environmental Medicine, Technical University of Munich, Helmholtz Zentrum München,UNIKA-T

2-Research Group Neurobiology of Stress Resilience, Max Planck Institute of Psychiatry, Munich, Germany

3- BRAINCITY, Nencki Institute, Warsaw, Poland

Corresponding author: Behnam Vafadari
Behnam.vafadari@helmholtz-muenchen.de

Abstract:

Stress, in particularly adolescence stress has been shown to result in long-term changes in brain and behavior of humans and rodents as well as predisposing individuals to multiple neuropsychiatric disorders like depression and schizophrenia. Stress-resulting in behavioral changes are often associated with structural changes of the neuronal dendritic spines, especially of the limbic system. Thus, analyzing alterations in structure of the dendritic spines in animals, which were subjected to adolescence stress, might provide useful information on pathogenesis of mental disorders. Herein, we analyzed the length, head width and area of dendritic spines of neurons from the hippocampal CA3, the central amygdala and the basolateral amygdala (BLA) regions in mature C57BL/6 mice, which were subjected to adolescent psychosocial stress. Results showed that stressed animals had longer spines in the hippocampus, larger spines in the BLA and shorter, smaller spines in the central amygdala (CeA). The latter finding is particularly intriguing, as it has been shown that central amygdala is not just the relay center for signals from the BLA in fear response, but is involved in antagonistic and inhibitory function to the BLA and active in appetitive and reward pathways.

Keywords: Psychosocial stress, adolescence, dendritic spines, hippocampus, amygdala

Introduction:

Stressful life events may lead to development of behavioral changes in humans and rodents like anxiety, along with plastic changes in the brain. These alterations can lead to states, which over time may cause mental illness and increase the risk of the neuropsychiatric disorders such as schizophrenia (Popovic et al. 2019). Hippocampus and amygdala of the limbic system have been implicated in adults with posttraumatic stress disorder, secondary to either childhood abuse or maltreatment (Ahmed-Leitao et al. 2016). Recent evidence points to dendritic spines as important substrates of pathogenesis in neuropsychiatric disorders such as autism spectrum disorders (ASD), schizophrenia and Alzheimer's disease (Penzes et al. 2011) apart from depression (Qiao et al. 2016). Dendritic spines alterations have been reported in schizophrenia in different brain areas, e.g., they were demonstrated in amygdala in postmortem tissue of schizophrenic patients and in animal models of schizophrenia (Glausier and Lewis (2013); Flores et al. 2016).

Herein, we focus on the effects of psychosocial stress on dendritic spines in the basolateral amygdala (BLA), central amygdala (CeA) and the hippocampus that are the key areas for stress response. We assessed the long-term effects of stress on the dendritic spines, which may lead to alterations in their functions and have implications in psychiatric disorders. It has been demonstrated that amygdala hyperactivity is associated with highly anxious individuals (Zhang et al. 2018). Stress and anxiety have significant consequences on morphological alterations of the dendritic spines within limbic regions as BLA- a region that has been historically associated with anxiety and has been shown to be sensitive to stress (Blume et al. 2019). Outputs from BLA neurons play important role in influencing the regulation and function of brain regions such as prefrontal cortex, nucleus accumbens and hindbrain, which are implicated in cognition and motivation (Sharp 2017). Therefore, understanding dendritic spines alterations in BLA is vital for uncovering the mechanisms underlying mental disorders caused by anxiety and stress

(Qiao et al. 2016; Leuner & Shors, 2013). The CeA has been well recognized as relay station for aversive behaviors and recently has also been shown to be involved in the appetitive responses as well, in contrast to the BLA which is mostly involved in aversive pathway and fear response (Kim et al. 2017). It is possible that this is mediated through local GABAergic interneurons and projections, which may inhibit the projections from other regions such as BLA. Being crucial to fear response and appetitive learning, CeA is thus an important region for analysis of neuronal dendritic spine changes. Hippocampus is also an important brain region, which has been implicated in stress response (Kim et al. 2015). Multiple reports have demonstrated that the hippocampal CA3 neurons exhibit structural changes and altered dendritic spines morphology under different stress conditions (Radley et al. 2013). Therefore, analyzing these regions for dendritic spine structure changes after psychosocial stress may shed light into the behavioral phenotypes associated with such stress events.

