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Inactivation of Basolateral Amygdala Prevents Stress-Induced Astroglial Loss in the Prefrontal Cortex

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Abstract

Repeated stress causes cognitive decline and decreases the expression of glial fibrillary acidic protein $(GFAP)^+$ astroglial cells in the prefrontal cortex (PFC). The stress-induced alterations in astroglial density and morphology might significantly contribute to cognitive impairments. Apart from PFC, a key region involved in modulation of repercussions of stress is basolateral amygdala (BLA), which undergoes hypertrophy following chronic immobilization stress (CIS) and has intense reciprocal connections to the PFC. Interestingly, inactivation of BLA precludes stress-induced learning deficits. However, the modulatory role of BLA on CIS-induced alterations in $GFAP^+$ astroglial density and associated learning deficits are presently unknown. Accordingly, we present two sets of experiments evaluating the effects of BLA inactivation either permanently or temporarily on CIS-induced changes in learning and astroglial expression in the PFC. CIS causes impairment in novel object recognition memory and astroglial loss in the PFC. In experiment I, we permanently inactivated the BLA by ibotenate lesion prior to CIS and observed a significant improvement in learning. Surprisingly, BLA lesion also prevented the stress-induced astroglial loss in the PFC. Furthermore, in the experiment II, we analyzed whether the effects of permanent inactivation could be mirrored by the temporary blockage of BLA specifically during stress. Interestingly, temporary inactivation of BLA mimics the effects of lesion. There was a notable prevention of learning impairment and astroglial loss in the PFC following BLA inactivation during stress. The present study emphasizes that stress-induced astroglial loss might contribute to cognitive deficits and modulation of BLA activity might be a viable strategy for management of stress-related PFC dysfunctions.

Keywords Chronic stress · Inactivation of basolateral amygdala · Prefrontal cortex · Astroglial plasticity · Cognitive deficits

Abbreviations

BLA	Basolateral amygdala
PFC	Prefrontal cortex
PrL	Prelimbic cortex
ACC	Anterior cingulate cortex
GFAP	Glial fibrillary acidic protein
CIS	Chronic immobilization stress
NOR	Novel object recognition
HPA axis	Hypothalamic pituitary adrenal axis
NMDA	*N*-methyl-D-aspartate

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Introduction

Repeated exposure to stressful conditions causes profound cognitive decline [1–3] which are often associated with the emergence of depressive [4–6] and anxiety [7] disorders. The stress-induced aberrant changes in neuronal functions such as dendritic debranching, spine loss, and altered electrophysiological properties are well studied in the prefrontal cortex (PFC) and are often associated with learning deficits [1, 2, 8]. In addition to neuronal changes in the PFC, growing body of evidence suggests that astrocytes in the PFC mediate several aspects of stress-related dysfunctions [9, 10]. Several lines of evidence from past decade show that chronic stress profoundly affects morphology and functioning of astrocytes in various stress-sensitive regions, particularly the PFC [11]. Given the role of astrocytes in the maintenance of synaptic function [12, 13], it is plausible that stress-induced astrocytic loss and disruption of morphology could mediate cognitive decline. For instance, inhibition of glial glutamine synthetase [14], glycogenolysis [15], or glutamate reuptake [16] impairs learning and memory.

Selective inhibition of astrocytes causes decreased gamma oscillations which were associated with impaired novel object recognition memory [17]. Further, astroglial loss during chronic stress has been associated with learning impairment [4] and the emergence of depressive behavior [10]. Additionally, astrocytic dysfunctions have been widely reported in neurodegenerative disorders associated with cognitive impairments such as Alzheimer's disease [18]. Together, these studies demonstrate that astrocytic dysfunctions might be crucial in the manifestation of stress-induced behavioral effects including cognitive decline.

Apart from PFC, the basolateral amygdala (BLA) is crucially involved in modulation of stress-induced cognitive deficits [8, 19, 20]. In an earlier study, we showed that chronic immobilization stress (CIS) causes dendritic hypertrophy and enhanced spinogenesis in the principal neurons of BLA [21]. In contrast to BLA, prefrontal cortical neurons undergo dendritic atrophy and spine loss following stress [2, 22, 23]. Importantly, detrimental effects of stress might be attributed to extensive reciprocal connections between prefrontal cortex (PFC) and BLA [24–27]. Several lines of research suggest that BLA can modulate the activity of PFC during stressful conditions [20, 28–30]. For instance, lesion of BLA blocked the detrimental effects of both systemic corticosterone and intra-PFC glucocorticoid receptor agonist administration on working memory [20, 28]. The lesion and pharmacological inactivation of BLA not only blocks detrimental effects of stress on the PFC [8, 20] but also in the hippocampus [29, 31–36]. In our earlier study, we demonstrated that inactivation of BLA prevents the stress-induced spatial learning deficit in a partially baited radial arm maze task. In addition, the silencing of BLA partially blocked the increase in plasma corticosterone levels following stress [37]. These findings suggest that BLA activity is crucial in mediating the stress-induced detrimental effects on PFC and hippocampal-dependent learning. However, to our knowledge, there are no studies investigating the effects of inactivation of BLA on stress-induced alterations in prefrontal astrocytic density and accompanying cognitive decline. This study will emphasize the role of cortical astrocytes in learning especially during chronic stress conditions. Further, it will elucidate a putative mechanism for prevention of cognitive deficits by amygdalar silencing during stress.

The primary aim of the study was to evaluate the modulatory role of BLA on stress-induced alterations in the density of cortical astrocytes and associated changes in learning. We hypothesized that inactivation of BLA could prevent stress-induced learning impairment and associated astroglial loss in the PFC. Accordingly, we evaluated the effects of BLA inactivation on CIS-induced alterations in novel object recognition memory and glial fibrillary acidic protein $(GFAP)^+$ astroglial density in the prelimbic (PrL) and anterior cingulate (ACC) subregions of the PFC. We estimated the $GFAP^+$ astrocytes using unbiased stereology, which is a gold standard method for estimation of cellular density [9]. In addition, we utilized two different strategies to inhibit BLA during stress. The experiment I was performed to determine whether ablation of the BLA using ibotenic acid prior to CIS would prevent the stress-induced astroglial loss and associated decline in recognition memory. Furthermore, experiment II was performed to assess whether temporary inactivation would mirror the effects of BLA lesion. Therefore, in experiment II, we temporarily silenced the BLA using lidocaine, a sodium channel blocker, prior to each session of immobilization stress for 10 days and then assessed the recognition memory and changes in GFAP density.

Materials and Methods

Subjects

Adult male Wistar rats (200–225 g; 2–2.5 months old) were obtained from the Central Animal Research Facility, NIMHANS, Bengaluru, and group housed in a climate-controlled vivarium with a 12:12-h dark/light cycle. The water and food were available ad libitum. The behavioral experiments were carried out during the light phase of the cycle between 09:00 and 15:00 h. All the experiments were in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India and approved by the Institutional Animal Ethics Committee.

Experimental Design

Figure 1 shows the outline for the experiment I (a) and II (b). Before the start of experiments, all animals were handled by the experimenter for 7 days. In experiment I, we evaluated the effect of BLA lesion on stress-induced cognitive deficits and associated changes in $GFAP^+$ astroglial density in the PFC. Animals were randomized to one of the groups: naïve rats either served as non-stressed control (NC) or subjected to 10 days of CIS, rats with intra-BLA phosphate-buffered saline (PBS, pH 7.4) infusion either served as non-stressed vehicle control (PBS) or were subjected to CIS and rats with intra-BLA ibotenate infusion either served as non-stressed lesion control (IBO) or were subjected to CIS.

Experiment II was performed to evaluate the effect of transient inactivation of BLA during stress on learning and GFAP expression in the PFC. The animals were randomly allocated to one of the following groups: rats receiving a single intra-BLA infusion of saline (4 min/day) on each day for 10 days, either served as non-stressed saline control (SAL) or were subjected to immobilization stress, 15 min following saline infusion, rats subjected to a single intra-BLA lidocaine (0.25 μl/ min for 4 min/day) infusion on each day for 10 days,

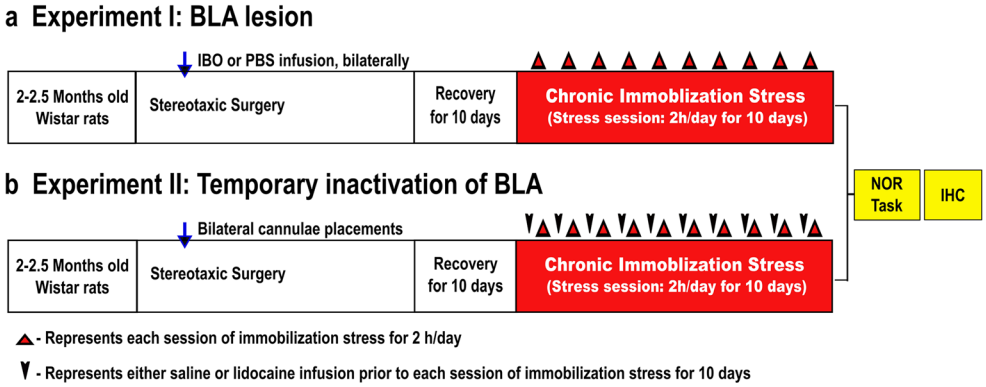

Fig. 1 Schematic representation of experiments I and II. In both experiments, 2–2.5-month-old adult male Wistar rats were subjected to handling by experimenter for 7 days prior to the onset of experiments. In experiment I (**a**), rats were subjected to stereotaxic bilateral intra-BLA infusion of either PBS (vehicle) or ibotenic acid. Following 10 days of postsurgical recovery, the animals were subjected to 10 days of immobilization stress for 2 h between 10:00 and 12:00 h. On 11th and 12th day, rats from all groups were subjected to assessment of novel object recognition (NOR) task, followed by perfusion and immunohistochemical localization of $GFAP^+$ cells in the PrL and ACC subregions of PFC. Experiment II (**b**) was performed in a separate set of animals. Rats were subjected to bilateral cannulae implantation into the BLA followed by 10 days of postsurgical recovery. These rats received an intra-BLA infusion of either saline or lidocaine 15 min prior to each session of stress for 10 days followed by NOR task and immunohistochemistry (IHC). The arrow indicates the bilateral infusion of PBS or ibotenic acid into the BLA during stereotaxic surgery; arrowhead indicates an intra-BLA infusion of either saline or lidocaine and the filled triangle indicates each session of 2 h of immobilization stress for 10 days

either served as non-stressed lidocaine control (LI) or exposed to immobilization stress, 15 min following lidocaine infusion.

The naïve rats which either served as non-stressed control (NC) or subjected to 10 days of CIS are the same group of animals for the experiments I and II. Further, the naïve non-stressed and stressed rats served as non-surgical control. For both experiments, rats underwent surgical manipulations followed by recovery for 10 days. Thereafter, one set of rats served as non-stressed control and another set was subjected to CIS. In experiment I, rats received either single intra-BLA infusion of ibotenic acid or PBS during surgery followed by recovery and exposure to CIS. In experiment II, animals received either lidocaine or saline infusion prior to each session of stress for 10 days. After that, non-stressed and stressed animals from both experiments were subjected to novel object recognition task followed by perfusion and immunohistochemistry experiments.

