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Title: Role of Amygdala-Infralimbic Cortex Circuitry in Glucocorticoid-Induced Facilitation of Auditory Fear Memory Extinction

Running Title: Extinction of Auditory Fear Conditioning

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Abstract

Purpose: The basolateral amygdala (BLA) and infralimbic area (IL) of medial prefrontal cortex (mPFC) are two inter-connected brain structures that mediate both fear memory expression and extinction. Besides the well-known role of the BLA in the acquisition and expression of fear memory, projections from IL to BLA inhibit fear expression and have a critical role in fear extinction. However, the details of IL-BLA interaction remain unclear. Here, we aimed to investigate the role of functional reciprocal interactions between BLA and IL in mediating fear memory extinction.

Methods: Using lidocaine (LID), male rats underwent unilateral or bilateral inactivation of the BLA and then unilateral intra-IL infusion of CORT, prior to extinction training of auditory fear conditioning paradigm. Freezing behavior was reported as an index for the measurement of conditioned fear. Infusions were performed before the extinction training, allowing to examine the effects on fear expression and also further extinction memory. Experiments 1-3 investigated the effects of left or right infusion of CORT into IL, and LID unilaterally into BLA on fear memory extinction.

Results: Results showed that intra-IL infusion of CORT in the right hemisphere reduced freezing behavior when administrated before the extinction training. Auditory fear memory extinction was impaired by asymmetric inactivation of BLA and CORT infusion in the right IL; however, the same effect was not observed with symmetric inactivation of BLA.

Conclusion: It is concluded that the IL-BLA neural circuit may provide additional evidence to contribution of this circuit in auditory fear extinction. This study demonstrate dissociable roles for right or left BLA in subserving the auditory fear extinction. Our finding also raise the possibility that left BLA-IL circuitry may contribute in mediating auditory fear memory extinction via underlying mechanisms, however further research is required.

Keywords: Auditory fear memory, Extinction, Inactivation, Infralimbic, Amygdala
Highlights

- CORT infusion in the right, but not left IL, facilitated auditory fear memory extinction.
- CORT infusion in the right IL following symmetric BLA inactivation had not any effect on auditory fear memory extinction.
- Asymmetric BLA inactivation prior to CORT infusion into the right IL impaired auditory fear memory extinction.

Plain Language Summary

Previous studies have established that glucocorticoids, which released in stressful conditions, enhance fear memory extinction. In this study, we found that CORT infusion in the right IL, but not left IL, facilitates auditory fear memory extinction. The effect of CORT infusion in the IL was not blocked by the intra-BLA injections of lidocaine when administrated in the ipsilateral hemisphere, but asymmetric BLA inactivation and CORT infusion into the right IL impaired auditory fear memory extinction.
1. Introduction

Accumulating evidence indicates that learned fear responses are reduced following repetitive exposure to the conditioned stimulus (CS) without the unconditioned stimulus (US), a process termed Fear extinction. This new inhibitory learning has been developed as a translationally valuable assay for studying anxiety and trauma-induced disorders (Sierra-Mercado, Padilla-Coreano et al. 2011, Hitora-Imamura, Miura et al. 2015).

Additionally, the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC), as involved areas in the encoding and expression of learned fear are part of the neuro-circuitry mechanism underpinning the fear extinction, highlight the importance of two neural structures in the encoding learned fear extinction (Sotres-Bayon, Cain et al. 2006, Herry, Ciocchi et al. 2008). The evaluation of specific roles of each area revealed that IL and BLA have opposite effects on fear extinction so that BLA is thought to involve in the initial acquisition of extinction (Vidal-Gonzalez, Vidal-Gonzalez et al. 2006, Sierra-Mercado, Padilla-Coreano et al. 2011, Kritman and Maroun 2013).

