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Basolateral amygdala regulation of adult hippocampal neurogenesis and fear-related activation of newborn neurons

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Abstract

Impaired regulation of emotional memory is a feature of several affective disorders, including depression, anxiety and post-traumatic stress disorder. Such regulation occurs, in part, by interactions between the hippocampus and the basolateral amygdala (BLA). Recent studies have indicated that within the adult hippocampus, newborn neurons may contribute to support of emotional memory, and that regulation of hippocampal neurogenesis is implicated in depressive disorders. How emotional information impacts newborn neurons in adults is not clear. Given the role of the BLA in hippocampus-dependent emotional memory, we investigated whether hippocampal neurogenesis was sensitive to emotional stimuli from the BLA. We show that BLA lesions suppress adult neurogenesis, while lesions of the central nucleus of the amygdala do not. Similarly, we show that reducing BLA activity through viral vector-mediated overexpression of an outwardly rectifying potassium channel suppresses neurogenesis. We also show that BLA lesions prevent selective activation of immature newborn neurons in response to a fear conditioning task. These results demonstrate that BLA activity regulates adult hippocampal neurogenesis and the fear context-specific activation of newborn neurons. Together, these findings denote functional implications for proliferation and recruitment of new neurons into emotional memory circuits.

Keywords

neurogenesis; hippocampus; fear conditioning; basolateral amygdala; emotion; stem cell

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Emotion strongly modulates memory function in adult mammals, altering the strength and longevity of memories and sometimes leading to memory dysfunction. Notably, disordered emotional memory contributes to several affective disorders, including depression, anxiety and post-traumatic stress disorder. Emotion-associated modulation of memory appears to rely largely on interaction between the memory processes of the hippocampus and emotional input provided by the basolateral complex of the amygdala (BLA)[1-4]. Behaviorally, BLA activity supports memory for emotionally salient stimuli in rodents and humans[1, 5, 6] while at the neurophysiological level, BLA activity supports hippocampal long-term potentiation (LTP)[7-10] and enhances hippocampal output to other brain areas[11]. BLA activity also modulates activation of hippocampal immediate early genes, another marker of hippocampal plasticity[12], as well as hippocampal responses to stress[13, 14], further suggesting a modulatory role for the BLA in hippocampal function.

Recent studies suggest a role for adult hippocampal neurogenesis in emotional memory function[15-19]. In adult mammals, new hippocampal neurons arise from a resident population of neural stem cells located in the dentate gyrus[20-23] and form a population of immature neurons that incorporate into existing networks within weeks of birth[24]. Ablation of adult neurogenesis by genetic knockout or irradiation impairs contextual fear memory[15, 16] and may also modulate the transfer of fear-related memories from the hippocampus to other neural structures for long-term storage[17]. The heightened plasticity of newly born neurons appears to be key, as acceleration of their maturation impairs fear learning[25]. The requirement of new neurons for fear memory suggests a clinical role for adult neurogenesis in several affective disorders[3, 26, 27]. However, the mechanisms by which adult neurogenesis responds to emotional stimuli to influence memory formation are not yet clear.

We explored the adult neurogenic response to emotional input from the BLA and its potential role in fear memory. Specifically, we investigated how BLA activity would affect adult neurogenesis and modulate activation of immature neurons in response to fear-associated context.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (Charles River) were pair-housed on a 12h light/dark cycle. All animal procedures were approved by the UC Berkeley and MIT Animal Care and Use Committees.

Stereotaxic surgery

Excitotoxic lesions of the BLA or CeA or sham surgeries were performed as per[28]. Coordinates for BLA infusion were: -2.8 mm anterior/posterior (A/P), +/-5.1 mm medial/lateral (M/L) relative to bregma; -6.8 mm (2 min) and -6.5 mm (1 min) relative to dura. Coordinates for CeA infusion were: -2.2 mm A/P and +/-4.4 mm M/L relative to bregma; -7.0 mm from dura (1 min). Six to 8 hrs after surgery all rats received an additional injection of buprenorphine (0.05 mg/kg, s.c.). For viral vector infusions, virus was infused 0.2 μl/min

for 10 min (2 μl total) at the same BLA coordinates. Viral vectors were prepared as per[29] and titers were 10^6-10^8 infectious particles/ml.

Bromodeoxyuridine injections

5-Bromo-2'-deoxyuridine (BrdU, Sigma) was dissolved in physiological saline and injected intraperitoneally for all experiments.

Immunohistochemical staining

Rats were anesthetized with Euthasol euthanasia solution and transcardially perfused with ice cold 4% paraformaldehyde in 0.1M PBS. Brains were post-fixed for 24 hrs at 4°C, equilibrated in 30% sucrose in 0.1M PBS and then stored at -80°C. Immunostaining was performed on a 1 in 6 series of free-floating 30 μm cryostat sections.