Surgery and Infusion Schedule

Rats were anesthetized with ketamine hydrochloride (90 mg kg^{-1}; Ketmin® 50, Themis Medicare Ltd., India) and xylazine (10 mg kg^{-1}; Xylaxin®, Indian Immunologicals Ltd., India) combination injected via intraperitoneal (i.p.) route and placed in a stereotaxic instrument (David Kopf Instruments, USA) after ensuring the surgical plane of anesthesia.

Experiment I

In experiment I, animals were subjected to an excitotoxic lesion of BLA using ibotenic acid. A 30-gauge infusion needle was targeted to the BLA (anteroposterior, − 2.8 to − 3.0 mm from bregma; mediolateral, 4.8 to 5 mm; and dorsoventral, 7.8 to 8.2 mm from dura [38]). Ibotenic acid solution (10 μg/ml) was prepared freshly in PBS, pH 7.4, and infused bilaterally (0.25 μl/per side) using a microinjector (Harvard Apparatus, MA, USA) through an infusion needle connected to a Hamilton syringe [37]. The infusion needle was left in place for an additional 2 min for diffusion. Animals of the vehicle control group received a bilateral intra-BLA infusion of PBS. The rats were allowed to recover for 10 days and then subjected to CIS.

Experiment II

A stainless-steel guide cannula was implanted bilaterally, 1 mm above the BLA (anteroposterior, − 2.8 to − 3.0 mm from bregma; mediolateral, 4.8 to 5 mm; and dorsoventral, 7.0 to 7.2 mm from dura [38]). A 30-gauge stylet was inserted to maintain the patency of the cannula. Following surgery, the rats were allowed to recover and then subjected to either intra-BLA lidocaine or saline infusion 15 min prior to each session of immobilization stress for 10 days. Lidocaine (50 μg/μl) was freshly prepared in isotonic saline and injected using 30-gauge infusion needle through the guide cannula by removing the stylet. Lidocaine (0.25 μl/min) was infused bilaterally over a period of 4 min. The needle was retained in place for an additional 2 min for diffusion [37].

Chronic Immobilization Stress

The CIS procedure was performed as described previously [37, 39, 40]. Rats were subjected to 10 consecutive days of

immobilization stress for 2 h per day between 10:00 and 12:00 h. Animals were immobilized in a decapitation cone and secured using adhesive tapes. The decapitation cones were made up of a thick plastic sheet and tapered at one end to facilitate the entry of animals. The cones were trimmed at the tapered end to promote breathing [37]. Following each session of immobilization stress, the rats were released from the cones and returned to the home cage.

Novel Object Recognition Task

Following CIS on days 11 and 12, the novel object recognition (NOR) task was performed for the experiments I and II. The task was performed as described previously [41] with minor modifications. The apparatus was a wooden black open-field box (100 × 100 × 40 cm) with a paddy husk-covered floor, placed in a dimly illuminated room. The objects to be discriminated were made up of inert material and heavy enough not to be displaced by rats. We used identical copies of white marble pots and 1 kg iron weight. The task consists of three sessions namely habituation, familiarization, and test (Supplementary Fig. 1). The habituation was performed on day 11, whereas familiarization and test sessions were performed on day 12. During habituation, each rat was allowed to explore the arena in the absence of objects in two sessions of 10 min each with an interval of 1 h. In the familiarization session, the rat was placed in the arena and allowed to explore two identical marble pots for a duration of 5 min. The objects were placed in diagonally opposite corners of the apparatus. To avoid the olfactory cues in a particular location of the field, the objects were cleaned with 70% alcohol, counterbalanced, and the husk was mixed prior to initiation of each trial. The intra-trial interval between familiarization and test was 1 h. During the test, one of the familiar objects was replaced with a novel object having a different texture, color, and shape [42, 43]. The animal was allowed to explore the arena containing one novel and one familiar object for a duration of 5 min. The objects were cleaned, counterbalanced, and husk was mixed between the animals. The familiarization and test sessions were videotaped and analyzed using commercial tracking software (EthoVision® XT 8.0, Noldus Information Technology, The Netherlands). The time spent in exploring each object was recorded during familiarization and test sessions. Exploration was defined as the investigation of an object by touching with the nose and/or by pointing the nose from a distance of not more than 2 cm. Discrimination index (DI) was calculated as a percentage of difference in time spent with novel and familiar object to total time spent exploring the objects.

$$\mathrm{DI} = \frac{\text{Time spent with novel object}(N) - \text{Time spent with familiar object}(F)}{\text{Time spent with novel object}(N) + \text{Time spent with familiar object}(F)} \times 100$$

Rats showing a total exploration time of less than 10 s in either familiarization or test session were excluded from the study. The number of animals utilized for NOR task was 8 rats per group.

Perfusion and Fixation

On day 13, rats from both experiments were deeply anesthetized and transcardially perfused with cold 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were harvested and post-fixed in the same fixative for 72 h.

Histology

For stereological analysis, every sixth 40-μm-thick coronal section [5, 39, 44–47] was obtained through rostrocaudal levels of the PFC extending from bregma + 1.8 to + 5.8 mm [38] using a vibratome (Leica, Wetzlar, Germany). After collection of PFC sections, 50-μm-thick sections, spanning entire BLA, were collected and stained with 0.1% cresyl violet [37, 48, 49]. The cresyl violet-stained sections were examined for needle tip/cannula placement or extent of lesions under a bright field microscope (BX-51, Olympus, Tokyo, Japan). The rats with misplaced cannulae, excessive lesion, or with extensive tissue damage at the site of injection were excluded from the analysis.

Immunohistochemistry

Free-floating sections were equilibrated in 0.1 M phosphate buffer saline (PBS) and then incubated for 30 min in sodium citrate buffer at 40 °C, followed by washing with PBS containing 0.3% Triton X-100 (PBS-Tx). To eliminate endogenous peroxidases, sections were incubated in 1:1 PBS/methanol containing 3% H_2O_2 for 30 min followed by blocking with 3% normal horse serum in 0.1 M PBS-Tx (pH 7.4) for 2 h. The sections were incubated overnight at 4 °C with rabbit anti-GFAP (1:500; Sigma-Aldrich, St. Louis, MO, USA). The sections were incubated with biotinylated universal secondary antibody for 3 h at room temperature followed by incubation with avidin-biotin complex (Vector Laboratories Inc., Burlingame, CA, USA). The immunolabeled sections were developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO, USA). The DAB-stained sections were mounted onto gelatin-coated slides, lightly counterstained with cresyl violet, dehydrated, cleared in xylene, and coverslipped.

Unbiased Stereology

All the slides were coded and analyzed by a trained observer, blind to experimental conditions. The number of $GFAP^+$ cells in the PrL and ACC was quantified throughout the rostrocaudal axis using an optical fractionator in

Stereoinvestigator software (MBF Bioscience, Williston, Vermont, USA). The optical fractionator probe enables the three-dimensional unbiased counting of cells using an optical dissector distributed over a fraction of section selected by systematic random sampling [50]. Live images of GFAP$^+$ cells were visualized using a BX51 Olympus microscope, attached to a MAC 5000 XYZ motorized stage with Ludl's joystick, focus control (Ludl Electronic Products, Hawthorn, NY, USA), and an imaging video camera (MBF Bioscience, Williston, VT, USA). The anatomical boundaries of PrL and ACC were contoured at a low magnification (×4, N.A. 0.1). The immunoperoxidase-stained GFAP$^+$ cells were then counted at high magnification (×100, N.A. 1.3) on every sixth section with a guard zone of 2 μm. The GFAP$^+$ astrocytes were counted, only if the cell body came into focus within the dissector height. The total number of GFAP$^+$ cells (N) in the PrL and ACC was estimated by multiplying the total number of cells per section by the section periodicity and expressed as the total number of GFAP$^+$ cells per region as described earlier [39, 44–47].

$$N = \sum Q^- \times {}^1/_{ssf} \times {}^1/_{asf} \times {}^t/_h$$

where N is the number of GFAP$^+$ cells estimated within the region of interest; $\sum Q^-$ is the actual number of GFAP$^+$ cells counted; *ssf* is the section sampling fraction, i.e., 6; *asf* is area sampling fraction which consists of area of X, Y steps, i.e., 375 μm × 375 μm divided by area of counting frame, i.e., 80 μm × 80 μm; t is thickness of the section, i.e., ~18–19 μm; and h is the height of dissector, i.e., 10 μm. The cellular density of GFAP$^+$ cells in the PrL (n = 5–6 per group) and ACC (n = 5–6 per group) was calculated by dividing the total number of cells with the estimated planimetry volume.

Statistical Analyses

The time spent with the novel and familiar object during test session was analyzed using a paired t test. The data of discrimination indices and stereological estimates were analyzed with a two-way ANOVA with treatment groups (three levels) as column factor and stress (two levels) as row factor. Further, we have used Tukey's post hoc test to determine the source of significance detected by the ANOVA. Cohen's d was calculated for pairwise estimation of effect size. Pearson's correlation coefficient was calculated to find the relationship between learning and GFAP expression. Data are expressed as mean ± SEM. $p < 0.05$ was considered as statistically significant. The number of animals utilized in each group for different parameters is indicated in the figure legends.

Results

Experiment I

Basolateral Amygdalar Lesions

The extent of lesion in the BLA across different atlas plates ranging from bregma −1.60 to −3.80 mm is shown in Fig. 2a. Representative photomicrographs of an intact BLA in non-stressed PBS control rat (a–d) and lesioned BLA following ibotenic acid infusion (e–h) are shown in Supplementary Fig. 2. The animals with bilateral BLA lesions of 50% or more were included in the analysis. The data of seven animals were excluded from the analysis due to the misplaced infusion of ibotenic acid leading to lesions outside the BLA.

Lesion of Basolateral Amygdala Prevents Chronic Stress-Induced Impairment in Novel Object Recognition Memory

In experiment I, we first evaluated the effect of BLA lesion on stress-induced alterations in recognition memory using NOR task. Figure 3 shows the representative tracks (a), time spent with novel and familiar objects (b), and discrimination indices (c) during the test phase of the task. On the 12th day, the non-stressed control and stressed rats were subjected to familiarization and test phase of NOR task. A paired Student's t test revealed that non-stressed control rats spent significantly more time with the novel object ($t_{(7)} = 4.77$, $p < 0.01$ vs. familiar object, $n = 8$). Stressed animals were unable to discriminate between the novel and familiar object ($t_{(7)} = 0.80$, $p > 0.05$ vs. familiar object, $n = 8$), suggesting deficits in recognition memory. The performance of PBS-infused rats subjected to stress was similar to that of stressed animals ($t_{(7)} = 0.67$, $p > 0.05$ vs. familiar object, $n = 8$). BLA lesion prior to stress blocked the impairment in recognition memory. The lesioned animals subjected to stress spent more time in exploring novel object than the familiar object ($t_{(7)} = 5.22$, $p < 0.01$, $n = 8$; Fig. 3b). PBS infusion or BLA lesion in the absence of stress did not affect the recognition memory (PBS: $t_{(7)} = 4.61$, $p < 0.01$, $n = 8$; IBO: $t_{(7)} = 3.99$, $p < 0.01$ vs. familiar object, $n = 8$). Further, total exploration time during test phase was similar between non-stressed control and stressed rats (Table 1, $p > 0.05$). The stressed animals and their respective non-stressed control spent almost equal time exploring identical copy of the objects during familiarization phase (for all groups, $p > 0.05$ vs. object 2; data not shown).