Previous evidence from functional disconnection studies investigate how memory processing regulating is linked to interactions between BLA and mPFC (Fuchs, Eaddy et al. 2007, Mashhoon, Wells et al. 2010, Fineberg, Haddad et al. 2013). Recent studies have been showed that neuronal connections between the BLA and IL region and also reciprocal connections from BLA to IL are important for extinction. On the other hand, plasticity in both the IL and BLA also be involved in the inhibition of fear (Akirav, Raizel et al. 2006, Myers and Davis 2007, Rahman, Shukla et al. 2018).

Synchronized neural activity in both BLA and mPFC due to reciprocal connections between two brain areas refers the consolidation of fear memory (Popa, Duvarci et al. 2010). Furthermore, by using functional disconnection investigations regulation of memory processing by interactions between BLA and mPFC has been well documented (Mashhoon, Wells et al. 2010). By the way, any deficits in memory processing that will come after BLA inactivation in one hemisphere and mPFC in the contralateral hemisphere might be caused by perturbing functional communication within BLA–mPFC circuitry. For example asymmetric inactivation of BLA–mPFC circuitry leads to disruption in serial information flow between these regions and expression of contextual fear memory (Stevenson 2011).

A converging body of evidence has established that glucocorticoids can facilitate fear extinction but GR antagonists impair extinction of conditioned fear (Ninomiya, Martynhak et al. 2010,
Blundell, Blaiss et al. 2011, de Quervain, Wolf et al. 2019). Similarly, by using cortisol extinction based psychotherapy will be enhanced (Aerni, Traber et al. 2004, de Quervain, Schwabe et al. 2017). Furthermore animal studies have shown that facilitating the extinction of contextual fear and fear potentiated startle can be occur following administration of GR agonists (Cai, Blundell et al. 2006, Yang, Chao et al. 2006, Ninomiya, Martynhak et al. 2010, Blundell, Blaiss et al. 2011). These effects are mediated partly through the IL since intra IL administration of glucocorticoid receptor (GR) agonist was established to facilitate extinction learning (Dadkhah, Abdullahi et al. 2018). In addition, multiple series of human studies indicated decreased cortisol level in post-traumatic stress disorder (PTSD) patients (Yehuda 2009), suggesting that low levels of cortisol may contributed in inability to extinguish the fear memory (de Quervain, Wolf et al. 2019). Although much of our understanding of fear extinction comes from inactivation studies, the role of functional interactions between BLA and contralateral or ipsilateral IL in mediating the expression of fear memory extinction is not clear. Taken together, these data support the hypothesis that GRs in both IL and BLA have a role in extinction but it remains unclear if activation of GRs in IL is depend on BLA.

2. Materials and Methods
This present study investigated the role of functional interactions between BLA and IL in mediating the extinction of auditory fear memory. Although the BLA–IL circuitry is well known as a critical circuit in fear memory extinction (Bloodgood, Sugam et al. 2018), reversible inactivation studies should be down to investigate the reciprocal interconnection of BLA–IL circuitry, as well as modulatory effect of glucocorticoids in these brain structures in regulating the fear memory extinction.

2.1. Animals
Adult male Wistar rats (weighing 250–280 g) at the time of surgery were used for conducting experiments. Rats were housed, four per cage (60 × 40 × 20 cm), maintained in a room with normal light cycle and temperature (24 ± 2 °C) and free access to food and water. All behavioral tests were carried out between 8:00 –15:00 AM. The experimental protocol were conducted with internal ethical approval and was approved by the Ethical Review Board of Semnan University of Medical Sciences (Iran).
2.2. Surgery and cannulae implantation

Anesthesia was induced with ketamine hydrochloride (60 mg/kg) and xylazine (10 mg/kg) intraperitoneally. At the time of surgery rats were placed in a stereotaxic frame and one stainless steel guide cannulae of 23-gauge were implanted unilaterally above the IL (anterior, 2.9 mm; lateral, 1.0 mm; ventral, 5.0 mm) and BLA (anterior, 2.8 mm; lateral, 5.0 mm; ventral, -7.0 mm below the cortical surface). Dental acrylic was used to fix cannulas to the skull (Fig. 1).