Methods:

We have analyzed dendritic spines of the BLA, CeA and hippocampus of 6 months old C57BL/6 mice. Mice were obtained from an in-house breeding facility at the Animal House at Nencki Institute. These mice were subjected to psychosocial stress after weaning at 1 month of age during their adolescence (Laviola et al. 2003). The experiments were performed on C57BL/6 gender randomized (both males and females) mice - 4 control mice and 4 mice subjected to psychosocial stress. All procedures were performed in accordance with the Animal Protection Act in Poland, Directive 2010/63/EU, and were approved by the first Local Ethics Committee (Permission No 148/2016, 257/2012 and 507/2018). After stressing, mice were maintained individually in ventilated cages with dimensions of 38 cm (length) x 23 cm (width) x 20 cm (height) with continuous access to water and food. The animals were provided with nesting material. The following conditions were provided for the mice: temperature of 21-23 °C, humidity 50-60%, daily cycle 12 / 12h (light phase from 7a.m. to 7p.m.).

Stressing procedure

Mice were assigned to either control treatment or psychosocial stress. For the second group, mice were introduced into the cages of gender matched FVB mice (aggressive strain, 3 months old). The detailed protocol of stressing is already explained (Vafadari et al. 2019). Briefly, the experimental mice are exposed to the FVB mice till the first attack on day1. For the subsequent days the experimental mice were put inside a perforated metal-cup cage such that there was visual and olfactory contact but no direct contact with FVB mice. A Latin Square approach was followed to prevent habituation. The duration of stress lasted 1 hour each day for 21 days and was performed in second half of the day cycle. 8 mice (4 wild type control and 4 wild type were under chronic stress) were chosen to check the dendritic spines changes after 6 months from birth.

Perfusion, cutting and staining the brain

The animals were perfused by 1.5 % paraformaldehyde (PFA) and then the brains were extracted, and for post-fixation they were placed in 1.5 % PFA for 25 minutes. The brains were cut into sections with desired regions and collected in PBS for slices recovery. Then the slices were placed on glass slides and were put inside the humid chamber. The dendritic spines morphology was assessed based on images of DiO-labelled neurons. Dioctadecyloxacarbocyanine (DiO) crystals (Thermofisher, Catalog #: D275) were sonicated for 30 minutes and the crystals were placed on desired regions of the brain slices by the micro-capillary. Afterwards the brain slices were incubated at room temperature for 20 minutes in humid chambers in dark. Finally, the slices were mounted.

Confocal microscopy and analyzing the data:

Images of dendritic spines (4 dendrites per region in 4 animals per group) of the CA3 region of the hippocampus (pyramidal neurons), BLA (pyramidal neurons) and CeA (mostly medium spiny neurons) were acquired by 488 nm fluorescent illumination 40x objective, using a Leica confocal microscope. The pictures resolutions were 1024x1024, 70-nm pixel size.

The dendritic spines shape (length, head-width) and spines are was analysed using SpineMagick software (Ruszczycki et al. 2012).

Statistical analysis:

The data was analyzed using Graph Pad Prism version 8. T-test and ANOVA. The statistical values are expressed as mean ± standard error of the mean (SEM). Indications of significance correspond to $P < 0.05$.

Results:

Alterations in morphology of dendritic spines were analysed in BLA, CeA and hippocampus of mice that underwent psychosocial stress during adolescence. The mice were sacrificed 6 months after birth to assess the long-term effects of adolescence psycho-social stress. After perfusion, the brain cut into slices and then the desired brain regions were stained by lipophilic dye, DiO to reveal cellular morphology.

In the hippocampus, longer spines were observed in stressed mice as compared to control mice, whereas no significant differences were observed in 'head width' and 'area' of the spines (Fig.1). In BLA the spines were longer with smaller head widths in stressed mice as compared to controls, whereas the spine area remained the same. In general, shape factor can be measured using spine neck width and length, as well as spine head (Adrian et al. 2017). Herein, we also used length/head-width (head and neck) to estimate the 'spine shape'. As a result, we can see larger spine shape in stressed mice in comparison to control animals (Fig.2). Finally, in CeA, in contrast to hippocampus and BLA, stressed mice showed comparatively shorter spines and smaller head-width in comparison to controls. A larger spine area was also seen in control mice as compared to the stressed mice (Fig.3).

Discussion:

Stressful life events may produce a state of anxiety and may lead to a variety of mental health issues. These might be associated with alterations in dendritic and synaptic structures particularly within brain regions known to be involved in anxiety, such as amygdala and hippocampus (Leuner and Shors, 2013). The present study was focused on analyzing long-term structural changes in the spines in these two regions in mice subjected to psycho-social stress in adolescence. While in the stressed mice, the BLA and hippocampus showed increased spine length with the BLA also showing bigger ‘spine, the CeA exhibited decreased spine length, head-width and area.