A two-way ANOVA of discrimination index revealed a significant effect of stress ($F_{(1, 42)} = 42.08$, $p < 0.001$), group ($F_{(2, 42)} = 9.63$, $p < 0.001$) and interaction between stress × lesion ($F_{(2, 42)} = 8.81$, $p < 0.001$). Post hoc Tukey's

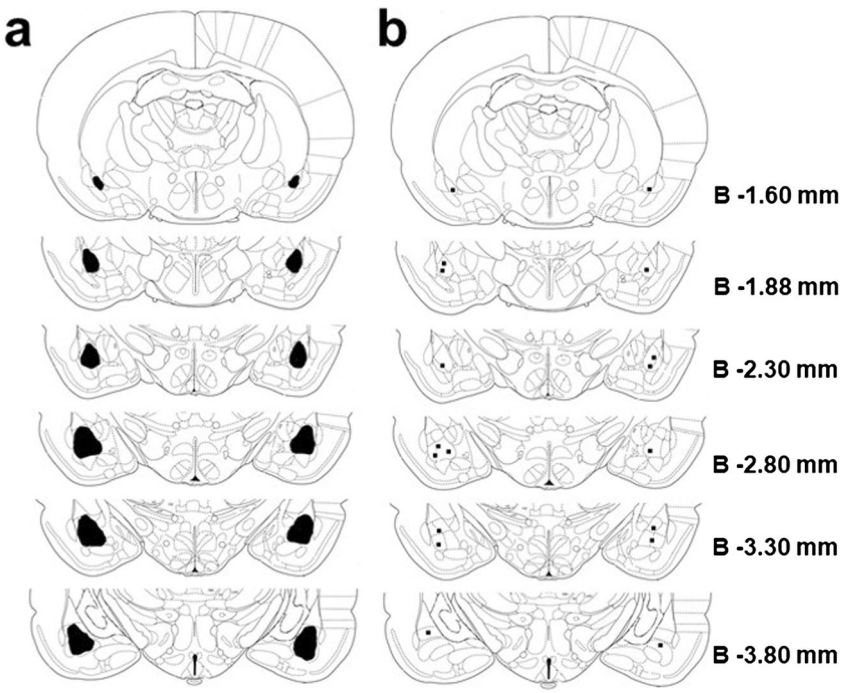

Fig. 2 Schematic diagram showing the extent of lesion and cannulae placements in the BLA. Schematic representation of the average extent of bilateral damage to the BLA following ibotenic acid infusion ($n = 4$, **a**). The areas in black indicate neuronal loss across different levels of atlas plates ranging between bregma − 1.60 and − 3.80 mm. Bilateral mapping of the site of injection and cannulae placements within the BLA for temporary inactivation ($n = 9$, **b**). Each filled square represents the location of the cannula between bregma − 1.60 and − 3.80 mm [38]

comparisons suggested that CIS significantly decreases the discrimination index ($p < 0.001$ vs. NC non-stressed control, $d = 2.71$; Fig. 3c). The decrease in the discrimination index of PBS-infused rats subjected to stress was similar to that of stressed rats ($p > 0.05$ vs. NC rats subjected to stress). BLA lesion prior to stress abolished the impairment in recognition memory ($p < 0.001$ vs. PBS-infused rats subjected to stress, $d = 2.33$). In the absence of stress, the discrimination indices for lesioned or PBS-infused rats were similar to that of NC non-stressed control ($p > 0.05$).

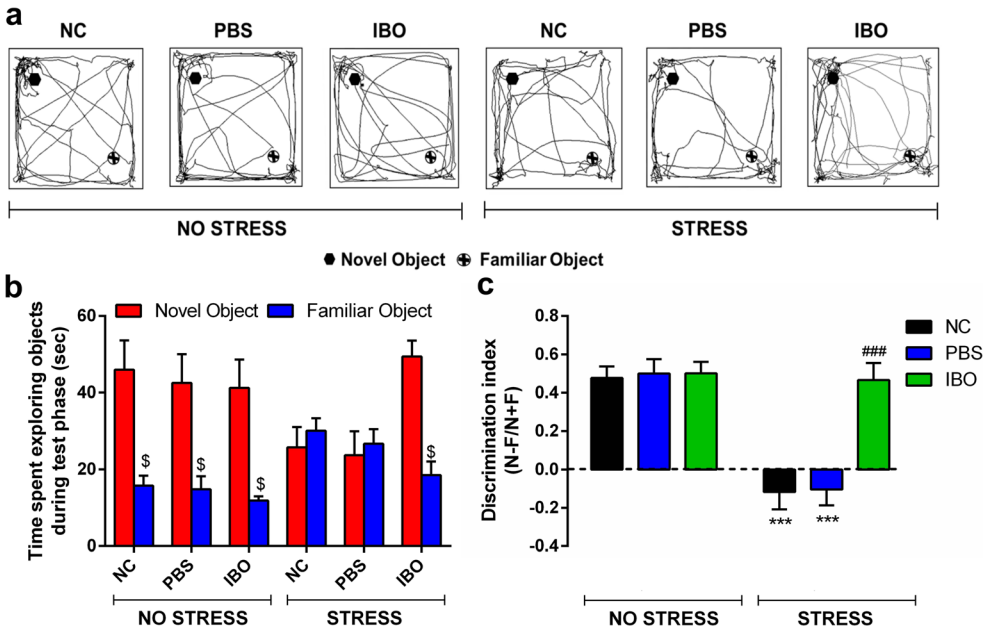

Fig. 3 BLA lesion prevents stress-induced impairment in novel object recognition memory. Representative tracks of stressed rats and their respective non-stressed control (**a**), time spent with novel and familiar objects during test phase (**b**), and discrimination index (**c**). Data are expressed as mean ± SEM. NC: naïve rats either served as non-stressed control or were subjected to chronic immobilization stress; PBS: rats with intra-BLA PBS infusion either served as non-stressed PBS control or were subjected to chronic immobilization stress; IBO: rats with intra-BLA ibotenate infusion either served as non-stressed lesion control or were subjected to chronic immobilization stress. $^{\$}p < 0.001$ vs. familiar object, paired t test; $^{***}p < 0.001$ vs. corresponding non-stressed control, $^{\#\#\#}p < 0.001$ vs. PBS-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test ($n = 8$ per group)

Table 1 BLA lesion did not affect the total time spent in exploring objects during the test session of novel object recognition task

Total object exploration time (s)					
No stress			Stress		
NC ($n = 8$)	PBS ($n = 8$)	IBO ($n = 8$)	NC ($n = 8$)	PBS ($n = 8$)	IBO ($n = 8$)
61.72 ± 9.55	58.30 ± 10.17	47.07 ± 6.63	55.92 ± 6.92	50.40 ± 9.40	67.92 ± 4.95

Data are expressed as mean ± SEM. The number of animals in each group is indicated in the parenthesis

NC naïve rats either served as non-stressed control or were subjected to chronic immobilization stress, *PBS* rats with intra-BLA PBS infusion either served as non-stressed PBS control or were subjected to chronic immobilization stress, *IBO* rats with intra-BLA ibotenate infusion either served as non-stressed lesion control or were subjected to chronic immobilization stress

Basolateral Amygdalar Lesion Prevents Chronic Stress-Induced Loss of GFAP$^+$ Astroglial Cells in the Prefrontal Cortex

The number of GFAP$^+$ cells in PrL and ACC was estimated using an optical fractionator probe. To substantiate the existing literature, we first sought to find out whether there is any loss of GFAP$^+$ astroglial cells within the PFC in our stress model. Thereafter, we evaluated the effect of BLA lesion prior to CIS on alterations in GFAP$^+$ astroglial cells.

1. *Lesion of basolateral amygdala prevents stress-induced loss of GFAP$^+$ astroglial cells in the PrL*

Figure 4 (top panel) shows the region of investigation spanning between bregma + 5.16 and + 2.52 mm (a), representative photomicrographs of GFAP-stained section of stressed groups and respective non-stressed control (b–g), stereological quantification of GFAP$^+$ number (h), and density (i) of astroglial cells in the PrL cortex. A two-way ANOVA of GFAP$^+$ astroglial numbers revealed a significant effect of stress ($F_{(1, 29)} = 62.35$, $p < 0.0001$), BLA lesion ($F_{(2, 29)} = 9.63$, $p < 0.001$), and interaction ($F_{(2, 29)} = 12.19$, $p < 0.001$). Similarly, a two-way ANOVA of GFAP$^+$ astroglial density revealed a significant effect of stress ($F_{(1, 29)} = 18.60$, $p < 0.001$) and interaction ($F_{(2, 29)} = 5.61$, $p < 0.01$).

Post hoc comparisons indicated a decrease in the number of GFAP$^+$ astrocytes ($p < 0.001$, $d = 2.84$, $n = 5$) as well as density ($p < 0.01$, $d = 1.94$, $n = 5$) in the PrL region of stressed rats as compared to non-stressed control. Decreased GFAP$^+$ astroglial number and density (both, $p > 0.05$, $n = 6$) in the PBS-infused rats subjected to stress were similar to that of stressed rats. Lesion of BLA prior to stress prevented the decrease in GFAP$^+$ astroglial number ($p < 0.01$, $d = 3.67$, $n = 6$) and density (both, $p < 0.01$, $d = 2.85$, $n = 6$) as compared to PBS-infused rats subjected to CIS. PBS infusion or BLA lesion in the absence of stress did not affect GFAP$^+$ astroglial number and density (both, $p > 0.05$ vs. NC non-stressed control, $n = 6$) in the PrL region of PFC. Further, the improvement in novel object recognition memory following lesion was correlated with the prevention of decrease in GFAP$^+$ astroglial density in the PrL cortex ($p < 0.001$, $r^2 = 0.87$, Fig. 9a).

Remarkably, chronic exposure to stress causes region-specific changes in the astroglial cell density. The number and density of GFAP$^+$ astroglial cells within the reference area, i.e., primary motor cortex, remained unaltered between non-stressed control and stressed rats ($p > 0.05$ vs. NC rats subjected to stress, $n = 4$; Supplementary Fig. 3).

2. *Permanent inactivation of basolateral amygdala prevents stress-induced loss of GFAP$^+$ astroglial cells in the ACC*

Figure 4 (bottom panel) shows the anatomical boundaries of ACC from bregma + 4.20 to + 2.16 mm (j), representative photomicrographs of GFAP-stained astroglia cells in stressed groups and respective non-stressed control (k - p), number (q), and density (r) of GFAP$^+$ astroglial cells within ACC. Similar to PrL, we found that CIS causes profound loss of astroglial cells in the ACC. A two-way ANOVA of GFAP$^+$ astroglial numbers revealed a significant effect of stress ($F_{(1, 30)} = 51.89$, $p < 0.001$), BLA lesion ($F_{(2, 30)} = 10.30$, $p < 0.001$), and interaction ($F_{(2, 30)} = 9.28$, $p < 0.001$). Similarly, a two-way ANOVA of GFAP$^+$ astroglial density revealed a significant effect of stress ($F_{(1, 30)} = 28.11$, $p < 0.001$), BLA lesion ($F_{(2, 30)} = 3.89$, $p < 0.05$), and interaction ($F_{(2, 30)} = 4.19$, $p < 0.05$).

