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INFRALIMBIC CORTEX CONTROLS THE ACTIVITY OF THE HYPOTHALAMUS-PITUITARY ADRENAL AXIS AND THE FORMATION OF AVERSIVE MEMORY: EFFECTS OF ENVIRONMENTAL ENRICHMENT

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HIGHLIGHTS

- Infralimbic stimulation increases basal plasma levels of corticosterone.

- Environmental enrichment enhances the effects of infralimbic stimulation.

-Infralimbic inhibition reduces stress-induced corticosterone in control animals.

- Infralimbic cortex contributes to HPA activation during stress and aversive memory.

ABSTRACT

The aim of the present study was to investigate the effects of the stimulation and inhibition of the ventral part of the medial prefrontal cortex (infralimbic cortex) on basal and stress-induced plasma levels of corticosterone and on the acquisition of aversive memory in animals maintained in control and environmental enrichment (EE) conditions. Intracortical microinjections of the $GABA_A$ antagonist picrotoxin and agonist muscimol were performed in male Wistar rats to stimulate and inhibit, respectively, the activity of the infralimbic cortex. Injections were performed 60 min before foot shock stress and training in the inhibitory avoidance task. Picrotoxin injections into the infralimbic cortex increased basal plasma levels of corticosterone. These increases were higher in EE rats which suggest that EE enhances the control exerted by infralimbic cortex over the hypothalamus-pituitary-adrenal (HPA) axis and corticosterone release. Muscimol injections into the infralimbic cortex reduced the stress-induced plasma levels of corticosterone and the retention latency 24 h after training in the inhibitory avoidance performance in control and EE animals, respectively. These results further suggest that the infralimbic cortex is required for the activation of the HPA axis during stress and for the acquisition of contextual aversive memories.

Keywords: prefrontal cortex, picrotoxin, muscimol, stress, corticosterone, emotional memory.

1. INTRODUCTION

Growing evidence shows that the prefrontal cortex (PFC) regulates the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis and the release of corticosterone (cortisol in humans) in response to stress [1, 2]. Yet how the PFC exerts this regulation is a matter of debate. Previous studies in rodents suggest that the dorsal (prelimbic) and ventral (infralimbic) parts of the medial PFC exert opposite control on the HPA axis activity and plasma levels of corticosterone in response to stress [2-4]. However, most pharmacological and lesion studies are limited to the dorsal part of the medial PFC or do not distinguish between the dorsal and ventral parts [5-8] and, therefore, the role played by the infralimbic cortex in the regulation of the HPA axis and corticosterone concentrations is still uncertain. A dysfunctional regulation of the HPA axis as well as other limbic areas by the infralimbic cortex (ventral medial PFC in humans) is associated with stress mal adaptation and stress-related disorders [9-12].

Infralimbic cortex also contributes to stress adaptation by modulating emotional learning and memory [13, 14]. Studies focused on the prelimbic cortex suggest a negative control by this prefrontal area on the acquisition of contextual fear-related memories [6, 8]. However, and despite its well established role in extinction memory [15, 16], few studies have investigated whether infralimbic cortex contributes to the acquisition of aversive memory. In fact, lesion studies report controversial results suggesting that infralimbic cortex facilitates [13] or does not change [17] the acquisition of contextual aversive memories.

Animals housed in an enriched environment show a lower reactivity to stress that is reflected by a reduced anxiety and a faster habituation to novelty in the open field [18-23]. These behavioural effects produced by environmental enrichment (EE) are, in part, mediated by changes in the activity of the HPA axis and the release of corticosterone [24]. Studies from our laboratory and others suggest that changes in the activity of the PFC, presumably infralimbic cortex, explain the reduced reactivity to stress that is observed in enriched animals [9, 25, 26].

The aim of the present study is twofold. Firstly, to investigate the effects of the stimulation and inhibition of the infralimbic cortex on basal and stress-induced plasma

levels of corticosterone, and on the acquisition of aversive memory evaluated by the inhibitory avoidance task. And secondly, to investigate whether housing animals in EE changes the role played by the infralimbic cortex in these variables. Intracortical microinjections of the $GABA_A$ antagonist picrotoxin and the $GABA_A$ agonist muscimol were performed to stimulate and inhibit, respectively, the activity of the infralimbic cortex [8]. In this study we also evaluate whether EE changes spontaneous motor activity in the open field as observed in previous studies from our laboratory [20].