![Diagram](image)

Surgical procedures included the placement of one stainless steel guide cannulae above the IL and BLA in one hemisphere. Stainless steel inserted into guide cannulae to extend 0.5 mm beyond the guide cannula to prevent clogging. Finally rats were given 7 days of postoperative recovery time.

2.3. Drug infusions

Drugs were obtained from Sigma Co., England. Lidocaine (LID) (10 µg/0.5 µl) were infused with microinjection apparatus (Stoelting, USA) into the BLA in one hemisphere to inactivate this region before extinction training. Corticosterone (CORT) (20 ng, /0.5 µl) or vehicle (VEH) were infused into the IL in one hemisphere 10 min after administration of LID or VEH in right or left BLA. CORT and LID were dissolved in saline and injected in one hemisphere (20 ng, /0.5 µl), 10 min prior to the fear extinction training. This concentration has been used in our previous study to examine the effects of CORT on fear extinction (Dadkhah, Abdullahi et al. 2018). Saline solution as a VEH was injected to animals in the control group.

After removing the stylus from the guide cannulae, CORT and/or VEH were infused in a volume of 0.5 µl over 1 min via injector cannulae 30-gauge extending 1.0 mm from the tip of the guide cannulae. The polyethylene tubing (PE10) which was connected to a Hamilton microsyringe was inserted to cannulae for microinjection. Animals received 0.3 µl volume of drug bilaterally in 1
min. After infusion, cannulae were left in their place for additional 1 min, preventing reverse flow of the microinfused drug.

2.4. Experimental groups
In experiments 1, animals divided randomly into the 4 groups: R/IL/SAL, L/IL/SAL (received SAL in right or left IL), R/IL/CORT, and L/IL/CORT (received CORT in right or left IL) groups. In experiment 2, animals divided randomly into the 4 groups which received infusions unilaterally into IL and BLA: IL/SAL-BLA/SAL (received SAL in right IL and unilateral BLA), IL/CORT-BLA/SAL (received CORT in right IL and SAL in unilateral BLA), IL/SAL-BLA/LID (received SAL in right IL and LID in unilateral BLA), and IL/LID-BLA/LID (received LID in right IL and LID in unilateral BLA).

In experiment 3, animals divided randomly into the 4 groups which received infusions contralaterally into IL and BLA: IL/SAL-BLA/SAL (received SAL in right IL and contralateral BLA), IL/CORT-BLA/SAL (received CORT in right IL and SAL in contralateral BLA), IL/CORT-BLA/LID (received SAL in right IL and LID in contralateral BLA), and IL/LID-BLA/LID (received LID in right IL and LID in contralateral BLA).

2.4. Histology
Following behavioral examination, rats sacrificed and the brains were removed and immersed in fresh 10% formaldehyde, followed by sectioning into 40-µm coronal slices with a microtome, and the cannulae location was examined under a light microscope by hematoxylin and eosin staining (Fig.1B).

2.5. Behavioral test
2.5.1. Auditory fear conditioning apparatus
Auditory fear conditioning and extinction was carried out in the conditioning chamber inserted in the sound-attenuating box. The chamber containing a floor equipped with steel bars that delivered a electric footshock, which served as the US. A video camera inserted inside the chamber to record animal behaviors. Freezing behavior was measured as a fear index, referred to the absence of all movements except for respiration (Fanselow 1994). At the end of each experimental trial, shock grids and trays under the box were cleaned with wet towel.
2.5.2. Fear conditioning and extinction

Briefly, habituation were conducted on the first day of behavioral test (day 0) [rats underwent 12 tones for 30s, without shock, 3 min intertrial interval (ITI) after the first 6 tones] for 9 minutes. On conditioning day (day 1), each rat housed in the grid box were received three conditioning trials in which a tone (Tone: 4 kHz; 80 dB sound pressure level, ITI of 2 min) immediately followed by the final tone co-terminated with a mild foot-shock (0.8 mA, 1 s) (Fig. 2). Freezing time during the 30 s tone were reported. The results of extinction training for the 15 tones in the extinction training are represented as 5 blocks (the average of 3 trials).