Further, intergroup comparisons indicated a decrease in GFAP$^+$ astrocyte number ($p < 0.001$, $d = 3.15$, $n = 6$) and density ($p < 0.01$, $d = 2.55$, $n = 6$) within the ACC region of stressed rats as compared to non-stressed control. PBS infusion or BLA lesion in the absence of stress did not affect GFAP$^+$ astroglial number and density (both, $p > 0.05$ vs. NC non-stressed control, $n = 6$). PBS-infused rats subjected to stress also showed a decrease in GFAP$^+$ astroglial number and density ($p > 0.05$ vs. NC rats subjected to stress, $n = 6$). Interestingly, lesion of BLA prior to stress prevented the decrease in GFAP$^+$ astroglial number ($p < 0.001$, $d = 3.97$, $n = 6$) and density ($p < 0.05$, $d = 2.06$, $n = 6$) as compared to PBS-infused rats subjected to stress. In addition, the prevention of decrease in GFAP$^+$ astroglial density in ACC following BLA

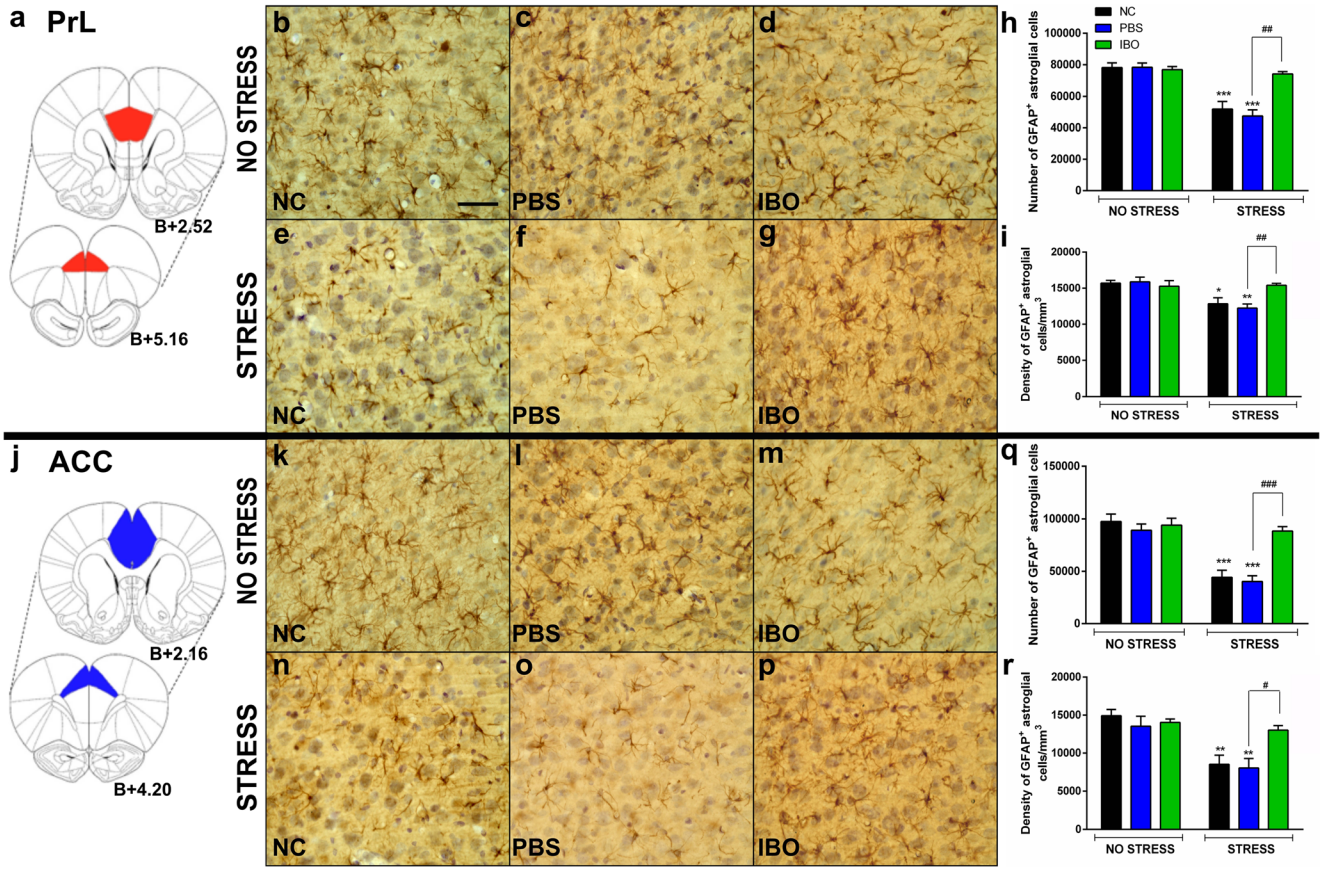

Fig. 4 Lesion of BLA prior to stress precludes GFAP$^+$ astroglial loss in the prefrontal cortex. The anatomical boundaries of PrL cortex from bregma + 5.16 to + 2.52 mm (**a**) and ACC from bregma + 4.20 to + 2.16 mm (**j**) [38]. Representative micrographs of GFAP-stained section of PrL (**b**–**g**) and ACC (**k**–**p**) of stressed groups and respective non-stressed control at ×40 magnification. Scale bar = 25 μm in **b** and is applicable to all. Stereological quantification of GFAP$^+$ astroglial number and density within the PrL (**h** and **i**, respectively) and ACC (**q** and **r**, respectively). Data is expressed as mean ± SEM. Groups are as described in Fig. 3. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ vs. corresponding non-stressed groups, $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ vs. PBS-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test (n = 5–6 per group)

lesion was significantly correlated with restoration of deficits in novel object recognition memory ($p < 0.001$, $r^2 = 0.90$, Fig. 9b).

Basolateral Amygdalar Lesion Prevents Stress-Induced Volume Loss in the Prefrontal Cortex

Figure 5 shows the planimetry volumes of PrL (a) and ACC (b) in stressed groups and respective non-stressed control. The planimetry volumes of PrL and ACC were estimated during the acquisition of astroglial numbers and density. We found that exposure to CIS decreases the volume of both PrL and ACC. A two-way ANOVA of PrL volumes indicated a significant effect of stress ($F_{(1,\ 29)} = 26.20$, $p < 0.001$) and BLA lesion ($F_{(2,\ 29)} = 5.11$, $p < 0.05$). Similar to PrL, two-way ANOVA of ACC volumes indicated a significant effect of stress ($F_{(1,\ 30)} = 16.07$, $p < 0.001$), BLA lesion ($F_{(2,\ 30)} = 6.40$, $p < 0.01$), and interaction ($F_{(2,\ 30)} = 5.47$, $p < 0.01$). The post hoc analysis revealed that exposure to CIS decreases the volume of both PrL ($p < 0.05$, $d = 2.16$, $n = 5$) and ACC ($p < 0.05$, $d = 3.07$, $n = 6$) as compared to NC non-stressed control. Neither infusion of PBS nor ibotenic acid into the BLA in non-stressed rats affected the volumes of PrL and ACC ($p > 0.05$ vs. NC non-stressed control, $n = 6$). The volume loss in both regions was persistent in PBS-infused rats subjected to stress (both, $p > 0.05$ vs. NC rats subjected to stress, $n = 6$). Interestingly, the BLA lesion prior to stress precluded the loss of volume in PrL cortex ($p < 0.01$, $d = 2.09$ vs. PBS-infused rats subjected to stress, $n = 6$) and ACC ($p < 0.01$, $d = 2.06$ vs. PBS-infused rats subjected to stress, $n = 6$).

Experiment 2

The preventive effects of BLA lesion prior to stress motivated us to investigate whether these effects could be replicated by inactivation of BLA specifically during each session of stress.

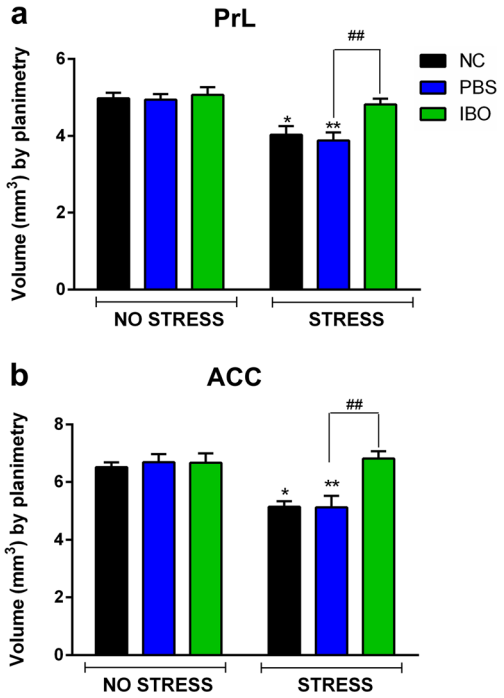

Fig. 5 Effect of BLA lesion on the stress-induced decrease in volumes of the PFC. Planimetry volumes of the PrL (**a**) and ACC (**b**). Data are expressed as mean ± SEM. Groups are as described in Fig. 3. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. corresponding non-stressed groups, $^{\#\#}p < 0.01$ vs. PBS-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test ($n = 5$–6 per group)

Cannulae Placements

The site of the infusion needle into the BLA across different atlas plates ranging from bregma − 1.60 to − 3.80 mm is shown in Fig. 2b. Data of five animals with infusion needle outside the BLA were excluded from analysis in the lidocaine-infused rats subjected to stress. Data from seven animals with misplaced cannulae were excluded from analysis in the chronic lidocaine- and saline-infused groups.

Temporary Inactivation of Basolateral Amygdala Precludes Deficits in Novel Object Recognition Memory Following Chronic Stress

Following intra-BLA cannulation and postsurgical recovery, animals were subjected to either saline or lidocaine infusion into the BLA prior to each session of stress for 10 days, and thereafter, object recognition memory was assessed. Figure 6 shows the representative tracks of stressed and respective non-stressed control (a), time spent with novel and familiar objects during test phase (b), and discrimination indices (c). The non-stressed control rats spent more time with novel object as compared to familiar object ($t_{(7)} = 4.77$, $p < 0.01$, $n = 8$). The saline or lidocaine infusion in the absence of stress did not affect the recognition memory (SAL: $t_{(7)} = 5.93$, $p < 0.001$ vs. familiar object, $n = 8$; LI: $t_{(7)} = 5.16$, $p < 0.001$ vs. familiar object, $n = 8$). However, stressed animals were unable to discriminate between novel and familiar object ($t_{(7)} = 0.80$, $p > 0.05$, $n = 8$). The performance of saline-infused rats subjected to stress was similar to that of stressed animals ($t_{(7)} = 0.92$, $p > 0.05$, $n = 8$). Interestingly, the temporary inactivation of BLA specifically during stress blocked the impairment in recognition memory. The intra-BLA lidocaine-infused rats subjected to stress spent more time in exploring novel object than the familiar object ($t_{(7)} = 6.77$, $p < 0.001$, $n = 8$). The total exploration time between stressed and non-stressed groups remained unaltered during the test session ($p > 0.05$ vs. NC, Table 2). During familiarization phase, the stressed animals and their respective non-stressed control spent almost equal time exploring both the objects (for all groups, $p > 0.05$ vs. object 2; data not shown).