We chose adolescent psychosocial stress as our model to study spine structural changes as this apparently mimics bullying, and, societal and racial adversities in humans without being as drastic as the chronic social defeat model which has been already established as a model of depression (Pryce and Fuchs, 2017). Chronic juvenile restraint stress has been shown to affect dendritic architecture in hippocampus, amygdala and prefrontal cortex (PFC), associated with altered behavior in male and female mice (Eiland et al. 2012). Adolescence is a very critical period of brain development during which adverse experiences tend to have long lasting effects (Fuhrmann et al. 2015; Rajaleid et al. 2016). Present world scenario places a large population of adolescent individuals (10-20%) at risk, particularly in the developing world, with most being underdiagnosed and under-treated leading to growing cases of depression and suicide (Kessler et al. 2007). In our previous work (Vafadari and Mitra et al. 2019) we had reported that wild-type mice that underwent adolescence psychosocial stress exhibited increased locomotion in open field and after treatment with MK-801 providing evidence for behavioral implications of the stress paradigm. We had further shown that reduced levels of synaptic, extracellularly-operating matrix metalloproteinase-9 (MMP-9) known to affect dendritic spine morphology (Michaluk et al, 2011), further accentuates the phenotype and leads to

schizophrenia like symptoms (Vafadari and Mitra et al. 2019). Hence, we investigated whether there are any long-term structural changes in wild-type mice due to stress that might have acted as a pre-cursor to the behavioral changes and gene x environment (GXE) affects.

Stress has been known to affect hippocampus, particularly the CA3 pyramidal neurons, leading to reduction in dendritic arborizations and spine density (Conrad et al. 1999). These has been negatively associated with affected memory and cognitive abilities. It is noteworthy that in a study on tree shrews, psycho-social stress *per se* did not result in any significant changes in the spine density, but reduced branching in CA3 neurons (Magarinos et al. 1996).

A longer dendritic spine is usually associated with learning but can be unstable, whereas larger spines are usually stable and are associated with memory (Kasai et al. 2003). Our study showed in the hippocampus increased spine length in stressed mice without any significant differences in area or head width. Since hippocampus is associated with spatial information processing and episodic learning and memory, these changes along with changes observed in other similar studies might correlate to the altered behaviors observed in chronically stressed animals. It must be noted here that since the used stress paradigm is milder than social defeat, it is possible that resulted in less severe changes in hippocampus unlike as observed in other stress paradigms (Qiao et al. 2016).

Amygdala has been traditionally regarded as the center for emotional processing and the stress responsive region of the brain that plays a key role in the fight or flight response (Ressler 2010). The amygdala is quite heterogeneous in terms of its function and neuronal populations. The BLA consists mostly of glutamatergic neurons, whereas the CeA contains mainly GABAergic neurons. In the accepted model of amygdala circuitry and fear response, the BLA receives inputs from different brain regions and relays it to the CeA that is instrumental in multiple output pathways that elicit responses (Ressler 2010). However, it should be noted that CeA by itself receives inputs from different brain regions and in contrast to the BLA, particularly the

anterior BLA, which is involved in aversive responses, is actually broadly associated with appetitive responses (Kim et al. 2017). This makes it more interesting to learn about changes in dendritic spine structure in these two regions after subjection to stress, which has not been studied in details yet (Zhang et al. 2018). Chronic stress has been shown to enhance dendritic length, branching and spine density on neurons of the BLA (Vyas et al. 2002, 2003, 2006; Pawlak et al. 2003; Mitra et al. 2005; Hill et al. 2011; Qin et al. 2011; Monsey et al. 2014; Qiao et al. 2016; Montalban et al. 2019). Interestingly, Padival et al. (2015) showed that adolescent restraint stress resulted in reduced dendritic spine density in BLA as opposed to adult stress indicating possible different mode of action(s) and long-term consequences. These differences based on the age of the stressed rodent may partially explain our finding that spine shape is increased in BLA with possibly lesser mushroom shaped spines, after adolescent psycho-social stress, as opposed to those observed in BLA of rodents subjected to adult traumatic stress (Zhang et al. 2019). Our study also finds that the spines of the neurons in the CeA exhibit decreased length, decreased head width and decreased area indicating towards immature spines. This also suggests that neurons in this region are highly affected by stress. Since it has been shown that CeA neurons also inhibit aversive neuronal projections from the BLA (Kim et al. 2017) apart from acting as a relay center, it can be speculated that the observed morphological changes may be associated with such gating resulting in exhibition of fear and anxiety. It is interesting to note that chronic stress has been shown to have no effect on dendritic branching and length in neurons of the central amygdala (Vyas et al. 2003). This places stronger emphasis on the importance of studying spine morphology and thus a further detailed analysis on the spine changes in different neuronal subpopulations in CeA would provide more answers to stress response and affected aversive and reward pathways.