For PCNA and BrdU staining, sections were rinsed in 0.1M Tris-buffered saline (TBS) and pretreated for 10min with 0.3% H_2O_2 in TBS. For BrdU only, sections were incubated in 2N HCl at 37°C for 30 min. All sections were incubated in blocking solution (1% normal donkey solution, 0.3% triton-X 100 in TBS) for 30 min followed by overnight incubation at 4°C on rotation in primary antibody (mouse anti-PCNA, 1:500 in blocking, Abcam, Cambridge, MA; mouse anti-BrdU, 1:500, BD Biosciences, San Jose, CA). Sections were rinsed and transferred to secondary antibody (biotin anti-mouse, 1:500, Jackson ImmunoResearch, West Grove, PA) for 2 hrs at room temperature. Following rinsing, sections were incubated in ABC reagent (Vector, Burlingame, CA) and then developed with DAB (Vector). Sections were mounted on gelatin-coated slides, dehydrated in alcohol and coverslipped with permount mounting medium.

Triple immunohistochemistry was performed similarly. Primary antibodies were: goat anti-DCX (1:200, Santa Cruz Biotechnology), mouse anti-S100β (1:200, Abcam), rat anti-MBP (1:100, Abcam), rabbit anti-cfos (1:100; Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were: Cy5 anti-goat, Cy3 anti-mouse, Cy3 anti-rat, biotin anti-rabbit (1:500; Jackson ImmunoResearch). For the cFos/BrdU/DCX triple stain, a tertiary incubation for 1 hr at room temperature was included with streptavidin-Alexa488 (1:1000 in TBS, Invitrogen, Carlsbad, CA) after secondary incubation. All sections were then incubated in 4% paraformaldehyde for 10 min, rinsed and incubated in primary antibody against BrdU as above (1:500, rat anti-BrdU, Abcam; mouse anti-BrdU, BD Biosciences). The next day, sections were rinsed and incubated in secondary antibody: FITC anti-rat; Cy3 anti-rat; biotin anti-rat (1:500, Jackson ImmunoResearch). For MBP/BrdU staining, a tertiary incubation for 1 hr at room temperature was added with streptavidin-Alexa488 (1:1000 in TBS, Invitrogen). Sections were mounted on gelatin-coated slides and coverslipped with DABCO anti-fading medium.

BrdU, PCNA and cFos quantification

BrdU, PCNA or cFos positive cells were counted in the dentate gyrus and subgranular zone using a 40x air objective (Zeiss). The area sampled was calculated using StereoInvestigator software (MicroBrightfield, Williston, VT) and used to calculate the number of positive cells per m^2.

Confocal analysis

25-50 BrdU or cFos positive cells were located in the dentate gyrus for each animal (or each hemisphere for unilateral lesion) and assessed in z-series of <1.0 um slices to determine if other markers (DCX, S100β, MBP, cFos, BrdU) were co-expressed. Confocal images were captured on a Zeiss 510 META/NLO confocal microscope with a 40x oil objective and adjusted for brightness and contrast using LSM Image Browser software.

Lesion and viral vector assessment

One series of sections was mounted and counterstained with cresyl violet for lesion assessment. If a lesion did not cover at least 75% of the area of interest without affecting surrounding areas, that rat was removed from analysis (Figure 1). For virus expression, if GFP expression was not found in the BLA or if expression was focused outside the BLA, the rat was removed from analysis.

Fear conditioning

Two weeks after unilateral lesion of the BLA or sham surgery, rats received 4 daily injections of BrdU (100 mg/kg). Two weeks after the last BrdU injection, rats were exposed to fear conditioning. Fear conditioning chambers were 12 w × 10 l × 12 h inch boxes with an electrified grid floor inside a sound attenuating chamber (Coulbourn Instruments, Whitehall, PA). Chambers had two house lights and a house fan that were on at all times during testing and training. Rats were placed in the box and allowed 5 min to acclimate. The animals then received 10 unsignaled, 1mA, 1s duration shocks with an intertrial interval ranging from 10 to 120 s. Rats were left in the chamber for 3 min after the last shock and then returned to their home cage. Chambers were cleaned with 70% ethanol between trials. The next day, rats were placed back in the fear context or a novel context for 15 min without any shock delivery. The novel context was the fear conditioning chamber with the grid floor removed and laminated cork boarding covering three of the walls. Freezing behavior was tracked and analyzed with FreezeFrame software (Coulbourn). After context exposure, rats were returned to their home cages for 45 min and then perfused as in other studies. This time delay was chosen because it coincides with the elevation in IEG expression following context memory activation[30].

Statistical analysis

Unpaired t-tests were used to assess the effects of bilateral lesion on proliferation and cell differentiation, context exposure on freezing behavior, and context exposure on BrdU co-labeling of sham operated rats (unequal variances assumed). Paired t-tests were used to assess proliferation differences in the unilateral lesion study, with an unpaired t-test to compare control PCNA/BrdU levels to that contralateral to BLA lesion. Repeated measures analysis of variance (ANOVA) was used to compare the effect of unilateral BLA versus CeA lesion on cell proliferation as well as the effect of novel versus fear context exposure on BrdU co-labeling in unilaterally BLA lesioned rats. Bonferroni post-hoc tests were used to assess the effect of hemisphere within each lesion group in both cases. $p<0.05$ was considered significant for all studies.