A two-way ANOVA of discrimination index revealed a significant effect of stress ($F_{(1, 42)} = 38.26$, $p < 0.001$), temporary inactivation of BLA ($F_{(2, 42)} = 13.96$, $p < 0.001$) and interaction between stress × BLA inactivation ($F_{(2, 42)} = 12.51$, $p < 0.001$). Tukey's post hoc comparisons suggested that CIS significantly impairs recognition memory ($p < 0.001$ vs. NC, $d = 2.71$). The performance of rats subjected to saline infusion prior to each session of stress was comparable to stressed rats ($p > 0.05$ vs. NC rats subjected to stress). The temporary inactivation of BLA during stress abolished the impairment in recognition memory ($p < 0.001$, $d = 3.11$ vs. saline-infused rat subjected to stress). The discrimination indices for saline or lidocaine-infused group in absence of stress were similar to that of NC non-stressed control ($p > 0.05$).

Temporary Inactivation of Basolateral Amygdala Precludes Prefrontal Astroglial Loss Following Chronic Stress

Similar to BLA lesion experiments, we performed the in-depth stereological quantification of GFAP $^{+}$ astroglial cells within the volumes of PrL and ACC.

1. *Temporary inactivation of basolateral amygdala prevents stress-induced astroglial loss in the PrL*

The top panel of Fig. 7 shows the anatomical boundaries of PrL cortex from bregma + 5.16 to + 2.52 mm (a), representative micrographs of GFAP-stained sections of stressed group and respective non-stressed control (b–g), and stereological quantification of GFAP^{+} astroglial number (h) and density (i) within the PrL. A two-way ANOVA of GFAP^{+} astroglial numbers revealed a significant effect of stress ($F_{(1, 29)} = 41.07$, $p < 0.001$), temporary inactivation of BLA ($F_{(2, 29)} = 8.61$, $p < 0.001$), and interaction ($F_{(2, 29)} = 11.01$, $p < 0.001$). Similarly, a two-way ANOVA of GFAP^{+} astroglial density revealed a significant effect of stress ($F_{(1, 29)} = 22.18$,

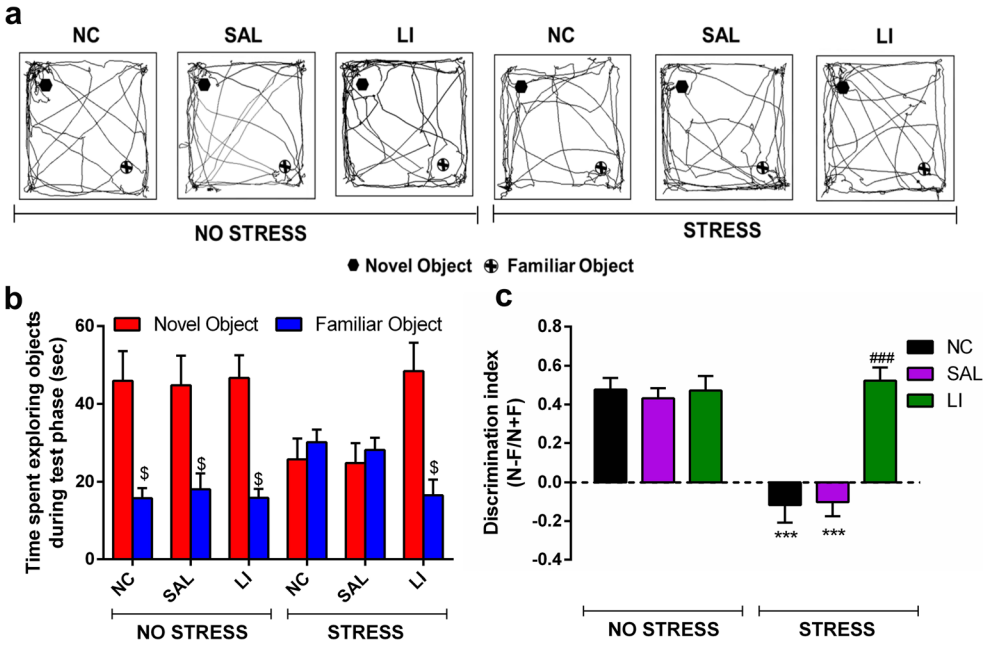

Fig. 6 Temporary inactivation of BLA abolishes stress-induced impairment in novel object recognition memory. Representative tracks of stressed and respective non-stressed control (**a**), time spent with novel and familiar objects during test phase (**b**), and discrimination index (**c**). Data are represented as mean ± SEM. NC: naïve rats either served as non-stressed control or were subjected to chronic immobilization stress; SAL: rats received a single intra-BLA infusion of saline on each day for 10 days, either served as non-stressed saline control or were subjected to immobilization stress; LI: rats subjected to a single intra-BLA lidocaine infusion on each day for 10 days, either served as non-stressed lidocaine control or were exposed to immobilization stress. $^{\$}p < 0.01$ vs. familiar object, paired *t* test; $^{***}p < 0.001$ vs. corresponding non-stressed control, $^{\#\#\#}p < 0.001$ vs. saline-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test ($n = 8$ per group)

$p < 0.001$), interaction ($F_{(2, 29)} = 9.10$, $p < 0.001$), and group effect ($F_{(2, 29)} = 5.79$, $p < 0.001$).

Post hoc comparisons indicated a decrease in GFAP$^+$ astrocyte number ($p < 0.001$, $d = 2.84$ vs. NC non-stressed control, $n = 6$) as well as density ($p < 0.05$, $d = 1.94$ vs. NC non-stressed control, $n = 6$) in the PrL region of stressed rats. Reduction in GFAP$^+$ astroglial number and density were persistent following saline infusion during each session of stress (both, $p > 0.05$ vs. NC rats subjected to stress, $n = 6$). The stress-induced decrease in GFAP$^+$ astroglial number ($p < 0.001$, $d = 2.56$ vs. saline-infused rat subjected to stress, $n = 6$) and density ($p < 0.001$, $d = 2.32$ vs. saline-infused rat subjected to stress, $n = 6$) were precluded by temporary inactivation of BLA. In the absence of stress, neither saline nor lidocaine infusion affected GFAP$^+$ astroglial number and density (both, $p > 0.05$ vs. NC non-stressed control, $n = 6$) in the PrL. Furthermore, the astroglial loss within PrL was correlated with deficits in novel object recognition memory ($p < 0.001$, $r^2 = 0.92$, Fig. 9c), which were significantly restored by inactivation of BLA.

2. *Temporary inactivation of basolateral amygdala prevents stress-induced astroglial loss in the ACC*

Figure 7 (bottom panel) shows the boundaries of ACC spanning from bregma + 4.20 to + 2.16 mm (j), representative

Table 2 Temporary inactivation of BLA did not affect the total time spent in exploring objects during the test session of novel object recognition task

Total object exploration time (s)					
No stress			Stress		
NC ($n = 8$)	SAL ($n = 8$)	LI ($n = 8$)	NC ($n = 8$)	SAL ($n = 8$)	LI ($n = 8$)
61.72 ± 9.55	62.87 ± 11.42	62.62 ± 6.57	55.92 ± 6.92	52.92 ± 7.63	64.97 ± 10.87

Data are expressed as mean ± SEM. The number of animals in each group is indicated in the parenthesis

NC naïve rats either served as non-stressed control or were subjected to chronic immobilization stress, *SAL* rats received a single intra-BLA infusion of saline on each day for 10 days, either served as non-stressed saline control or were subjected to immobilization stress, *LI* rats subjected to a single intra-BLA lidocaine infusion on each day for 10 days, either served as non-stressed lidocaine control or were exposed to immobilization stress

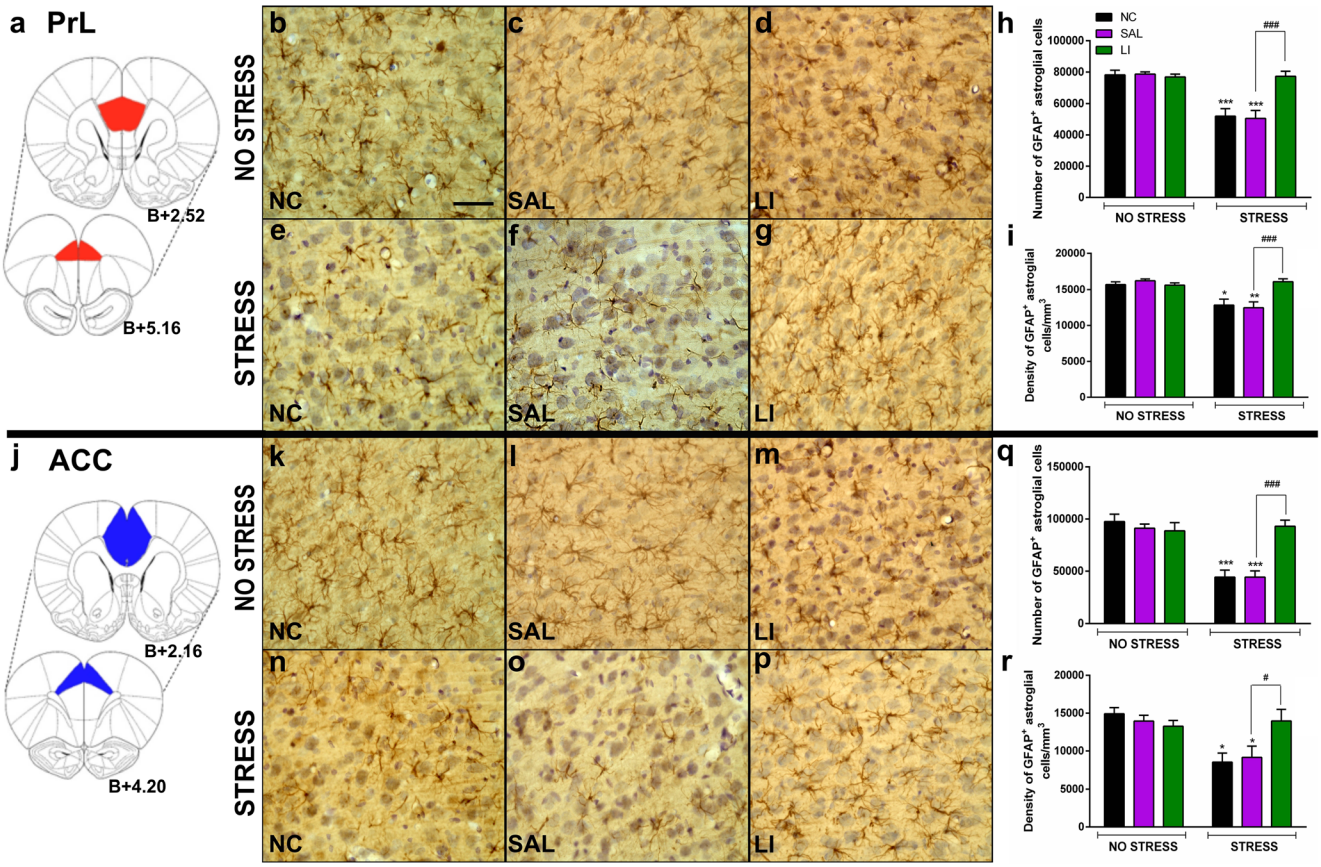

Fig. 7 Temporary inactivation of BLA was sufficient to preclude stress-induced loss of GFAP$^+$ astroglial cells in the prefrontal cortex. The anatomical boundaries of PrL cortex from bregma + 5.16 to + 2.52 mm (**a**) and ACC from bregma + 4.20 to + 2.16 mm (**j**) [38]. Representative micrographs of GFAP-stained section of PrL (**b**–**g**) and ACC (**k**–**p**) of stressed groups and respective non-stressed control at ×40 magnification. Scale bar = 25 μm in **b** and is applicable to all. Stereological quantification of GFAP$^+$ astroglial number and density within the PrL (**h** and **i**, respectively) and ACC (**q** and **r**, respectively). Data is expressed as mean ± SEM. Groups as described in Fig. 6. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ vs. corresponding non-stressed control, $^{###}p < 0.001$, $^{#}p < 0.05$ vs. saline-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test ($n = 6$ per group)

photomicrographs of GFAP-stained sections of stressed group and respective non-stressed control (k–p), and quantification of GFAP$^+$ astroglial number (q) and density (r). A two-way ANOVA of GFAP$^+$ astroglial numbers within the ACC revealed a significant effect of stress ($F_{(1, 30)} = 37.43$, $p < 0.001$), temporary inactivation of BLA ($F_{(2, 30)} = 7.65$, $p < 0.01$), and interaction ($F_{(2, 30)} = 12.22$, $p < 0.001$). Similarly, a two-way ANOVA of GFAP$^+$ astroglial density in the ACC revealed a significant effect of stress ($F_{(1, 30)} = 14.12$, $p < 0.001$) and interaction ($F_{(2, 30)} = 5.34$, $p < 0.05$).