In conclusion, this work provides new information on spine structural changes in the hippocampus and amygdalar subregions in mice subjected to psycho-social stress. The

observed spine structure changes are important in light of understanding pre-disposition and pathogenesis of several psychiatric disorders like schizophrenia and depression.

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Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Author Contributions: BV and LK designed the study. BV performed the experiments and analyzed the data. BV and SM wrote the manuscript.

Data Availability: The datasets generated for this study are available.

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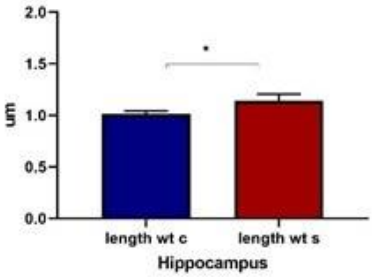
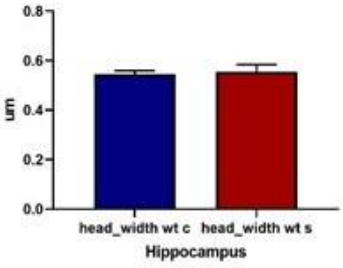
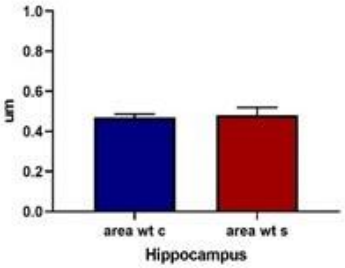






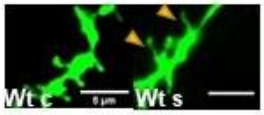
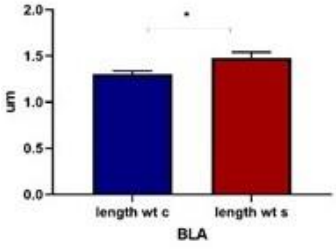
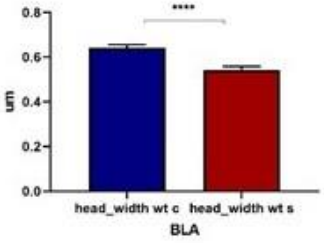
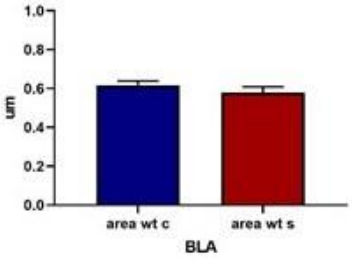


Fig.1 Dendritic spines morphology alterations in the hippocampus following stress in mice: After DiO staining and microscopy, the dendritic spines with 488 nm emission were visualized. After imaging, the spines analyzed by SpineMagick software. Dendritic spines were longer in wt s mice than wt c mice. wt c: control, wt s: stressed ($P < 0.05$ *). Analyzed by unpaired t-test.







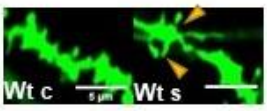
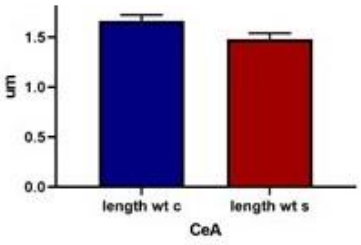
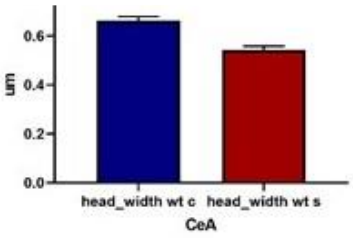


Fig.2 Dendritic spines morphology alterations in the BLA following stress in mice: After DiO staining and microscopy, the dendritic spines with 488 nm emission were visualized. After imaging, the spines analyzed by SpineMagick software. The spines are longer in wt s than controls whereas this difference is opposite in headwidth. wt c: control, wt s: stressed ($P < 0.05$ *, $P < 0.0001$ ****). Analyzed by unpaired t-test.