2. **Methods**

2.1. Animals and housing conditions

Male Wistar rats (3 months of age) (Harlan, Netherland) were housed in two different conditions during 2 months: the environmental enrichment (EE) group (weight 486 ± 5 g, n= 48) was housed in large cages (120 x 100 x 52 cm) 10-12 animals per cage, containing 2 running wheels, a rearrangeable set of plastic tunnels, a elevated platform, and toys changed every 5-6 days. The control group (weight 504 ± 6 g, n= 47) was housed in standard cages (35 cm x 50 cm x 20 cm) 2 animals per cage. Animals were provided with food and water *ad libitum* and maintained in a temperature-controlled room (22 ± 2 ºC) under an inverted light/dark cycle (lights on 20:00). The experiments were carried out during the dark phase of the cycle (15:00 - 18:00). All experiments were carried out in our laboratory at the University Complutense of Madrid following the Spanish regulations for the protection of laboratory animals (RD1201/2005; RD 53/2013).

2.2. Spontaneous motor activity

Spontaneous motor activity of the animals was evaluated in open field arenas (MED Associates Inc., St. Albans, USA). The open field apparatus consisted of a Plexiglas box (80 x 80 x 45 cm) equipped with two horizontal rows of eight infrared light sensitive photocell beams located at 5 and 15 cm, respectively, from the basement, allowing the detection of horizontal and vertical (rearing) motor activity. Interruptions of the photocell beams (activity counts) were registered automatically by computer software connected to the open field apparatus (MED Associates Inc., St. Albans, USA). Open field arena was wiped with 70 % alcohol between rats [20].

2.3. Microinjections into the infralimbic cortex

Three-five days after being tested in the open field, animals were anesthetized with equithesin (2.5 mg/kg, i.p.) and stereotaxically implanted in the brain with bilateral guide-cannulae to reach the infralimbic region of the ventromedial PFC using the following coordinates from bregma: + 2.5 mm rostral, +0.7 mm medial and -3.5 mm from the top of the skull and with the incisive bar set at -3 mm [27]. Guide cannulae, 23-gauge stainless-steel (PlasticsOne, Roanoke, VA, USA) were fixed to the skull surface with dental acrylic and three stainless-steel anchorage screws (Agntho´s,

Stockholm, Sweden). Dummy cannulae, 28-gauge stainless-steel, were inserted into the guide to keep it clean and prevent occlusion. Six-seven days after surgery bilateral injections into the infralimbic cortex were performed by means of injection cannulae, 28-gauge stainless-steel, protruding 1 mm below the tip of the guide and attached to a micropump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 0.25 µl per minute. A total volume of 0.25 µl per side was injected maintaining the injection cannulae in place for 60 seconds to allow the diffusion of the drug or vehicle. Slow flow rate and low volume injections try to avoid diffusion of the drug to neighbour brain regions. The animals received the microinjections in their home cages 60 min before collecting blood samples (see below) to minimize the effects of the injection procedure on corticosterone plasma levels. In addition, a sham injection was performed the day before experiments in order to habituate animals to the injection procedure.

The $GABA_A$ receptor antagonist picrotoxin 0.20 µg per side (Sigma-Aldrich, Spain) and the agonist muscimol 0.25 µg per side (Tocris Bioscience, UK) were freshly dissolved in artificial cerebrospinal fluid consisting of (in millimolar): NaCl 137, $CaCl_2$ 1.2, KCl 3, $MgSo_4$ 1, NaH_2PO_4 0.5, Na2HPO4 2, glucose 3, pH= 7.3. The artificial cerebrospinal fluid was also used as vehicle. Drugs and doses were selected based on previous studies from our laboratory which performed microinjections of these compounds into the prelimbic PFC [8]. Microinjections of picrotoxin and muscimol are well-established methods to stimulate and inhibit, respectively, transiently the activity of specific brain areas [6].