Post hoc comparison indicated that stress causes a significant decrease in GFAP$^+$ astrocyte number ($p < 0.001$, $d = 3.15$, $n = 6$) as well as density ($p < 0.05$, $d = 2.55$, $n = 6$) in the ACC as compared to non-stressed control. The decreases in GFAP$^+$ astroglial number and density (both, $p > 0.05$ vs. NC rats subjected to stress, $n = 6$) were persistent following saline infusion during stress. Similar to PrL, temporary inactivation of BLA prevented the decrease in GFAP$^+$ astroglial number ($p < 0.001$, $d = 3.31$, $n = 6$) and density ($p < 0.05$, $d = 1.30$, $n = 6$) within the ACC as compared to saline-infused rats subjected to stress. The infusion of saline or lidocaine in the absence of stress did not affect GFAP$^+$ astroglial number and density (both, $p > 0.05$ vs. NC non-stressed control, $n = 6$). Also, we observed a significant correlation between the astroglial loss within ACC and deficits in novel object recognition memory ($p < 0.001$, $r^2 = 0.91$, Fig. 9d), which were significantly precluded by inactivation of BLA.

Temporary Inactivation of Basolateral Amygdala Prevents Stress-Induced Loss of PrL and ACC Volumes

Figure 8 shows the planimetry volumes of PrL (a) and ACC (b) in stressed groups and respective non-stressed control. A two-way ANOVA of PrL volumetric data indicated a significant effect of stress ($F_{(1, 29)} = 22.60$, $p < 0.001$), inactivation of BLA ($F_{(2, 29)} = 3.98$, $p < 0.05$), and interaction ($F_{(2, 29)} = 3.58$, $p < 0.05$). Similar to PrL, two-way ANOVA of ACC volumes showed a significant effect of stress ($F_{(1, 30)} = 13.81$, $p < 0.001$), temporary inactivation of BLA ($F_{(2, 30)} = 6.19$, $p < 0.001$), and interaction ($F_{(2, 30)} = 4.28$, $p < 0.001$). The post hoc analysis revealed that exposure to CIS decreases

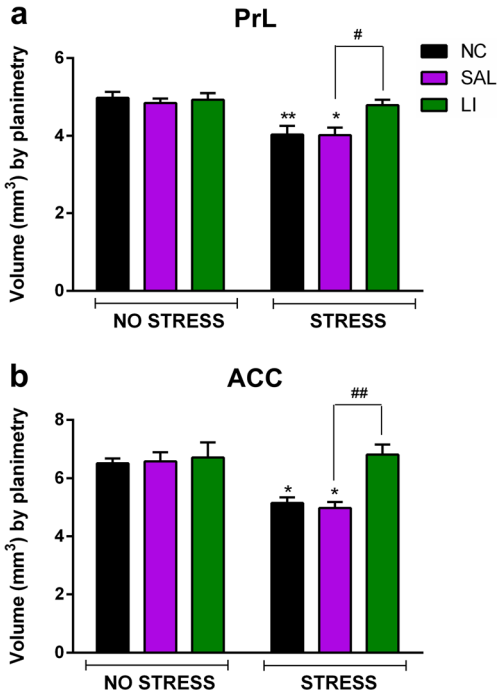

Fig. 8 Stress-induced loss of PFC volumes were prevented by temporary inactivation of BLA. Planimetry volumes of the PrL (**a**) and ACC (**b**). Data are represented as mean ± SEM. Groups as described in Fig. 6. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. corresponding non-stressed control, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. saline-infused rats subjected to stress, two-way ANOVA followed by Tukey's post hoc test ($n = 6$ per group)

the volume of both PrL ($p < 0.01$, $d = 2.16$, $n = 6$) and ACC ($p < 0.05$, $d = 3.07$, $n = 6$) as compared to non-stressed control. In the absence of stress, neither intra-BLA infusion of saline nor lidocaine affected the volumes of PrL and ACC (both, $p > 0.05$ vs. NC non-stressed control, $n = 6$). The volume loss in both regions was persistent following saline infusion prior to each session of stress (both, $p > 0.05$ vs. NC rats subjected to stress, $n = 6$). Surprisingly, the temporary inactivation of BLA was sufficient to preclude the volume loss of PrL ($p < 0.05$, $d = 1.87$, $n = 6$) and ACC ($p < 0.001$, $d = 2.66$, $n = 6$) as compared to saline-infused rats subjected to stress (Fig. 8). Based on the correlation between astroglial density and recognition memory, it is possible that volume loss in the PFC might be associated with cognitive deficits (Fig. 9).

Discussion

The present study provides the first experimental evidence that inactivation of BLA during stressful conditions prevents loss of $GFAP^{+}$ astrocytes in the PFC. Our results demonstrate that CIS causes impairment in NOR task which was associated with a decrease in astroglial density in the PrL and ACC region of PFC. Surprisingly, stress-induced learning deficit and astroglial loss in the PFC were prevented by BLA lesion. The second set of experiments sought to examine whether reversible inactivation of BLA specifically during stress would recapitulate the effects of BLA lesion. Interestingly, the temporary inactivation of BLA mimics the effects of BLA lesion. We found that temporary inactivation of BLA during stress prevents the impairment in NOR task. Further, inactivation of BLA was sufficient to prevent the astroglial loss in the PrL and ACC region of the PFC. Together, our results indicate that beneficial effects of BLA inactivation during stress might involve the prevention of astroglial dysfunction in the PFC.

In this study, we utilized the NOR task which is a novelty-based paradigm and relies on the ability of animals to discriminate between a novel object and familiar object [51, 52]. This task is relatively free from the involvement of any positive or negative reinforcer and thus comparable to memory testing paradigms used in humans [53]. We found that CIS causes impairment in NOR memory. CIS rats had a significantly lower discrimination index as compared to non-stressed control rats. Earlier studies have shown that CIS [34] as well as other forms of chronic stress [1, 33, 54] significantly decrease the discrimination index in NOR task. The results of present study are in accordance with the findings that chronic stress impairs prefrontal [1, 2, 55] as well as hippocampal [37, 56–60] dependent learning. Previous studies from our lab and others have demonstrated that CIS impairs learning and memory in partially baited radial arm maze task [4, 37] and object displacement test [59]. Further, stress-induced impaired learning was associated with aberrant neuronal [61] and glial [11, 62] plasticity in the PFC and hippocampus.

Repeated exposure to immobilization stress differentially affects the PFC and BLA. Variety of cognitive deficits involves prefrontal cortical dysfunctions, which are also widely reported following chronic stress [1, 55, 63]. In contrast to PFC, chronic stress induces hypertrophy, spinogenesis, and enhanced arborization in the pyramidal neurons of BLA [21, 64]. This enhanced spinogenesis [64], trophic support [65], and dendritic hypertrophy in BLA parallel with stress-induced cognitive deficits [8, 33, 37]. In experiment I, we found that BLA lesion prior to CIS prevented the stress-induced decrease in discrimination index. Previous findings have consistently reported that BLA lesion precludes stress-induced impairment in spatial [20, 35, 37] as well as non-spatial [20, 33] learning. For instance, memory-impairing effects of hippocampal glucocorticoid infusions in a PFC-dependent task were abolished by BLA lesion [20]. Interestingly, the glucocorticoid-mediated memory consolidation depends on the interaction between medial PFC and BLA [28]. In addition, BLA lesion blocks the CIS-induced impairment in the rate of learning and reference memory [37]. In the present study, BLA lesion did not affect the exploratory behavior or locomotor activity during NOR task. Indeed, lesion or inactivation of BLA during an anxiety task causes anxiolytic behavior [66–69]. However, the locomotor activity

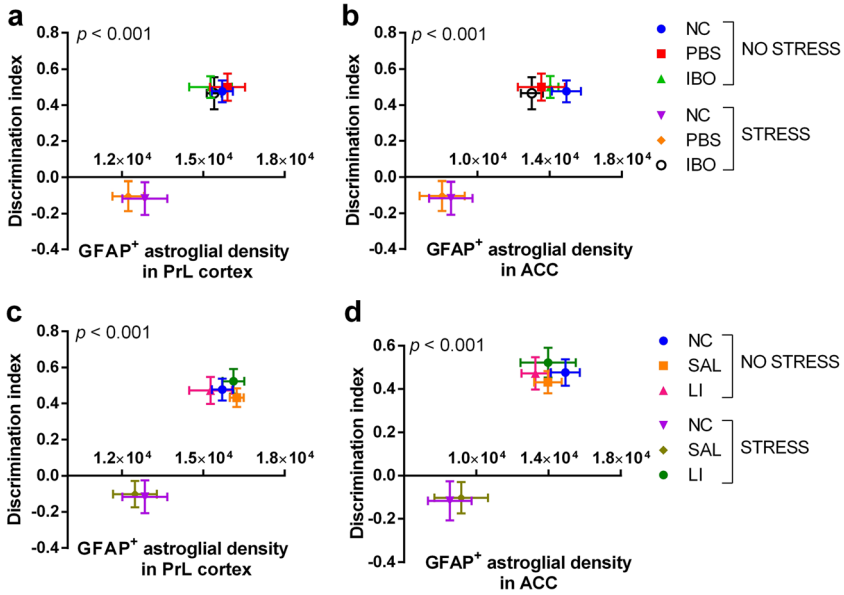

Fig. 9 Correlation plots illustrating the relationship between the discrimination index and density of GFAP$^+$ astroglial cells in the PrL or ACC of BLA lesioned (**a** and **b**, respectively) and temporary inactivated (**c** and **d**, respectively) groups. The decrease in recognition memory during novel object recognition task was associated with loss of the GFAP$^+$ astroglial cell in PrL and ACC. Groups as described in Figs. 3 and 6

remains unaltered following lesion or photoinhibition of BLA [66, 67, 69] with an exception of Parent et al. [68]. The observed changes in locomotor activity by Parent et al. [68] are due to exceptionally large and non-specific lesions which are mostly beyond BLA. In a seminal study by Felix-Ortiz et al. [67], they showed that photoinhibition of BLA terminals in PFC produces an anxiolytic effect with enhanced social interaction. However, they observed no significant change in exploratory behaviors such as walking, sniffing, rearing, self-grooming, and freezing [67]. Further, in the present study, the total distance traveled remains unaltered for BLA lesion group during the familiarization and test phase of NOR task. This is also possible due to habituation to the arena prior to actual NOR task. Additionally, the lesion-induced changes in anxiety behavior are specifically present in anxiogenic settings which do not exist during NOR task. Together, our data extend and support the observations of other studies suggesting a crucial modulatory role of BLA on stress-induced deficits in prefrontal as well as hippocampal-dependent learning.