Results

Bilateral BLA lesions suppressed hippocampal neurogenesis

Adult hippocampal neurogenesis is a multi-phase process regulated by the proliferation, differentiation, migration, and survival of new cells[23]. To assess the effect of loss of BLA input on the neurogenic process, we excitotoxically lesioned the BLA of adult male rats bilaterally[28, 31] (Figure 1) and investigated cell proliferation as well as differentiation (Figure 2a). BLA lesion reduced the number of proliferation cell nuclear antigen (PCNA) positive cells by 55.9% compared to rats who received bilateral sham surgery (Figure 2b). BLA-lesion also led to a 45.3% reduction in the number of BrdU positive dentate gyrus cells 5-10 days after proliferative cells were labeled by BrdU injection (Figure 2c). These results indicate suppression of cell proliferation following BLA lesion, resulting in a persistent reduction in immature cells. BLA lesion did not affect cell fate, with approximately 85-90% of new cells expressing the neural marker doublecortin (DCX) and less than 5% expressing the astrocytic marker S100β or the oligodendrocyte marker myelin basic protein (MBP) regardless of lesion (Figure 2d-e). These data indicate that BLA lesions cause a reduction in the pool of immature neurons and glia three weeks after lesion.

Unilateral BLA lesions suppressed hippocampal neurogenesis

We next investigated whether the suppression of adult hippocampal neurogenesis following BLA lesion is mediated by ipsilateral neural connections or by possible systemic changes (such as a change in circulating hormone levels). Because ipsilateral connections mediate the influence of BLA activity on hippocampal LTP[10, 32], we predicted that BLA lesion-induced suppression of adult hippocampal neurogenesis would similarly rely on ipsilateral neural connections and be hemisphere-specific. To investigate this hypothesis, BrdU and PCNA positive cells were quantified three weeks following unilateral BLA lesion (Figure 3a).

Unilateral BLA lesion suppressed the number of PCNA-labeled proliferative hippocampal cells by 32.2% ipsilateral to the lesion compared to contralateral (Figure 3b). Similar results were obtained with BrdU-labeled cells (11.29±1.40 cells ipsilateral, 22.35±4.09 cells contralateral, 49.6% suppression, n = 7). The number of proliferative cells ipsilateral to BLA lesion was both similar to that in bilaterally lesioned rats and significantly lower than that in bilateral sham-operated rats (Figure 3b). Together, these results imply that BLA-associated regulation of neurogenesis is hemisphere-specific, most likely suppressing cell proliferation through ipsilateral neural connections.

CeA lesions did not suppress hippocampal neurogenesis

We next investigated the anatomical specificity of BLA lesion-induced suppression of hippocampal neurogenesis. Previous studies show that while BLA lesions reduce hippocampal LTP, lesions of the central nucleus of the amygdala (CeA) do not[32]. We therefore predicted that CeA lesions would also have no effect adult hippocampal neurogenesis.

Three weeks after surgery to lesion either the BLA or the CeA (Figure 1; Figure 3a), we found that while there were 26.8% fewer BrdU positive cells ipsilateral to BLA lesion compared to contralateral, there was no difference in the number of proliferative BrdU positive cells ipsilateral versus contralateral to CeA lesion (Figure 3c). We found a similar suppression in the number of proliferative PCNA positive cells in the dentate gyrus ipsilateral to BLA, but not CeA, lesion (Figure 3d). Over all the experiments of figure 3, both BrdU and PCNA cell number ipsilateral to BLA lesion was suppressed relative to control while cell number contralateral to lesion was not different from that in controls. Hippocampal proliferation levels in CeA lesioned rats, however, did not differ from controls either ipsilateral or contralateral to lesion. These results indicate that BLA lesions ipsilaterally suppress hippocampal neurogenesis while CeA lesions do not, suggesting that the BLA influence over hippocampal neurogenesis is specific to loss of BLA input, and does not result non-specifically from ipsilateral excitotoxic cell death or surgical damage *per se*.

Viral vector-mediated reduction of BLA activity suppressed adult hippocampal neurogenesis

We next determined whether suppression of neural activity in the BLA without excitotoxic lesion is sufficient to modulate neurogenesis. To reduce BLA neural activity, we ectopically expressed the outwardly rectifying potassium channel Kv1.1 or a GFP-only control from a herpes viral vector in BLA neurons. This transgene construct has been shown to reduce basal neural firing[29, 33]. GFP-Kv1.1 overexpression reduced proliferative BrdU positive cell number by 36.5% and 30.5% (Figure 4) as compared to GFP-only viral vector infusion and sham-operated controls, respectively. These results indicate that reduction of BLA activity via Kv1.1 overexpression is sufficient to suppress hippocampal neurogenesis ipsilaterally.

Fear memory activates newly born neurons

To investigate the functional importance of BLA regulation of adult hippocampal neurogenesis, we examined how new neurons participate in BLA-dependent fear memory. Previous studies show that ablation of adult hippocampal neurogenesis causes impairments in BLA-dependent fear conditioning[15-17], suggesting a functional role for new neurons in fear-associated memory networks. Previous work also shows that new neurons may be particularly prone to integration into memory networks as they are preferentially activated by exposure to previously experienced contexts such as enriched environment, water maze and fear conditioning[34-36].

To test the activation of newborn neurons by fear memory, we exposed sham-operated rats to a contextual fear conditioning task, which is dependent on both BLA and hippocampal activity[3, 5, 6, 28], and then assessed expression of the immediate early gene (IEG) cFos in newly born neurons. cFos expression reflects neuronal activation and incorporation into hippocampal memory circuits[25, 34, 35]. It is also critical to hippocampal mediation of contextual fear conditioning specifically[37, 38].