2.4. Inhibitory avoidance task

Six-seven days after the surgical implantation of guide cannulas, animals were evaluated in the inhibitory avoidance apparatus consisting of a shuttle-box divided into 2 compartments separated by a guillotine door [8, 28]. The starting compartment (light compartment, 50 x 50 x 20 cm) was made of white opaque plastic; it had an open roof and was well lit by 1 overhead 60W bulb. The shock compartment (dark compartment, 25 x 25 x 20 cm) was made of black plastic; it had a closed (removable) roof, no illumination and an electrified grid floor. The inhibitory avoidance test was carried out as follows (Figure 1). On the training day, animals were placed in the light compartment and allowed to explore the whole apparatus (guillotine door open) over a period of 300 seconds (habituation). Five hours later, animals received an infralimbic microinjection

of vehicle, muscimol or picrotoxin in their home cages. One hour after the injection each rat was re-exposed to the apparatus and latency to enter in the dark compartment was measured (training latency). When animals stepped their four paws on the dark compartment, the door was lowered and a foot-shock (0.6 mA, 2 s) was delivered. After 10 seconds animals were removed from the dark compartment and returned to their home cages. On the testing day (24 hours after the foot-shock), rats were re-exposed to the light compartment and retention of the inhibitory avoidance response was recorded as the retention latency, up to a maximum of 600 seconds to enter the dark compartment. Animals that reached 600 seconds were removed from the light compartment by the experimenter.

2.5. Blood sampling and corticosterone assays

Blood samples (100 µl) were taken by tail-nick [29]. Animals were gently restrained and a small incision with a blade was made 1-2 mm above the tip of the tail. Blood samples were collected in heparinized vials in less than 2 minutes during the inhibitory avoidance performance. The experimental protocol was as follows (Figure 1): One hour after infralimbic microinjections, animals were re-exposed to the inhibitory avoidance apparatus and received a foot-shock; immediately after the foot shock, rats were taken to their home cages and the first blood sample (basal corticosterone) was collected; 30 min later, the second blood sample (stress-induced corticosterone) was collected. Vials containing blood samples were centrifuged for 10 minutes at 3,000 r.p.m. to obtain plasma samples. Plasma samples were storage at -80 ºC until the measure of corticosterone levels.

Total corticosterone levels in plasma were measured using a radioimmunoassay kit (MP Biomedicals Inc., Solon, OH, USA) following the instructions provided by the manufacturer. The inter- and intra-assay coefficient of variance was 6.5 % and 4.4 % respectively.

2.6. Histology

All animals were anesthetized with an overdose of anaesthesia and perfused intracardially with 0.9 % saline and 0.2 % heparin followed by 10% formalin. Bilateral injections into the infralimbic cortex of methylene blue were performed just before intracardial perfusions to better visualize the location of the injection cannulae. The

brain was removed and the placement of the injection cannulae was verified in sections cut with a cryostat microtome and viewing lens (Figure 2). Animals with incorrect placement of injection cannulae were not included in this study.

2.7. Statistical analysis

The spontaneous motor activity of control and EE rats was evaluated by a two-way analysis of the variance (ANOVA) (Group x Time) with repeated measures followed by planed comparisons. Three- and two-way ANOVAs with repeated measures followed by planed comparisons were use to evaluate the effects of muscimol and picrotoxin on basal and stress-induced corticosterone levels. A three-way ANOVA (Group x Treatment x Time) was performed to compared control and EE rats. Added to that, two-way ANOVAs (Treatment x Time) were performed to analyze the effects of treatments (Treatment) and stress (Time) in control and EE rats independently. Retention latencies 24 hours after training in the inhibitory avoidance performance were evaluated by non-parametric tests. A Kruskal-Wallis test was performed to compare the effects of vehicle, muscimol and picrotoxin in control and EE rats. A Mann-withney *U* test was performed to further discriminate drug effects.

3. RESULTS

3.1. Spontaneous motor activity in the open field.

Figure 3 shows the spontaneous motor activity during 60 min in the open field of both control and EE rats. The Two-way ANOVA (Group x Time) analysis showed a significant effect of Group ($F_{(1,93)}$= 48.70; p< 0.001), Time ($F_{(11,1023)}$= 147.60; p< 0.001) and Group x Time ($F_{(11,1023)}$= 12.55; p< 0.001) indicating that EE reduced total locomotion. Planned comparisons also showed that locomotion during the first 5 minutes was higher in EE rats compared to control rats ($F_{(1,93)}$= 10.51; p= 0.002) [30].

3.2. Effects of muscimol and picrotoxin microinjections into the infralimbic cortex on basal and stress-induced concentrations of corticosterone.