In experiment II, we observed that temporary inactivation of BLA using lidocaine prior to each session of stress significantly improved the discrimination index in NOR task. Previous studies show that pharmacological silencing of the BLA prevents the effect of both chronic [8, 31, 33, 37] and acute [19, 36] stressors on learning and memory. Our finding is supported by a recent report which demonstrates that 8 days of restraint foot shock (RFS)-induced impairment of NOR memory was abolished by photoinhibition of BLA [33]. In addition, several studies demonstrate that muscimol or β-adrenergic antagonist-induced inactivation of BLA precludes the stress or glucocorticoid-induced learning impairments [32, 34, 36, 41]. In our earlier study, we show that lidocaine-induced transient inactivation of BLA prevents stress-induced spatial learning deficits in a partially baited radial arm maze task [37]. Interestingly, the prevention of stress-induced cognitive deficits by BLA inactivation was associated with the reversal of aberrant changes in glucocorticoids receptor [33, 34, 39], plasma corticosterone levels [37], and electrophysiological alterations [8, 36].

Although numerous studies have focused on the alteration of neuronal functions in the PFC following stress, it is surprising that reports only from last decade emphasize on chronic stress-induced changes in cortical astrocytes. Earlier studies demonstrated that chronic stress induces astroglial loss and morphological alterations in the PFC [10, 11]. Here, we used astrocyte-specific GFAP protein to estimate the specific changes in the astroglial density following stress. Another astrocytic marker is S100β, a calcium binding protein which only stains the cell body. However, S100β should be used with caution as it is also present in oligodendrocytes and O2a glial progenitor cells [70, 71]. Due to the non-specific localization of S100β, we did not select it as a marker for astroglial cells. Consistent with the literature, we found that chronically stressed rats had a substantial decrease in GFAP$^+$ cell density, with an effect size of 1.94 in PrL and 2.55 in ACC as compared to non-stressed control rats. Indeed, astroglial loss in the PFC causes depressive-like behavior in rodents [10]. Further, there are numerous post-mortem studies which demonstrate that the number of astroglia within the PFC is significantly reduced in patients with the history of depression [72–74]. Apart from changes in the density, debranching of astroglial cells has been reported in infralimbic cortex following 21 days of restraint stress [11]. In addition, these changes in astrocytes were region-specific. For instance, in the present study, stress did not affect the astroglial number and density in the motor cortex, which is supported by previous observation [11].

It is plausible that these changes in astrocytic density, morphology, and functioning might reflect cognitive dysfunctions

associated with depression. Indeed, clinical depression is often associated with cognitive decline [75, 76]. Interestingly, infusion of astroglial-specific toxin into the PFC causes deficits in reversal learning, working memory, and attentional set-shifting [77]. Further, ablation of astrocytes in the PFC caused neuronal atrophy [77] which was associated with depressive behavior [10] and cognitive decline [77]. Chronic stress-induced depression is often comorbid with cognitive deficits [4, 45]. A recent study from our lab demonstrates that CIS precipitates depressive-like behavior and cognitive dysfunction [4]. Further, metabolic changes in prefrontal astrocytes following stress precipitate depression [10, 78, 79]. In addition, astrocytes are responsible for removal of extracellular glutamate from synaptic cleft and thus play a significant role in the prevention of glutamate excitotoxicity [80]. Stress causes a decrease in glutamate transporter-1 (GLT-1) expression, which is associated with impaired functioning of the glutamate-glutamine cycle and excitotoxicity [78, 81]. Reduction of GLT-1 in an animal model of Alzheimer's disease hastens cognitive decline [82]. Chronic stress-induced enhanced level of glutamate, astroglial loss, and decreased expression of GLT-1 in the PFC causes increased phosphorylation of GluN2B, a subunit of *N*-methyl-D-aspartate (NMDA) receptor [83]. Similarly, repeated exposure to stress causes a decrease in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor in the PFC leading to cognitive decline [1]. Furthermore, a recent study shows that optogenetic activation of astrocytes in ACC promotes ACC-BLA coupling and decision making [84]. Interestingly, in our study, we observe a significant correlation between performance in object recognition task and astroglial loss in the PFC. Further, loss of astroglial cells is often associated with glutamate excitotoxicity [78, 85], which might be a potential cause of cognitive decline and depressive-like behavior following stress.

Several lines of research suggest that the crosstalk between BLA and PFC are crucial in the manifestation of stress effects [8, 27]. Therefore, we evaluated the effects of BLA inactivation on prefrontal astrocytic density. A significant finding of this study is the prevention of decreased astroglial density in the PrL and ACC by BLA lesion prior to stress. Interestingly, the inactivation of BLA during stress also prevents the decrease in $GFAP^+$ astroglial density in both PrL and ACC regions of the PFC. To our knowledge, this is the first study which demonstrates that lesion or inactivation of BLA prevents stress-induced astroglial loss in the PFC. At this point in time, it is only possible to speculate about the regulation of cortical astrocytic functions by BLA during stress. We propose that inactivation of hyperactive BLA during stress might prevent the metabolic and structural changes in prefrontal astrocytes. It is plausible that the prevention of stress-induced astroglial loss in the PFC following BLA inactivation might be associated with restoration of aberrant changes in the glutamate-glutamine cycle, GLT-1 expression, and lactate production. Normalization of the astrocytic metabolic profile by inactivation of BLA might prevent glutamate excitotoxicity leading to amelioration of stress-induced cognitive deficits. However, further studies are required to establish this working hypothesis and to elucidate the mechanisms by which inactivation of BLA prevent the astroglial malfunctions following stress.

In addition to the astroglial loss, we also observed a significant decrease in volumes of PrL and ACC following chronic stress. On similar lines, several studies show that chronic stress or glucocorticoid administration causes PFC volume loss [55, 63, 86]. The astroglial loss in PFC also causes dendritic atrophy in neurons [77], which might be a potential cause of volume loss. Furthermore, stress-induced atrophy in the PFC was precluded by both lesion and temporary inactivation of BLA.

In conclusion, we demonstrate that CIS causes profound loss of $GFAP^+$ astroglial cells in the PFC. The astroglial loss following stress was correlated with learning deficit in NOR task. Furthermore, we show that both ablation and pharmacological inactivation of BLA during stress prevent the deficit in novel object recognition memory and loss of $GFAP^+$ astroglial cells in the PFC. Our results support and extend the concept of amygdalar silencing during stress, which might preclude the behavioral consequences and associated astroglial loss. Finally, we speculate that developing strategies to combat amygdalar hyperactivity might mitigate the astroglial dysfunctions in various psychiatric disorders including anxiety and depression.

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Contributions S.J.T., T.R.R., and B.S.S.R. conceptualized and designed the experiments; S.J.T. and S.C. performed the experiments and analyzed the data; B.N.S, T.R.R., and B.S.S.R. contributed to reagents/materials/analysis tools; and S.J.T., S.C., B.N.S, T.R.R., and B.S.S.R. wrote the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

References

1. Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z (2012) Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. Neuron 73(5):962–977

2. Ramkumar K, Srikumar BN, Venkatasubramanian D, Siva R, Shankaranarayana Rao BS, Raju TR (2012) Reversal of stress-induced dendritic atrophy in the prefrontal cortex by intracranial self-stimulation. J Neural Transm (Vienna) 119(5):533–543
3. Pawlak R, Shankaranarayana Rao BS, Melchor JP, Chattarji S, McEwen B, Strickland S (2005) Tissue plasminogen activator and plasminogen mediate stress-induced decline of neuronal and cognitive functions in the mouse hippocampus. Proc Natl Acad Sci U S A 102(50):18201–18206
4. Shilpa BM, Bhagya V, Harish G, Srinivas Bharath MM, Shankaranarayana Rao BS (2017) Environmental enrichment ameliorates chronic immobilisation stress-induced spatial learning deficits and restores the expression of BDNF, VEGF, GFAP and glucocorticoid receptors. Prog Neuro-Psychopharmacol Biol Psychiatry 76:88–100
5. Veena J, Srikumar BN, Raju TR, Shankaranarayana Rao BS (2009) Exposure to enriched environment restores the survival and differentiation of new born cells in the hippocampus and ameliorates depressive symptoms in chronically stressed rats. Neurosci Lett 455(3):178–182
6. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93(3):358–364
7. Pawlak R, Magarinos AM, Melchor J, McEwen B, Strickland S (2003) Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat Neurosci 6(2):168–174
8. Wei J, Zhong P, Qin L, Tan T, Yan Z (2017) Chemicogenetic restoration of the prefrontal cortex to amygdala pathway ameliorates stress-induced deficits. Cereb Cortex. https://doi.org/10.1093/cercor/bhx104
9. Czeh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31(8):1616–1626
10. Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry 64(10):863–870
11. Tynan RJ, Beynon SB, Hinwood M, Johnson SJ, Nilsson M, Woods JJ, Walker FR (2013) Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol 126(1):75–91
12. Newman EA (2003) New roles for astrocytes: Regulation of synaptic transmission. Trends Neurosci 26(10):536–542
13. Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32(8):421–431
14. Kant D, Tripathi SS, Qureshi MF, Tripathi S 2nd, Pandey S, Singh G, Kumar T, Mir FA et al (2014) The effect of glial glutamine synthetase inhibition on recognition and temporal memories in the rat. Neurosci Lett 560:98–102
15. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144(5):810–823
16. Gibbs ME, Hertz L, Ng KT (2004) Inhibition of short-term memory formation in the chick by blockade of extracellular glutamate uptake. Neurobiol Learn Mem 81(2):115–119
17. Lee HS, Ghetti A, Pinto-Duarte A, Wang X, Dziewczapolski G, Galimi F, Huitron-Resendiz S, Pina-Crespo JC et al (2014) Astrocytes contribute to gamma oscillations and recognition memory. Proc Natl Acad Sci U S A 111(32):E3343–E3352
18. Orr AG, Hsiao EC, Wang MM, Ho K, Kim DH, Wang X, Guo W, Kang J et al (2015) Astrocytic adenosine receptor A2A and Gs-coupled signaling regulate memory. Nat Neurosci 18(3):423–434
19. Roozendaal B, Portillo-Marquez G, McGaugh JL (1996) Basolateral amygdala lesions block glucocorticoid-induced modulation of memory for spatial learning. Behav Neurosci 110(5):1074–1083
20. Roozendaal B, McReynolds JR, McGaugh JL (2004) The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. J Neurosci 24(6):1385–1392
21. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 22(15):6810–6818
22. Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, Morrison JH, Wearne SL et al (2008) Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. J Comp Neurol 507(1):1141–1150
23. Wellman CL (2001) Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J Neurobiol 49(3):245–253
24. Amaral DG, Price JL (1984) Amygdalo-cortical projections in the monkey (Macaca fascicularis). J Comp Neurol 230(4):465–496
25. Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212(2):149–179
26. Krettek JE, Price JL (1977) The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171(2):157–191
27. Little JP, Carter AG (2013) Synaptic mechanisms underlying strong reciprocal connectivity between the medial prefrontal cortex and basolateral amygdala. J Neurosci 33(39):15333–15342
28. Roozendaal B, McReynolds JR, Van der Zee EA, Lee S, McGaugh JL, McIntyre CK (2009) Glucocorticoid effects on memory consolidation depend on functional interactions between the medial prefrontal cortex and basolateral amygdala. J Neurosci 29(45):14299–14308
29. Maroun M (2006) Stress reverses plasticity in the pathway projecting from the ventromedial prefrontal cortex to the basolateral amygdala. Eur J Neurosci 24(10):2917–2922
30. Maroun M, Richter-Levin G (2003) Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. J Neurosci 23(11):4406–4409
31. Roozendaal B, Sapolsky RM, McGaugh JL (1998) Basolateral amygdala lesions block the disruptive effects of long-term adrenalectomy on spatial memory. Neuroscience 84(2):453–465
32. Roozendaal B, Nguyen BT, Power AE, McGaugh JL (1999) Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. Proc Natl Acad Sci U S A 96(20):11642–11647
33. Rei D, Mason X, Seo J, Graff J, Rudenko A, Wang J, Rueda R, Siegert S et al (2015) Basolateral amygdala bidirectionally modulates stress-induced hippocampal learning and memory deficits through a p25/Cdk5-dependent pathway. Proc Natl Acad Sci U S A 112(23):7291–7296
34. Jeon B, Hwang YK, Lee SY, Kim D, Chung C, Han JS (2012) The role of basolateral amygdala in the regulation of stress-induced phosphorylated extracellular signal-regulated kinase expression in the hippocampus. Neuroscience 224:191–201
35. Kim JJ, Lee HJ, Han JS, Packard MG (2001) Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J Neurosci 21(14):5222–5228
36. Kim JJ, Koo JW, Lee HJ, Han JS (2005) Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. J Neurosci 25(6):1532–1539
37. Tripathi SJ, Chakraborty S, Srikumar BN, Raju TR, Shankaranarayana Rao BS (2017) Inactivation of basolateral