Two weeks after labeling proliferative cells with BrdU, rats were exposed to a series of 10 unpredictable shocks in a conditioning chamber (Figure 5a). Re-exposure to the shock-associated context (the fear context) the next day led to greater freezing compared to

exposure to a novel context (Figure 5b), indicating robust memory for the fear-associated environment. Forty-five minutes after re-exposure, rats were perfused and assessed for BrdU co-labeling with cFos. BrdU positive cells at this point were 15-19 days old, an age characterized by preferential recruitment into memory networks and possibly enhanced importance for hippocampal memory function[18, 34]. Using DCX as a marker of immature neurons, we found that exposure to the fear context increased the proportion of BrdU cells co-expressing DCX and cFos compared to rats exposed to the novel context (Figure 5c and d). Exposure to fear context did not alter the total number of cFos-positive cells in the dentate gyrus (Figure 5e). These data suggest that new neurons are activated by fear-associated memory but not simply by exposure to a novel environment.

BLA lesions prevented activation of new neurons by fear memory

We next investigated whether BLA lesion could influence the activation of new neurons by fear memory. Unilaterally BLA lesioned rats showed greater freezing in the fear context than in the novel context, indicating robust fear memory despite the presence of lesion, as expected in the presence of one functional BLA (Figure 5f). We found that BLA lesion blocked the fear-context associated increase in BrdU/cFos/DCX co-expression ipsilaterally (Figure 5g). In fear context exposed rats, a lower proportion of BrdU cells expressed cFos/DCX+ ipsilateral versus contralateral to lesion. The proportion of triple labeled cells contralateral to lesion did not differ from that in sham-operated rats. In rats exposed to the novel context, by contrast, BLA lesion did not affect the proportion of cFos/DCX-labeled BrdU cells. These results suggest that BLA lesion blocks the selective activation of two week old immature neurons by a context associated with an aversive stimulus.

We also found that there were significantly fewer BrdU/DCX positive cFos cells ipsilateral to the BLA lesion compared to contralateral in rats exposed to the fear but not the novel context (Supplementary Figure 1). These results suggest that, of cells activated by exposure to a fear context (i.e. expressing cFos), the percentage representing immature neurons is reduced by ipsilateral BLA lesion. The overall percentage of BrdU cells expressing DCX was not affected by lesion (Supplementary Figure 2).

Discussion

We demonstrate for the first time that BLA activity regulates adult neurogenesis and potentially modifies the recruitment of new neurons into networks underlying emotional memory. Bilateral and unilateral BLA lesions suppressed hippocampal neurogenesis, suggesting that ipsilateral BLA input supports basal hippocampal neurogenesis. These results extend several previous lines of work showing that BLA activity can affect hippocampal plasticity and function. Previous work demonstrates, for example, that lesion or inactivation of the BLA suppresses dentate gyrus LTP ipsilaterally[32]. Activation of β-adrenoceptors in the BLA can also enhance hippocampal memory function in an inhibitory avoidance task while increasing expression of Arc, an immediate early gene and marker of hippocampal plasticity[12]. In addition, the BLA plays an integral role in how stress affects the hippocampus; the work of McGaugh et al. has shown the BLA is required for stress-

induced impairments of memory retrieval as well as stress-induced enhancements in memory consolidation[4, 13, 14].

CeA lesions did not suppress hippocampal neurogenesis, suggesting that reduction in hippocampal neurogenesis is not attributable to generalized ipsilateral amygdala damage, and is a specific response to loss of BLA activity. Previous work on dentate gyrus-perforant path LTP similarly shows no effect of CeA lesions on LTP[32]. The dependence of hippocampal plasticity on BLA but not CeA activity is consistent with the behavioral function of the BLA, which is required for hippocampus-dependent contextual fear conditioning. The CeA, by contrast, participates in hippocampus-independent behaviors such as cued fear conditioning[39]. Together with previous studies, our results suggest a general model wherein BLA input promotes multiple forms of hippocampal plasticity, thereby supporting hippocampal memory.

We further demonstrated regulation of hippocampal neurogenesis by BLA activity using a viral vector-mediated overexpression of the voltage gated outwardly-rectifying potassium channel, Kv1.1. Outwardly rectifying potassium channels such as Kv1.1 regulate neuronal excitability, with voltage gated subtypes aiding in repolarization after an action potential and thereby decreasing glutamate release probability[40]. Kv1.1 overexpression in neurons via HSV vector specifically causes reduced neural resting potential, decreases basal firing rates and is neuroprotective against excitotoxic insult (i.e. kainic acid and glutamate)[29, 41]. In the BLA, overexpression of outwardly rectifying potassium channels in neurons also reduces anxiety and stress responses in adult rats, suggesting that suppressing BLA activity by manipulating potassium channel expression has consequences for emotionality[33]. In the present study, we found that overexpression of Kv1.1 in the BLA suppressed hippocampal neurogenesis ipsilaterally, suggesting that neural activity in the BLA supports hippocampal neurogenesis.