2.6. Statistical Analysis

All data were reported as the mean ± S.E.M. Data were analyzed using a two-way analysis of variance (ANOVA) with repeated measure and independent sample t-test followed by Tukey’s post hoc comparisons. In all experiments, P < 0.05 was considered as statistical significance. Also, analyses of within-session extinction, the data are expressed as 5 blocks which each block considered as the average of 3CS presentations per block (extinction blocks).

3. Results

3.1. Experiment 1: CORT infusion in right IL, but not left IL, facilitate fear memory extinction.

3.1.1. Conditioning

No freezing behavior on the conditioning day was observed during the first CS presentation in the extinction test (prior to US presentation); however, all groups had the same freezing during the second and third CS presentations (Fig. 3A), suggesting the groups’ uniformity. A two-way ANOVA with repeated measures (group × conditioned stimulus) indicated a significant effect of CS (F_{2, 36} = 957.968, P = 0.0001), no significant effect of group (F_{3, 24} = 10.976, P = 0.396) and of between two factors (F_{1, 24} = 2.896, P = 0.081).

3.1.2. Extinction tests

The extinction data of all experimental groups are represented in Fig. 3. Analysis with repeated measures (group × days) indicated a significant effect of group (F_{1, 18} = 43.357, P = 0.0001), of days (3 testing days: Test1, Test 2 and Test 3) (F_{2, 36} = 48.248, P = 0.0001) and between two factors (F_{1, 12} = 2.997, P = 0.005). ANOVA with repeated measures analysis for the group comparisons as shown in Fig. 3B and C indicated significant difference between SAL/IL and
R/IL/CORT groups (P = 0.0001), indicating that unilateral CORT infusion in right IL decrease freezing levels compare to CORT administrated in left IL.

3.2. Experiment 2: Symmetric BLA inactivation and CORT infusion in IL had no effect on fear memory extinction.

3.2.1. Conditioning

On the conditioning day, freezing behavior did not observed during the first CS presentation in the extinction box (prior to US presentation); however, all groups showed similar freezing during the second and third CS presentations (Fig. 4A), which indicates the groups’ uniformity. A two-way analysis of variance (ANOVA) with repeated measures (group × conditioned stimulus) indicated a significant effect of CS (F₁, 24 = 238.225, P = 0.0001), no significant effect of group (F₃, 24 = 1.897, P = 0.157) and no significant effect between two factors (F₁, 24 = 05.775, P = 0.519).

3.2.2. Extinction tests

According to the extinction data of the experimental groups which presented in Fig. 4, and results from tree-way ANOVA with repeated measures (LID × CORT treatment × days), a significant effect of LID treatment (F₁, 24 = 65.764, P = 0.0001), of CORT treatment (F₁, 96 = 113.097, P = 0.0001), and a significant effect of test days (3 testing days: Test1, Test 2 and Test 3) were observed (F₂, 48 = 64.287, P = 0.0001), which further indicated no significant interaction between day and CORT treatments (F₁, 24 = 25.992, P = 0.0001), no significant interaction between the LID and days (F₁, 24 = 7.313, P = 0.012), of treatment and LID (F₁, 24 = 65.764, P = 0.0001), and significant interaction between CORT treatment and LID and days (F₁, 24 = 7.313, P = 0.012).

Post hoc comparisons indicated decrease in freezing behavior in all groups after 15 CS presentations on Test1, Test 2, and Test 3 (Fig. 3B and C). The SAL/IL–LID/BLA, CORT/IL-SAL/BLA groups continued to display decreased freezing during the three test sessions compare to the SAL/IL–SAL/BLA group (P = 0.0001), indicating that unilateral CORT infusion in IL or unilateral BLA inactivation in the same hemispher facilitated extinction. Furthermore there was no significant difference between the CORT/IL-SAL/BLA and CORT/IL-LID/BLA groups on the blocks of three tests. This indicates unilateral CORT infusion in IL have facilitatory effect on extinction and this effect is independent from BLA activation in the same hemisphere (Fig. 4B and C).
3.3. Experiment3: Asymmetric BLA inactivation and CORT infusion in IL impair fear memory extinction.