amygdala prevents chronic immobilization stress-induced memory impairment and associated changes in corticosterone levels. Neurobiol Learn Mem 142(Pt B):218–229
38. Paxinos G, Watson C (2006) The rat brain in stereotaxic coordinates. Academic Press, Amsterdam
39. Tripathi SJ, Chakraborty S, Srikumar BN, Raju TR, Shankaranarayana Rao BS (2017) Prevention of chronic immobilization stress-induced enhanced expression of glucocorticoid receptors in the prefrontal cortex by inactivation of basolateral amygdala. J Chem Neuroanat. https://doi.org/10.1016/j.jchemneu.2017.12.006
40. Nibuya M, Takahashi M, Russell DS, Duman RS (1999) Repeated stress increases catalytic TrkB mRNA in rat hippocampus. Neurosci Lett 267(2):81–84
41. Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. Proc Natl Acad Sci U S A 103(17):6741–6746
42. de Lima MN, Laranja DC, Bromberg E, Roesler R, Schroder N (2005) Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. Behav Brain Res 156(1):139–143
43. Karasawa J, Hashimoto K, Chaki S (2008) D-serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behav Brain Res 186(1):78–83
44. Veena J, Srikumar BN, Mahati K, Bhagya V, Raju TR, Shankaranarayana Rao BS (2009) Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. J Neurosci Res 87(4):831–843
45. Veena J, Srikumar BN, Mahati K, Raju TR, Shankaranarayana Rao BS (2011) Oxotremorine treatment restores hippocampal neurogenesis and ameliorates depression-like behaviour in chronically stressed rats. Psychopharmacology 217(2):239–253
46. Abhijit S, Tripathi SJ, Bhagya V, Shankaranarayana Rao BS, Subramanyam MV, Asha Devi S (2018) Antioxidant action of grape seed polyphenols and aerobic exercise in improving neuronal number in the hippocampus is associated with decrease in lipid peroxidation and hydrogen peroxide in adult and middle-aged rats. Exp Gerontol 101:101–112
47. Vrinda M, Sasidharan A, Aparna S, Srikumar BN, Kutty BM, Shankaranarayana Rao BS (2017) Enriched environment attenuates behavioral seizures and depression in chronic temporal lobe epilepsy. Epilepsia 58(7):1148–1158
48. Shankaranarayana Rao BS, Govindaiah, Laxmi TR, Meti BL, Raju TR (2001) Subicular lesions cause dendritic atrophy in CA1 and CA3 pyramidal neurons of the rat hippocampus. Neuroscience 102(2):319–327
49. Shankaranarayana Rao BS, Desiraju T, Raju TR (1993) Neuronal plasticity induced by self-stimulation rewarding experience in rats—a study on alteration in dendritic branching in pyramidal neurons of hippocampus and motor cortex. Brain Res 627(2):216–224
50. West MJ (1999) Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. Trends Neurosci 22(2):51–61
51. Ennaceur A, Neave N, Aggleton JP (1997) Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp Brain Res 113(3):509–519
52. Gaskin S, Tardif M, Cole E, Piterkin P, Kayello L, Mumby DG (2010) Object familiarization and novel-object preference in rats. Behav Process 83(1):61–71
53. Baxter MG (2010) "I've seen it all before": explaining age-related impairments in object recognition. Theoretical comment on Burke et al. (2010). Behav Neurosci 124(5):706–709
54. Nishimura KJ, Ortiz JB, Conrad CD (2017) Antagonizing the GABAA receptor during behavioral training improves spatial memory at different doses in control and chronically stressed rats. Neurobiol Learn Mem 145:114–118
55. Cerqueira JJ, Mailliet F, Almeida OF, Jay TM, Sousa N (2007) The prefrontal cortex as a key target of the maladaptive response to stress. J Neurosci 27(11):2781–2787
56. Srikumar BN, Raju TR, Shankaranarayana Rao BS (2006) The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats. Neuroscience 143(3):679–688
57. McLaughlin KJ, Gomez JL, Baran SE, Conrad CD (2007) The effects of chronic stress on hippocampal morphology and function: an evaluation of chronic restraint paradigms. Brain Res 1161:56–64
58. Bodnoff SR, Humphreys AG, Lehman JC, Diamond DM, Rose GM, Meaney MJ (1995) Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J Neurosci 15(1 Pt 1):61–69
59. Rahman MM, Callaghan CK, Kerskens CM, Chattarji S, O'Mara SM (2016) Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress. Sci Rep 6:29127
60. Sunanda, Shankaranarayana Rao BS, Raju TR (2000) Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. Curr Sci 79(11):1581
61. Shankaranarayana Rao BS, Madhavi R, Sunanda RTR (2001) Complete reversal of dendritic atrophy in CA3 neurons of the hippocampus by rehabilitation in restraint stressed rats. Curr Sci 80:653–659
62. Hinwood M, Tynan RJ, Charnley JL, Beynon SB, Day TA, Walker FR (2013) Chronic stress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline. Cereb Cortex 23(8):1784–1797
63. Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OF, Sousa N (2005) Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. J Neurosci 25(34):7792–7800
64. Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proc Natl Acad Sci U S A 102(26):9371–9376
65. Govindarajan A, Shankaranarayana Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, Chattarji S (2006) Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proc Natl Acad Sci U S A 103(35):13208–13213
66. Felix-Ortiz AC, Beyeler A, Seo C, Leppla CA, Wildes CP, Tye KM (2013) BLA to vHPC inputs modulate anxiety-related behaviors. Neuron 79(4):658–664
67. Felix-Ortiz AC, Burgos-Robles A, Bhagat ND, Leppla CA, Tye KM (2016) Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience 321:197–209
68. Parent MB, Avila E, McGaugh JL (1995) Footshock facilitates the expression of aversively motivated memory in rats given post-training amygdala basolateral complex lesions. Brain Res 676(2):235–244
69. Maren S, Aharonov G, Fanselow MS (1996) Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav Neurosci 110(4):718–726
70. Steiner J, Bernstein HG, Bielau H, Berndt A, Brisch R, Mawrin C, Keilhoff G, Bogerts B (2007) Evidence for a wide extra-astrocytic distribution of S100B in human brain. BMC Neurosci 8:2

71. Steiner J, Bernstein HG, Bogerts B, Gos T, Richter-Landsberg C, Wunderlich MT, Keilhoff G (2008) S100B is expressed in, and released from, OLN-93 oligodendrocytes: influence of serum and glucose deprivation. Neuroscience 154(2):496–503
72. Si X, Miguel-Hidalgo JJ, O'Dwyer G, Stockmeier CA, Rajkowska G (2004) Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. Neuropsychopharmacology 29(11):2088–2096
73. Miguel-Hidalgo JJ, Baucom C, Dilley G, Overholser JC, Meltzer HY, Stockmeier CA, Rajkowska G (2000) Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. Biol Psychiatry 48(8):861–873
74. Rajkowska G, Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets 6(3):219–233
75. Bremner JD, Vythilingam M, Vermetten E, Vaccarino V, Charney DS (2004) Deficits in hippocampal and anterior cingulate functioning during verbal declarative memory encoding in midlife major depression. Am J Psychiatry 161(4):637–645
76. Castaneda AE, Tuulio-Henriksson A, Marttunen M, Suvisaari J, Lonnqvist J (2008) A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. J Affect Disord 106(1–2):1–27
77. Lima A, Sardinha VM, Oliveira AF, Reis M, Mota C, Silva MA, Marques F, Cerqueira JJ et al (2014) Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. Mol Psychiatry 19(7):834–841
78. Banasr M, Chowdhury GM, Terwilliger R, Newton SS, Duman RS, Behar KL, Sanacora G (2010) Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry 15(5):501–511
79. Chowdhury GM, Zhang J, Thomas M, Banasr M, Ma X, Pittman B, Bristow L, Schaeffer E et al (2017) Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects. Mol Psychiatry 22(1):120–126
80. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA et al (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16(3):675–686
81. Rappeneau V, Blaker A, Petro JR, Yamamoto BK, Shimamoto A (2016) Disruption of the glutamate-glutamine cycle involving astrocytes in an animal model of depression for males and females. Front Behav Neurosci 10:231
82. Mookherjee P, Green PS, Watson GS, Marques MA, Tanaka K, Meeker KD, Meabon JS, Li N et al (2011) GLT-1 loss accelerates cognitive deficit onset in an Alzheimer's disease animal model. J Alzheimers Dis 26(3):447–455
83. Li SX, Han Y, Xu LZ, Yuan K, Zhang RX, Sun CY, Xu DF, Yuan M et al (2018) Uncoupling DAPK1 from NMDA receptor GluN2B subunit exerts rapid antidepressant-like effects. Mol Psychiatry 23(3):597–608
84. Wang J, Tu J, Cao B, Mu L, Yang X, Cong M, Ramkrishnan AS, Chan RHM et al (2017) Astrocytic l-lactate signaling facilitates amygdala-anterior cingulate cortex synchrony and decision making in rats. Cell Rep 21(9):2407–2418
85. Popoli M, Yan Z, McEwen BS, Sanacora G (2011) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci 13(1):22–37
86. Sheline YI (2000) 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. Biol Psychiatry 48(8):791–800