The BLA sends the majority of its ipsilateral input to the hippocampus through two indirect pathways, relayed through either the medial septum or the entorhinal cortex[10, 42]. Both of these pathways have been implicated in BLA support of adult hippocampal LTP and provide excitatory input to the dentate gyrus[10, 43]. While the medial septum provides input through cholinergic projections to the dentate gyrus granule neurons, the entorhinal cortex primarily sends glutamatergic projections[10, 43]. Importantly, both acetylcholine and glutamate increase proliferation of adult neural precursor cells[44-46], suggesting that input from either pathway could mediate BLA support of adult neurogenesis in the dentate gyrus. In addition, induction of dentate gyrus LTP itself stimulates hippocampal neurogenesis [47, 48], raising the possibility that suppressed LTP following BLA lesion could in part underlie suppression of neurogenesis. Future studies will address the extent to which these two ipsilateral pathways contribute to the support of hippocampal neurogenesis.

New neurons, particularly those around two weeks old, influence emotional memory function[15, 18]. New neurons are also context-sensitive, integrating strongly into memory networks for contexts initially experienced around two weeks of age[34]. This preferential integration may rely on the heightened plasticity of immature neurons compared to their older counterparts in the dentate gyrus[34]. Indeed, acceleration of maturation beyond this

plastic phase can interfere with both integration of new neurons into non-fear related hippocampal memory networks as well as behavioral performance in hippocampus-dependent emotional memory tasks[25].

We investigated whether new neurons in this highly plastic phase of their development were integrated into fear memory circuits using a contextual fear conditioning task two weeks after labeling newborn cells. Contextual fear conditioning relies on both the BLA and the hippocampus[3, 5, 6, 28] and appears to be partially mediated by adult hippocampal neurogenesis[15, 49]. We chose cFos expression as a marker of neuronal activation due to its role in memory for fear-associated context[37, 38]. We found that exposure to a fear-associated context activated two week old neurons more than exposure to a novel context, an effect that was blocked by ipsilateral BLA lesion. These results suggest that immature neurons are integrated into neural networks for fear-associated memory, but that this integration is dependent on BLA input. However, it is also possible that the new neuron activation could reflect participation in fear extinction. Future studies will further explore this possibility.

One recent study using mice suggests that while fear conditioning activates newly born neurons[36], this activation occurs in older neurons than those examined in our study (six weeks versus 2 weeks) and in a smaller proportion of new cells. A separate study further suggested that there was no cFos co-expression in two-week old neurons following exposure to a water maze task in mice[25]. Rather, IEG expression occurred only in new neurons 4-6 weeks old. Given that new neurons mature faster and potentially play a more influential role in hippocampal function in rats than mice[50], these differences in activation and integration of new neurons likely relate to species differences in how new neurons function and mature.

Our results demonstrate a novel mechanism for the influence of emotion over hippocampal memory function. By modifying adult hippocampal neurogenesis and altering the incorporation of new neurons into emotional memory networks, BLA input could shape how emotional stimuli influence memory. Whether these two impairments are the result of two independent processes or contingent one upon the other (such as through selective suppression of proliferation of cells that would later respond BLA input) is not clear and will require more research. To our knowledge, our results represent the first report of regulation of adult hippocampal neurogenesis and new neuron activation by BLA activity and fear-associated network activation. Future studies will further address the behavioral relevance of this phenomenon for emotional memory function as well as the underlying molecular mechanisms for emotion regulation of hippocampal proliferative capacity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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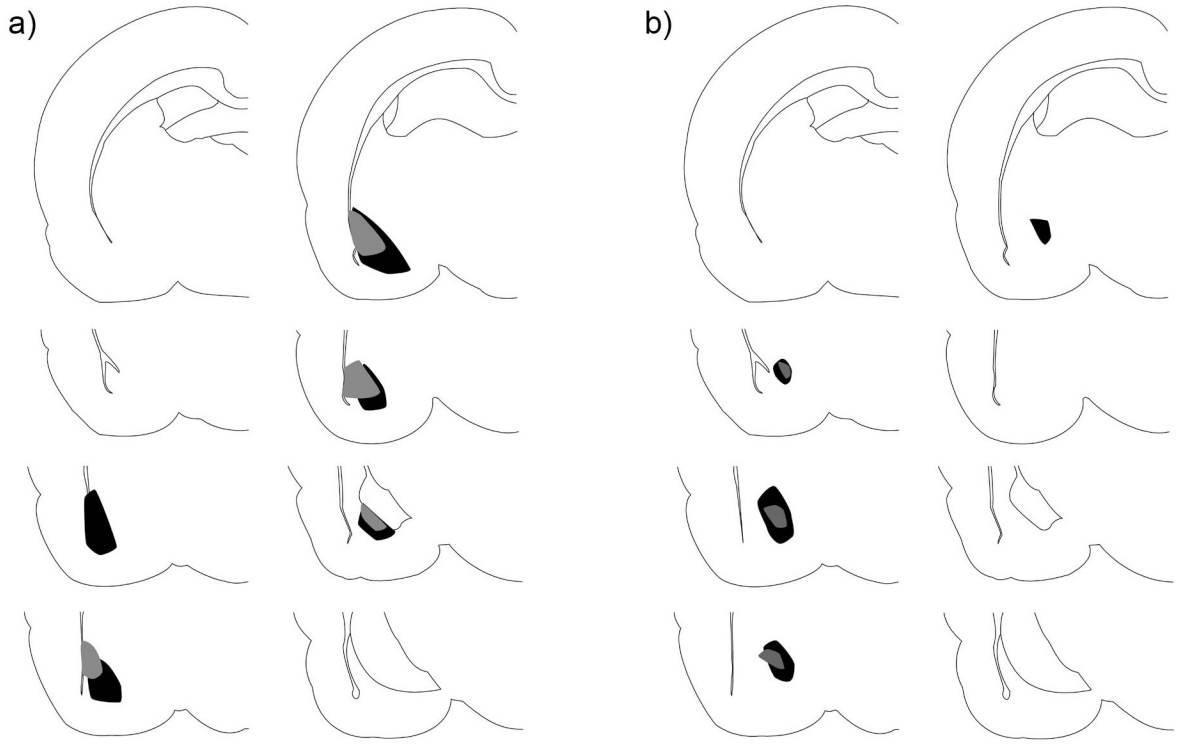