3.3.1. Conditioning

Similar to the previous experiments, no freezing behavior was observed during the first CS presentation in the extinction box (prior to US presentation); but similar freezing during the second and third CS presentations in groups were observed (Fig. 5A), which indicates the groups’ uniformity. A two-way analysis of ANOVA with repeated measures (group × conditioned stimulus) indicated a significant effect of CS (F$_{1, 24}$ = 788.667, P = 0.0001), no significant effect of group (F$_{3, 24}$ = 4.133, P = 0.017) and no significant effect between two factors (F$_{3, 24}$ = 3.275, P = 0.089).

3.3.2. Extinction tests

Three-way ANOVA with repeated measures (LID × CORT treatment × days) indicated a significant effect of LID (F$_{1, 24}$ = 18.458, P = 0.0001), a significant effect of CORT treatment (F$_{1, 24}$ = 33.829, P = 0.0001), and a significant effect of days (3 testing days: Test1, Test 2 and Test 3) (F$_{2, 48}$ = 101.428, P = 0.0001), which further indicated no significant interaction between day and CORT treatments (F$_{2, 48}$ = 2.772, P = 0.109), no significant interaction between the LID and days (F$_{2, 48}$ = 4.189, P = 0.052), no significant interaction between CORT treatment and LID (F$_{1, 24}$ = 96.504, P = 0.0001), and significant interaction between CORT treatment and LID and days (F$_{2, 48}$ = 0.572, P = 0.457) (Fig. 4).

Decreased freezing behavior was observed in all groups after 15 CS presentations on Test1, Test2, and Test3 (Fig. 5B and C). The SAL/IL–LID/BLA, CORT/IL-SAL/BLA groups continued to display decreased freezing during the test sessions than the SAL/IL–SAL/BLA group (P = 0.0001), indicating that both unilateral CORT infusion in IL and unilateral BLA inactivation, both had facilitatory effect on extinction. Furthermore a significant difference between the CORT/IL-SAL/BLA and CORT/IL-LID/BLA groups on the blocks of Test1, Test2 and Test3 (P = 0.003) was observed. This indicates while unilateral CORT infusion in IL (right IL) and contralateral BLA inactivation, both facilitate fear extinction, CORT infusion in IL following ipsilaterally inactivation of BLA impaired fear extinction (Fig. 5B and C). In other word CORT infusion in IL facilitate fear extinction via BLA activation in contralateral hemisphere (Fig. 5B and C).
4. Discussion

In the present study, we investigated the role of functional interactions between BLA and IL in mediating auditory fear memory extinction. Our results of experiment 1 suggest that unilateral CORT infusion into IL sub-region facilitate the extinction of auditory fear memory when administrated in right IL. In experiment 2, we showed that symmetric BLA inactivation prior to CORT infusion in right IL did not effective on auditory fear memory extinction. In experiment 3, we observed that asymmetric BLA inactivation and then CORT infusion in right IL impaired auditory fear memory extinction suggesting that right and left BLA play different roles in mediating the facilitatory effect of GRs of IL on fear memory extinction.

4.1. CORT infusion in right IL facilitates auditory fear memory extinction.

Glucocorticoids released from the adrenal cortex and have an important role in regulating different kinds of learning and memory including auditory and contextual fear memory and different brain regions such as hippocampus, basolateral amygdala (BLA), and mPFC (Abrari, Rashidy-Pour et al. 2009, Barsegyan, Mackenzie et al. 2010) mediates underlying mechanisms. Additionally, it is important to note that glucocorticoids play a key role in fear memory processing (Okuda, Roozendaal et al. 2004), and facilitate the consolidation of extinction of fear learning (Cai, Blundell et al. 2006, Abrari, Rashidy-Pour et al. 2008).