Figure 4 shows the effects of infralimbic injections (60 min before acute stress) of the $GABA_A$ agonist and antagonist muscimol and picrotoxin, respectively, on basal (A) and stress-induced (B) plasma levels of corticosterone in both control and EE rats. A three-way ANOVA analysis (Group x Treatment x Time) was performed to evaluate differences between control and EE rats (Group) on the effects of muscimol and picrotoxin (Treatment) as well as foot shock stress (Time) on corticosterone concentrations. The three-way ANOVA showed a significant effect of Group ($F_{(1,58)}$= 9.23; p= 0.004) and Treatment ($F_{(1,58)}$= 15.87; p< 0.001), but no Time ($F_{(1,58)}$= 0.13; n.s.). More specifically, planned comparisons showed that picrotoxin injections produced a higher increase of basal ($F_{(1,58)}$= 13.29; p< 0.001) (Fig. 4A) and stress-induced ($F_{(1,58)}$= 13.29; p< 0.001) (Fig. 4B) concentrations of corticosterone in EE compared to control rats.

Two-way ANOVAs (Treatment x Time) were also performed to evaluate the effects of muscimol and picrotoxin (Treatment) as well as foot shock stress (Time) on corticosterone concentrations in control and in EE rats independently. In control rats, a Two-way ANOVA analysis showed a significant effect of Treatment ($F_{(1,27)}$= 3.42; p= 0.047), but not Time ($F_{(1,27)}$= 0.06; n.s.), and Treatment x Time ($F_{(1,27)}$= 6.93; p= 0.004). More specifically, planned comparison analysis showed that picrotoxin injections increased basal concentrations of corticosterone ($F_{(1,27)}$= 6.56; p= 0.016) (Fig. 4A). This analysis also showed that foot shock stress (0.6 mA, 2 s) increased corticosterone concentrations in vehicle injected animals ($F_{(1,27)}$= 6,73; p= 0.015) and that muscimol

injections reduced the stress-induced concentrations of corticosterone compared to vehicle ($F_{(1,27)}$= 7.38; p= 0.011) (Fig. 4B). Picrotoxin did not significantly change the stress-induced concentrations of corticosterone compared to vehicle ($F_{(1,27)}$= 3.87; n.s.).

In EE rats, the Two-way ANOVA analysis showed a significant effect of Treatment ($F_{(1,31)}$= 14.46; p< 0.001), but not Time ($F_{(1,31)}$= 0.08; n.s.) or Treatment x Time ($F_{(1,31)}$= 1.97; n.s.). More specifically, planned comparison analysis showed that picrotoxin injections increased basal concentrations of corticosterone ($F_{(1,31)}$= 17.19; p< 0.001) compared to vehicle (Fig. 4A). This analysis also showed that foot shock stress (0.6 mA, 2 s) increased corticosterone concentrations in vehicle injected animals (Fig. 4B) although it did not reach statistical significance ($F_{(1,31)}$=1.36; p= 0.25). Picrotoxin did not significantly change the stress-induced concentrations of corticosterone compared to vehicle ($F_{(1,31)}$= 4.12; n.s.).

3.3. Effects of muscimol and picrotoxin microinjections into the infralimbic cortex on the inhibitory avoidance performance.

Figure 5 shows the effects of infralimbic injections (60 min before training) of the $GABA_A$ agonist and antagonist muscimol and picrotoxin, respectively, on retention latencies during the test 24 h after training in the inhibitory avoidance task in both control and EE rats. The training latencies, not represented in Figure 5, were: vehicle (Control= 12.75 ± 2.35 s; EE= 15.82 ± 5.12 s); Muscimol (Control= 10.50 ± 1.43 s; EE= 10.46 ± 2.10 s); Picrotoxin (Control= 11.10 ± 1.89 s; EE= 19.10 ± 7.12 s).

According to the non-parametric analysis Kruskall-Wallis, training latencies were not changed by treatment [control (χ^2= 1.71; df= 2; n.s.); EE rats (χ^2= 3.37; df= 2; n.s.)] or housing conditions (χ^2= 0.12; df= 1; n.s.). In contrast, this same analysis showed a significant effect of treatment in EE (χ^2= 10.65; *df*= 2; p= 0.049), but not control (χ^2= 5.43; *df*= 2; p= 0.066) rats, on Test retention latencies 24 h after training. More specifically, a Mann-Whitey analysis confirmed that muscimol injections into the infralimbic cortex reduced the Test retention latency in EE rats compared to vehicle (Z= 2.22; p= 0.034) (Fig. 5). The effects of muscimol were not statistically different between control and EE rats (Z= -0.27; n.s.).