Figure 1. Excitotoxic lesions

Minimum (grey) and maximum (black) extent of excitotoxic BLA (a) and CeA (b) lesions.

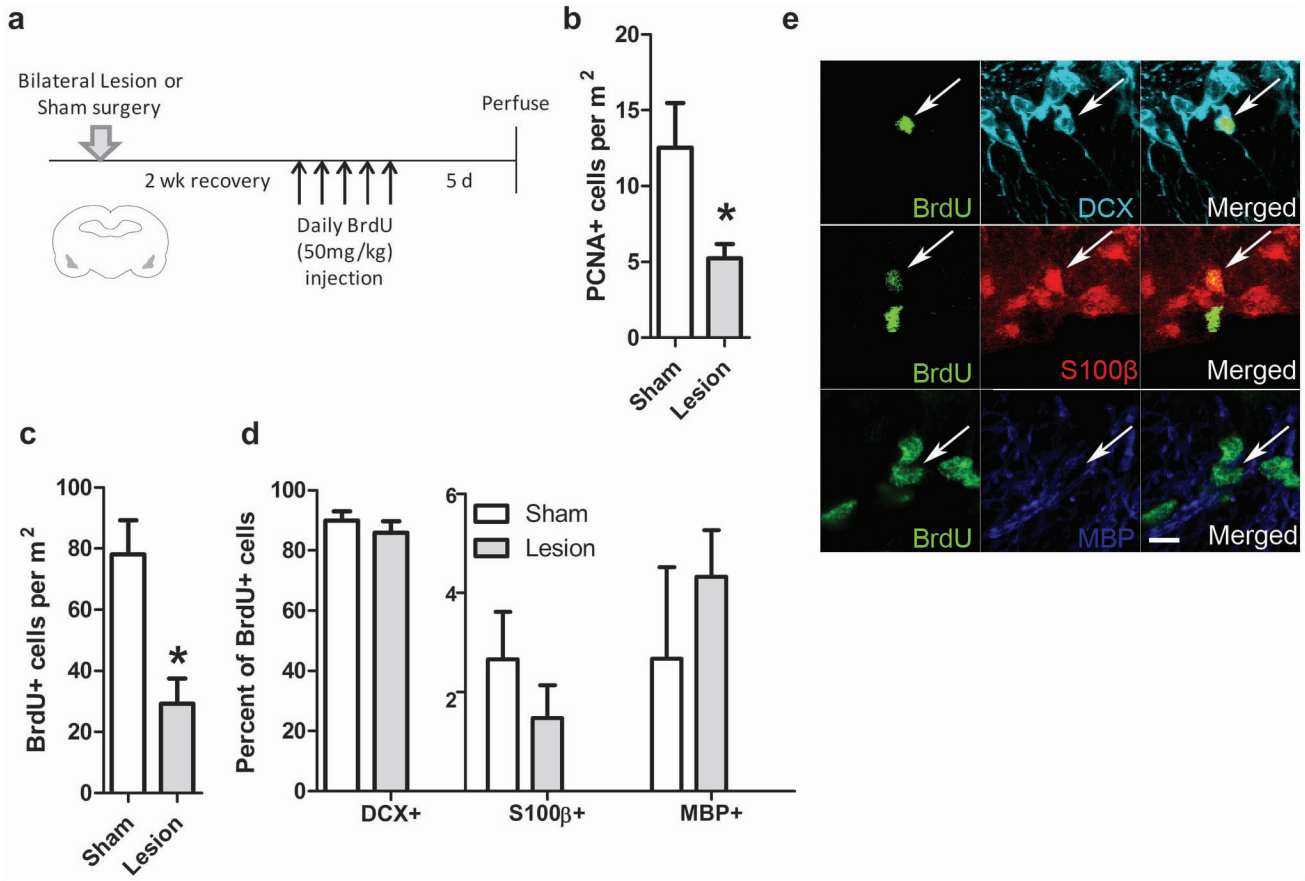

Figure 2. Bilateral BLA lesions suppressed hippocampal neurogenesis

(a) Experimental timeline. (b) Bilaterally lesioned rats (n = 5) had significantly fewer PCNA positive cells than sham operated rats (n =6). *p<0.05. (c) Bilaterally lesioned rats also had significantly fewer BrdU positive cells than sham operated rats, representing a reduction in the number of 5-10 day old cells. *p<0.05. (d) BLA lesion did not affect the percent of BrdU positive cells expressing one of three cell fate markers: doublecortin (DCX), S100β or myelin basic protein (MBP). (e) Representative confocal images showing colocalization of BrdU with DCX, S100β or MBP. Scale bar = 10 μm.