The present study indicated that whereas infusion of CORT in left had no effect on fear extinction, intra infusion of CORT in right IL reduced fear expression, similar to the pattern from our previous study indicating IL play an important role in CORT-induced facilitation of fear memory extinction (Dadkhah, Abdullahi et al. 2018). The extinction of shock-induced fear memories is depend on corticosterone synthesis since (Abrari, Rashidy-Pour et al. 2008). However, these effects are mediated similar to the previously observed via the IL subregion, since of a glucocorticoid receptor (GR) agonist into IL results in facilitation of extinction learning, while blocking of a GR antagonist suppress extinction learning (Dadkhah, Abdullahi et al. 2018). Consistent with this, a recent study implicated IL is contributed in decreased fear responses during extinction (Sierra-Mercado, Padilla-Coreano et al. 2011). Thus, IL is a key modulator for the extinction of fear memory and suggests that this area might be critical for the treatment of anxiety disorders through pharmacological manipulations.
4.2. CORT infusion in right IL following symmetric BLA inactivation, did not disturb fear memory extinction.

Another remarkable finding of the current study lies in clarifying the role of functional interactions between BLA and IL in mediating the extinction of auditory fear memory. Here, we found that symmetric inactivation of BLA and CORT infusion in right IL leaves fear memory extinction intact. In line with these findings, previous studies demonstrated that the finding of impairment expression of fear memory following reversible inactivation of BLA, which was in line with other studies (Laurent and Westbrook 2009).

Moreover, the effect of inactivating BLA and CORT infusion in IL area were examined separately. The logic of underlying strategy raised from the reciprocally connections between BLA and IL display increased firing associated with fear extinction or facilitation of memory extinction respectively (Senn, Wolff et al. 2014). Our finding support and extend the previous study have used functional disconnection to express unilateral or ipsilateral connectivity between these structures, assume that unilateral inactivation had no effect functionally in each area (Stevenson 2011). To our knowledge, this was the first study to directly examine whether projections from IL to BLA are predominantly ipsilateral or contralateral and facilitatory effect of CORT on memory extinction is mediated via ipsilateral or contralateral BLA. A considerable body of work using techniques, including inactivation or IL-targeted pharmacological manipulation, has shown that IL activity is critical to successful fear extinction but functionally disrupting this region impacting on fear memory impairment (Likhtik and Paz 2015, Tovote, Fadok et al. 2015). Furthermore, interactions between BLA-mPFC, and also reciprocal connections from BLA to IL might subserve fear memory extinction (Bloodgood, Sugam et al. 2018). Reciprocal projections between BLA and IL leads to plasticity in IL, suggesting the involvement of BLA and IL in mediating the extinction memory (Sierra-Mercado, Padilla-Coreano et al. 2011). Most importantly, synchronized neural activity in BLA – mPFC circuit after fear learning also indicate differential requirement of the IL-BLA circuit in the memory processing (Stevenson 2011). Given the well-established role for IL projections in the extinction (Hikind and Maroun 2008), it has been showed that fear extinction requires IL cortex projections to the BLA (Bloodgood, Sugam et al. 2018).

The glutamatergic projections from the mPFC interact with amygdala gamma-aminobutyric acid (GABA)ergic neurons (Paré, Quirk et al. 2004), and this synapsis may provide inhibitory input to the amygdala. So this projections to intercalated cells could be a possible pathway by which IL
tone responses can suppress the expression of conditioned fear (e.g., reduce freezing) (Rosenkranz and Grace 2002).

While IL sends descending projections to BLA, and BLA receives reciprocal connections, it’s unclear whether projections from IL to BLA are predominantly ipsilateral or bilateral. In addition, the strongest pathways from the BLA to the mPFC originated from different neurons (Reppucci and Petrovich 2016). Concerning functional connection between these regions and specially BLA-IL circuit (Pape and Pare 2010, Contreras and Gutiérrez-García 2019), the results obtained here showed that unilateral BLA inactivation alone has no effect on facilitatory effect of intra IL CORT infusion on fear extinction. One possibility is that the effect of CORT infused into IL on extinction memory expression, would act via BLA in different hemisphere. Taken that this finding, therefore, suggest that the processing in IL-BLA circuit involved in fear memory extinction is not parallel. Here, functional disconnection also suppose that unilateral inactivation of BLA alone has no effect on facilitatory effect of intra IL CORT infusion on fear extinction, supporting a lateralized function of amygdala and mPFC fear memory processing (Stevenson 2011). Together, functional interactions between BLA and IL might be an important issue for subserving extinction fear memory expression.