4. DISCUSSION

The present study shows that the stimulation and/or inhibition of infralimbic cortex modulates basal and stress-induced plasma levels of corticosterone as well as the formation of aversive memory. Specifically, the stimulation of infralimbic cortex by local injections of picrotoxin increased basal concentrations of corticosterone. This effect was enhanced by housing rats in an enriched environment which suggests that EE increases the control exerted by infralimbic cortex over the HPA axis. The inhibition of infralimbic cortex by local injections of muscimol reduced significantly the increases of corticosterone produced by acute stress and the memory acquisition of an aversive event (foot shock) in control and EE animals, respectively. These results suggest that infralimbic cortex is required for the activation of the HPA axis during acute stress and the acquisition of contextual aversive memories.

Despite the evidence showing that the PFC regulates the activity of the HPA axis [2, 10], previous studies had reported conflicting results regarding the role of the medial PFC on basal plasma levels of corticosterone [5, 6] probably because they did not distinguish well the prelimbic and infralimbic parts of the medial PFC. As shown, the stimulation of infralimbic cortex by local injections of picrotoxin increased the basal concentrations of corticosterone. Since picrotoxin injections increase the activity of cortical neurons [6], these results suggest that infralimbic cortex exerts a positive regulation over the HPA axis through the activation of cortical efferent projections. In contrast to picrotoxin, the inhibition of infralimbic cortex by muscimol injections did not change basal corticosterone concentrations which indicate that the control over the HPA axis by the infralimbic cortex is not tonic.

Housing animals in an enriched environment enhanced the effects of picrotoxin into the infralimbic cortex to increase basal plasma levels of corticosterone. These results suggest that EE increases the capability of infralimbic cortical outputs to activate the HPA axis and release corticosterone. An increase of cortical excitability could account for the enhanced activation of the infralimbic cortex found in enriched animals. In fact, previous studies have suggested that an increased cortical excitability facilitates whereas a decreased cortical excitability reduces, cortical activation involved in fear memory [31-33].

Studies from our laboratory and others report that enriched animals show a lower reactivity to stress [18, 19, 21, 22, 25, 26]. Based on the present study, we hypothesize that an enhanced capability of the infralimbic cortex to control the HPA axis contributes to the resilience of enriched animals to stress. In support of this hypothesis, it has been shown that EE increases the resilience of animals to social defeat stress by enhancing infralimbic cortical outputs to downstream limbic areas [9]. Also, in line with this possibility, it has been shown that an increased excitability in the PFC is associated with enhanced control over stress [33] and extinction learning [31]. Confirming our previous work [20, 23, 30], we also show in this study that EE reduces motor activity in the open field suggesting a faster habituation of enriched animals to novel environments (mild stress).

The inhibition of infralimbic cortex by muscimol injections reduced the increases of plasma levels of corticosterone produced by acute stress (foot shock) in control animals. This is in agreement with previous studies which show that infralimbic lesions attenuate stress-induced plasma corticosterone [3] and suggest that the infralimbic cortex facilitates the HPA axis activation and release of corticosterone produced by acute stressful stimuli. Interestingly, previous studies from our laboratory using a similar experimental protocol have shown that the stimulation of prelimbic cortex reduces stress-induced plasma levels of corticosterone [8]. These results are in agreement with the postulated idea that prelimbic and infralimbic cortices play opposite roles in the regulation of the HPA axis in response to stress [2, 3]. In fact, an indirect pathway from the PFC to the paraventricular nucleus of the hypothalamus has been proposed to regulate the HPA axis and the release of corticosterone [3, 34]. It is also shown in this study that injections of picrotoxin into the infralimbic cortex seem to increase and decrease the stress-induced corticosterone concentrations in EE and control animals, respectively. However, given the strong effect produced by picrotoxin increasing basal (pre-stress) concentrations of corticosterone, it is difficult to draw any conclusion regarding the role of infralimbic stimulation during stress. A similar controversy has been reported elsewhere [6].

Muscimol injections reduced the retention latency 24 h after training in the inhibitory avoidance test in control and EE animals (although it did not reach statistical

significance in control animals) which indicates that the acquisition of aversive memories is facilitated by the infralimbic cortex. These results together with the fact that the stimulation of prelimbic cortex inhibits the acquisition of aversive memory [8] support the idea of an opposite role of prelimbic and infralimbic cortices in emotional processing [35]. Our results are in agreement with studies in which infralimbic lesions reduced the retention latency using the same behavioural paradigm [13]. Interestingly, as shown here, muscimol injections into the infralimbic cortex also reduced stress-induced corticosterone concentrations as well as retention latency. These results suggest that the infralimbic cortex could contribute to the formation of aversive memory, in part, by increasing corticosterone concentrations. The involvement of corticosterone increases, immediately after training, enhancing the formation of aversive memory has been previously reported [36, 37].