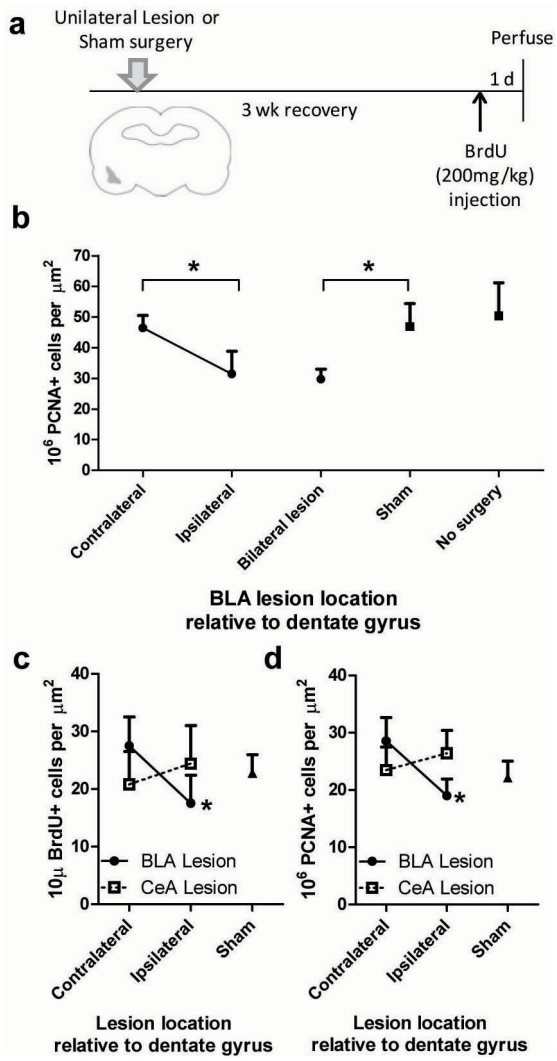

Figure 3. Unilateral lesions of the BLA, but not the CeA, ipsilaterally suppressed neurogenesis

(a) Experimental timeline. (b) There were significantly fewer dentate PCNA positive cells ipsilateral versus contralateral to unilateral BLA lesion (n = 5). Similar suppression of PCNA positive cells was found with bilateral BLA lesion (n = 6) as ipsilateral to unilateral lesion. The number of PCNA positive cells contralateral to lesion was similar to that found in the dentate gyrus of bilateral sham-operated animals (n = 3) and no surgery rats (n = 4). *p<0.05. (c) In BLA (n = 5), but not CeA (n = 6) lesioned rats, there were significantly fewer BrdU positive dentate cells ipsilateral versus contralateral to the lesion. Sham rats, n = 6. *p<0.01. (d) In BLA (n = 5), but not CeA (n = 6) lesioned rats, there were significantly fewer PCNA positive cells ipsilateral versus contralateral to the lesion. Sham rat, n = 6. *p<0.01.

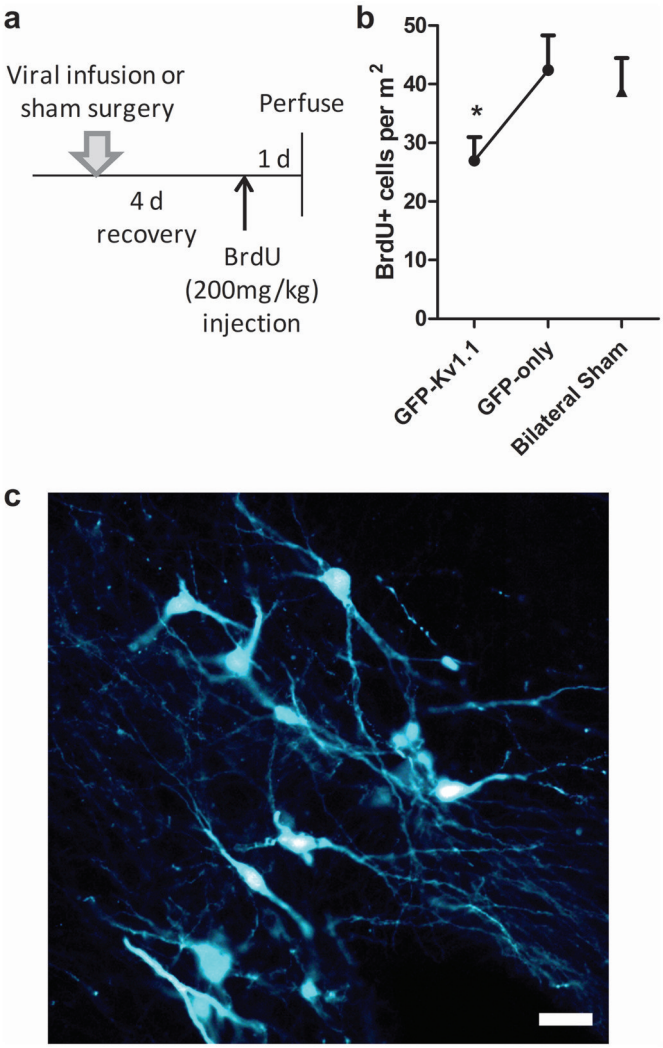

Figure 4. BLA silencing via potassium channel overexpression suppressed neurogenesis