As mentioned above, BLA and mPFC share ipsilateral connections. Consistent with the findings of this prior study, we found that while intra IL CORT infusion following unilateral BLA inactivation in the same hemisphere, leaves fear extinction intact compare to control group which received intra IL CORT alone. This finding indicate that asymmetric BLA inactivation impure that BLA and mPFC share ipsilateral connections between two areas. The current study found no effects of symmetric BLA inactivation and intra IL CORT infusion on extinction behavior in the auditory fear condition test, again confirm serial connection in previous findings.

4.3. Asymmetric BLA inactivation and CORT infusion in IL impair fear memory extinction.

The finding of impaired auditory fear memory extinction with intra IL infusion of CORT in right hemisphere following asymmetric BLA-inactivation provided the subserved functional interactions in BLA–IL circuit in processing the auditory fear memory extinction. This finding suggested the reciprocal nature of connections of BLA with IL in CORT-induced extinction memory facilitation via CORT administration in IL, differ in brain hemispheres.
This suggests that amygdalar stimulation-induced by IL activation during fear extinction connected to the facilitatory effect of CORT infusion in IL and this effect depends on inhibition of amygdala neurons which increasing freezing levels. As noted before, GABA-ergic mechanisms in the amygdala play a critical role in controlling the extinction of fear.

In addition, vmPFC is widely considered to inhibit the amygdala through activating the group of GABAergic intercalated cells ITC in BLA, which also have extinction neurons (Seo, Funderburk et al. 2016). However, the effects of inactivation of the BLA which impaired extinction memory is in agreement with other studies (Demetrio Sierra-Mercado). Moreover, glucocorticoid effects on memory processing is linked to bidirectional interactions between the BLA and mPFC. The memory-modulating properties of glucocorticoids depends on functional interactions between the mPFC and BLA (Roozendaal, McReynolds et al. 2009).

Furthermore, neurotransmitters are known to have a critical role in fear extinction since increases in dopamine (DA) and noradrenaline (NA) levels in the mPFC (Hugues, Garcia et al. 2007) have been observed, and increasingly appears the increase in DA in the mPFC results in greater reduction in fear after extinction training. Despite this, evidence reported changing flux of neurotransmitters including DA, NA, serotonin (5-HT), (GABA and glutamate in key brain regions during extinction of fear memories (Cahill and Milton 2019). Thus, it could be argued that impaired fear extinction following asymmetric BLA inactivation and CORT infusion in IL at least in part, due to changing flux of neurotransmitters in IL-BLA circuit.

Another reason for functional disconnecting between BLA and IL might be associated to inactivation of rostral or caudal BLA (Floresco and Ghods-Sharifi 2007, Fuchs, Eaddy et al. 2007). In support of this possibility, a similar effect was also reported after functionally disconnecting of posterior BLA and paralimbic sub-region of mPFC, results in which play a critical role in fear memory expression, resulting impairment by asymmetric inactivation of pBLA and PL (Stevenson 2011).