Interestingly, despite increasing corticosterone concentrations, injections of picrotoxin into the infralimbic cortex did not increase significantly the retention latency in the inhibitory avoidance test in control or EE animals. One possibility to explain this apparent contradiction is the timing of corticosterone increases. As shown, picrotoxin increased corticosterone concentrations few minutes (probably 15-30 min) before the training in the inhibitory avoidance task. This delay matters since studies have shown that increases of corticosterone before memory training do not enhance memory acquisition or even impair memory [38]. It is also interesting at this respect that other factors such as noradrenergic or amygdala activity are required to occur simultaneously to corticosterone increases to enhance memory acquisition during the inhibitory avoidance task [36].

EE did not significantly modify the stress-induced corticosterone concentrations in vehicle and muscimol treated animals. Likewise, the acquisition of aversive memory was not significantly changed by EE in none of the treatment groups studied. These results indicate that the contribution of the infralimbic cortex to modulate the HPA axis during acute stress and the acquisition of emotional memory is not substantially changed by EE.

Studies in humans and animals suggest that a reduced activity of infralimbic cortex (ventral medial PFC in humans) is associated with stress mal adaptation and stress-

related disorders (i.e. posttraumatic stress disorder) [11, 12]. In this study we show that the infralimbic cortex regulates basal and stress-induced concentrations of corticosterone as well as the acquisition of aversive memories. These results further suggest that a dysfunctional ventromedial PFC might contribute to stress-related disorders through impairing HPA axis regulation and emotional processing. Moreover, EE enhances the control that the infralimbic cortex exerts on the HPA axis. A stronger interaction between the infralimbic cortex and the HPA axis as well as other limbic areas might facilitate the extinction of fear-related memories [12, 31, 39] and protect enriched animals to stress-related disorders [9].

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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FIGURE LEGENDS

Figure 1. Schematic representation of the experimental design used in the present study for the behavioural experiments. After evaluating spontaneous motor activity in the open field (60 min), animals were implanted with guide cannulas for intracerebral injections and 6-7 days after surgery they were tested in the inhibitory avoidance apparatus. The foot shock (0.6 mA, 2 s) given during the training test was used to evaluate the stress-induced plasma levels of corticosterone in control and EE rats.

Figure 2. Schematic representation showing the approximate location of microinjections into the infralimbic cortex of control (left) and EE rats (right) (adapted from Paxinos and Watson, 1998).

Figure 3. Temporal profile of the spontaneous motor activity showed by control and EE rats exposed for the first time to the open field apparatus. Data (mean ± SEM) represent absolutes values of distance traveled (cm). The number of animals is shown in *parenthesis*. Insert shows the total distance traveled for both control (white bar) and EE (black bar) rats. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control rats.

Figure 4. Effects of infralimbic microinjections of muscimol and picrotoxin (60 min before foot shock stress; see Figure 1) on basal (A) and stress-induced (B) plasma concentrations of corticosterone in control and EE rats. Data (mean ± SEM) are shown in ng/ml. Number of animals is shown in *parenthesis*. $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ compared to vehicle group; $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control group; $^{\blacklozenge}p < 0.05$ compared to basal corticosterone.

Fig. 5 Effects of infralimbic microinjections of muscimol and picrotoxin (60 min before training test; see Figure 1) on Test retention latencies 24 h after training in control and EE rats. Data (mean ± SEM) represent retention latencies in seconds (s). The number of control (vehicle= 8; muscimol= 12; picrotoxin= 10) and EE rats (vehicle= 11; muscimol= 13; picrotoxin= 10) are shown in parenthesis. $^{\#}p < 0.05$ compared to vehicle EE group.