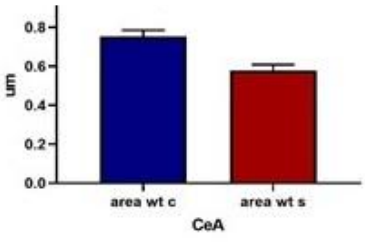


Fig.3 Dendritic spines morphology alterations in the CeA following stress in mice: After DiO staining and microscopy, the dendritic spines with 488 nm emission were visualized. After imaging, the spines analyzed by SpineMagick software. The spines are longer in wt c. The headwidth and area in wt c mice are larger than stressed group. wt c: control, wt s: stressed ($P < 0.05$ *, $P < 0.0001$ ****). Analyzed by unpaired t-test.

Figures

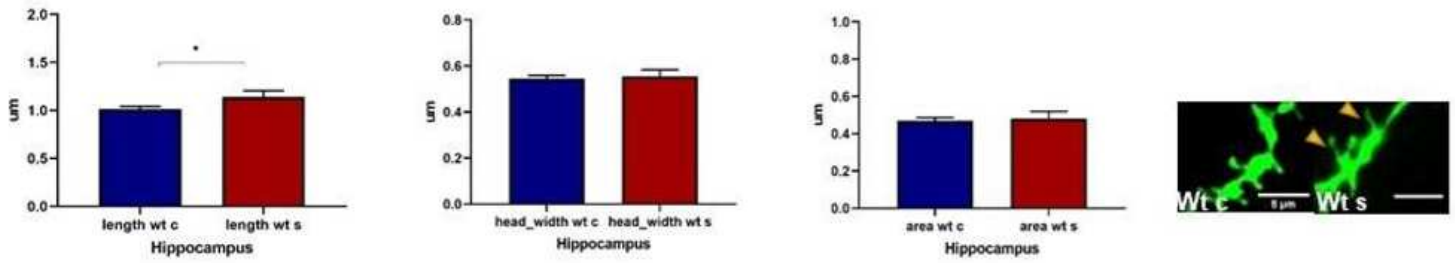


Figure 1

Please see the Manuscript PDF file for the complete figure caption

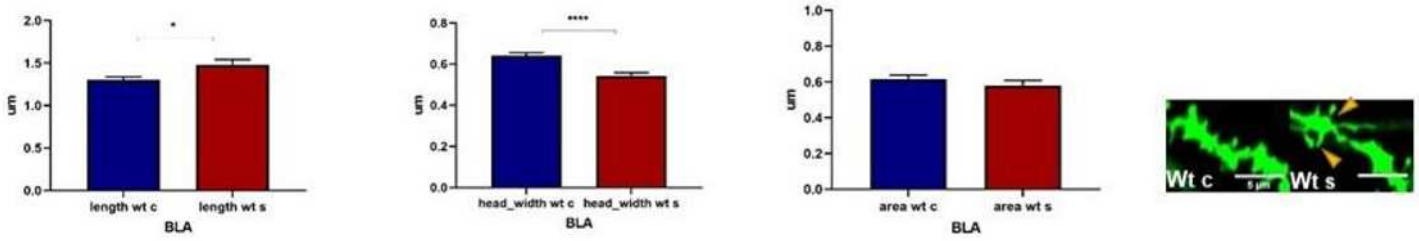


Figure 2

Please see the Manuscript PDF file for the complete figure caption

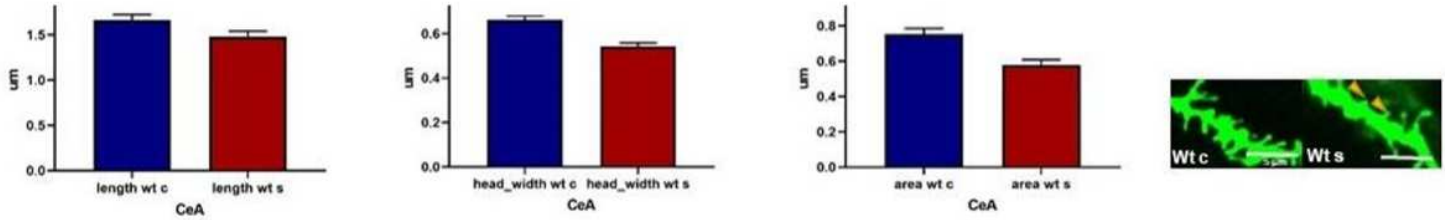


Figure 3

Please see the Manuscript PDF file for the complete figure caption