(a) Experimental timeline. (b) In rats infused with GFP-Kv1.1 and GFP-only viral vectors to reduce BLA activity (n=8), there were significantly fewer BrdU positive dentate cells ipsilateral to GFP-Kv1.1 infusion. Sham rats, n = 15. *p<0.05. (c) Representative images of GFP expression in virus-infected BLA neurons. Scale bar = 10 μm

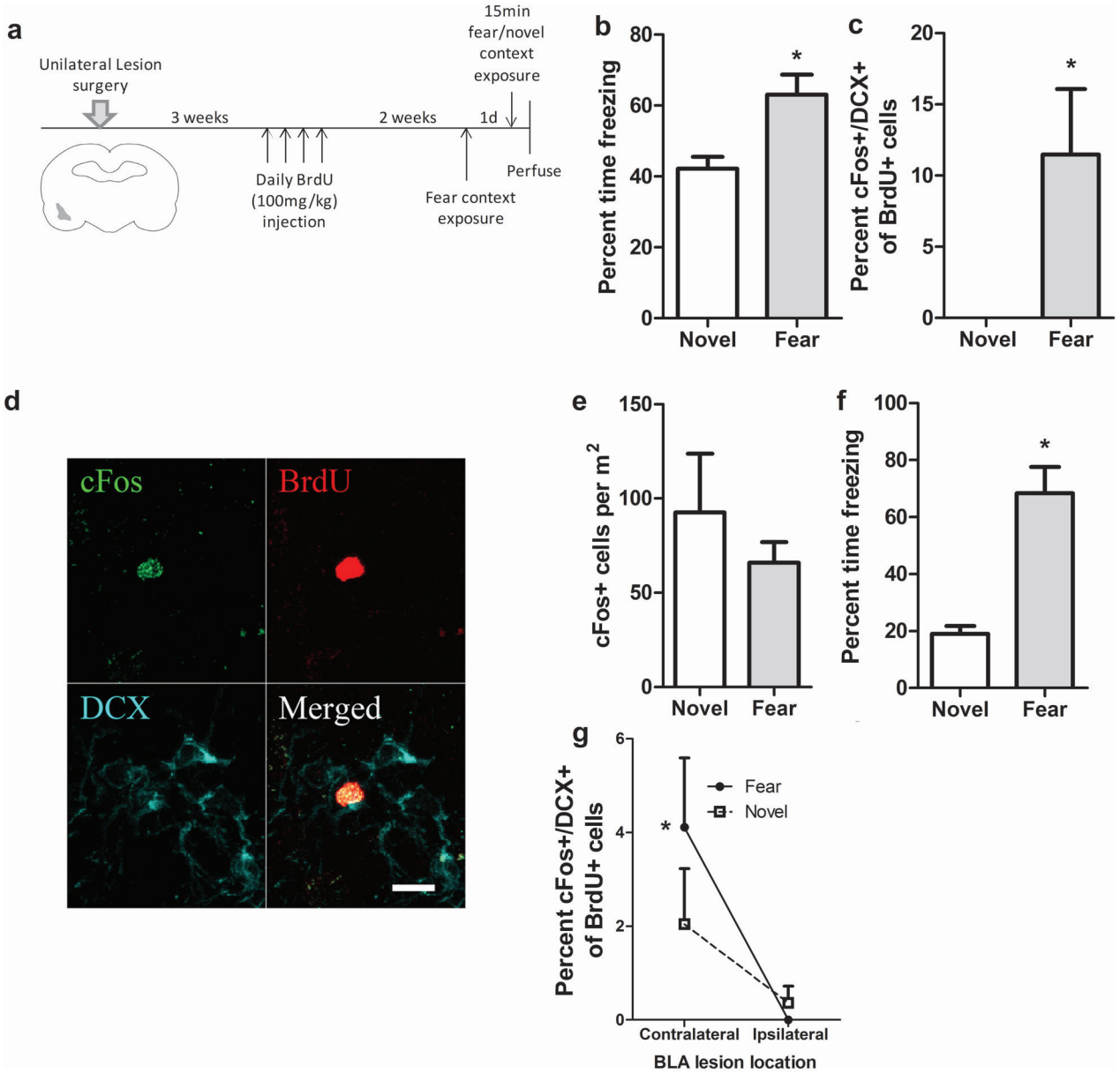

Figure 5. Unilateral BLA lesions blocked activation of immature neurons by exposure to a fear-associated context

(a) Experimental timeline. (b) Exposure to the fear context (n = 7) resulted in significantly more freezing than exposure to the novel context in sham operated rats (n = 5). (c) Sham-operated rats exposed to the fear context had a greater proportion of BrdU positive cells that co-expressed DCX and cFos. (d) Representative confocal image of a BrdU cell (red) expressing both DCX (blue) and cFos (green). Scale bar = 10 μm. (e) Sham-operated rats exposed to the fear context had similar numbers of cFos+ cells in the dentate gyrus as rats exposed to the novel context. (f) In unilaterally BLA lesioned rats, exposure to the fear context (n = 5) resulted in significantly more freezing than exposure to the novel context (n = 6). (g) In rats exposed to the fear but not the novel context, there was a lower percentage

of DCX/cFos labeled BrdU positive cells ipsilateral versus contralateral to the lesion. *$p<0.05$