It should be noted, earlier findings have shown that brain lateralization may have an effect on the function of memory (Baker and Kim 2004). We found that either the intra-IL administration of CORT in right hemisphere and contralateral or ipsilateral BLA inactivation had not similar effects again demonstrating an existence of lateralization. Un-similar effect of unilateral BLA inactivation in each hemisphere on freezing behavior in the present study, indicates that the asymmetric neural activity of the left BLA in auditory fear extinction which depends on intra IL infusion of CORT in
right hemisphere. Consistent with the findings of this experiment, earlier studies provide further support for the notion that inactivation of left or right BLA had not similar effects due to lateralization. Furthermore, it has been showed that right BLA is dominant in fear memory conditioning (Baker and Kim 2004). Reports from previous studies demonstrated interactions between BLA and mPFC are involved in regulation of memory processing by using functional disconnection (Fuchs, Eaddy et al. 2007, Mashhoon, Wells et al. 2010). In this procedure, any resulting deficits caused memory processing following BLA inactivation in one hemisphere and mPFC in the contralateral one, might therefore be related to perturbed communication within BLA-mPFC circuitry. One logical reason to the underlying strategy is that although unilateral inactivation which leaves each area functionally unimpacted, impairments in the serial information flow in BLA-mPFC circuitry are observed by asymmetric inactivation (Stevenson 2011).

Many more inputs originated from different areas project to the amygdala (Pape and Pare 2010). Thus, the activity of IL-projecting cells in the BLA which is related to extinction might emerge due to reciprocal connectivity from the IL back down onto these cells.

With respect to hemisphere-specific morphological changes in BLA, dendritic retraction has been shown in the left BLA in response to extinction (Moench, Maroun et al. 2016). Further, increased connectivity between BLA-vmPFC during extinction learning has been reported (Belleau, Pedersen et al. 2018). Previous studies have documented direct connections between the vmPFC and the BLA supporting a bottom-up control mechanism due to direct connections between the CeMA and vmPFC (Seo, Funderburk et al. 2016).

These findings propose a fundamental role of the left BLA in CORT-induced facilitation in fear memory extinction when administrated in right IL.

**Conclusion**

Functional connections between the IL and BLA have received less attention in the literature. The present study demonstrated the presence of contralateral IL and BLA connections, which have not been previously documented. We also confirms the well-known lateralization connections assume an obvious difference hemisphere-specific processing between the left and right amygdalae.
References


**Fig. 1.** Experimental timeline of behavioral training, testing design and drug injections.

**Fig. 2.** A histological verification of guide cannulae placements in the experimental subjects. (A) IL: Infralimbic, (B) BLA: Basolateral Amygdala. (See Materials and Methods for more detail).

**Fig. 3.** Effects of intra-IL (right or left IL) infusion of corticosterone on fear memory extinction. (A) There were no differences in freezing response between the groups during the conditioning. (B, C) Freezing during fear extinction test. Unilateral infusion of CORT into right IL significantly decreased freezing compared to saline (SAL) group (***P < 0.001). R: Right, B: Block, IL: Infralimbic, BLA: Basolateral amygdala, (CORT): Corticosterone, SAL: Saline, Ex: Extinction.

**Fig. 4.** Effects of symmetric BLA inactivation and then corticosterone infusion into right IL on fear memory extinction. (A) No significant difference in freezing between the groups during the conditioning was observed. (B, C) Freezing during fear extinction testing. Compared to IL/SAL-BLA/LID group, symmetric BLA inactivation and then corticosterone infusion into right IL had the same effect on freezing levels. ***P < 0.001 compare to IL/SAL-BLA/SAL group. R: Right, B: Block, IL: Infralimbic, BLA: Basolateral amygdala, LID: Lidocaine, CORT: Corticosterone, SAL: Saline, Ex: Extinction.

**Fig. 5.** Effects of asymmetric BLA inactivation and then corticosterone infusion into right IL on fear memory extinction. (A) There were no differences in freezing levels between the groups during the conditioning. (B, C) Freezing during fear extinction testing. Compared to IL/SAL-BLA/LID group, asymmetric BLA inactivation and then unilateral infusion of intra right IL CORT increased freezing. ***P = 0.0001 compare to IL/SAL-BLA/SAL group and ^^^P = 0.0001 compare to IL/SAL-BLA/LID group. R: Right, B: Block, IL: Infralimbic, BLA: Basolateral amygdala, LID: Lidocaine, CORT: Corticosterone, SAL: Saline, Ex: Extinction.