Figure 1

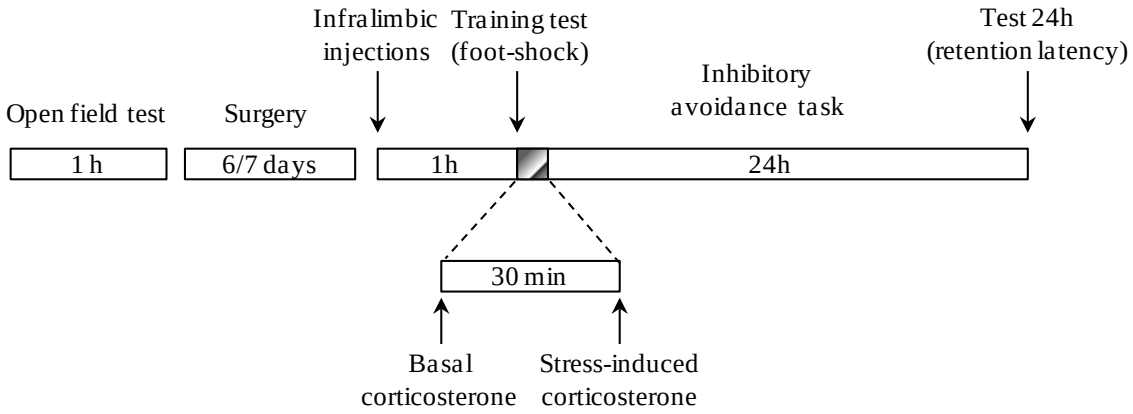

Infralimbic injections
Training test (foot-shock)
Test 24h (retention latency)
Inhibitory avoidance task
Open field test
Surgery
1 h
6/7 days
1h
24h
30 min
Basal corticosterone
Stress-induced corticosterone

Figure 2

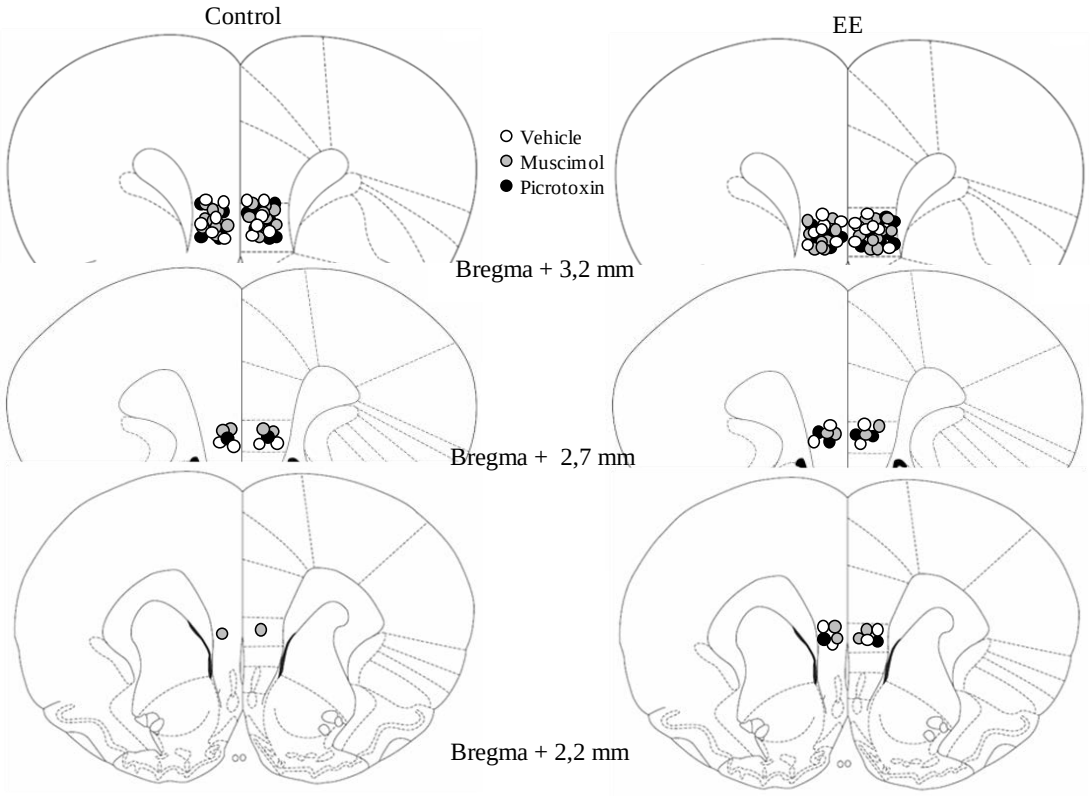

Control
EE
Vehicle
Muscimol
Picrotoxin
Bregma + 3,2 mm
Bregma + 2,7 mm
Bregma + 2,2 mm

Figure 3

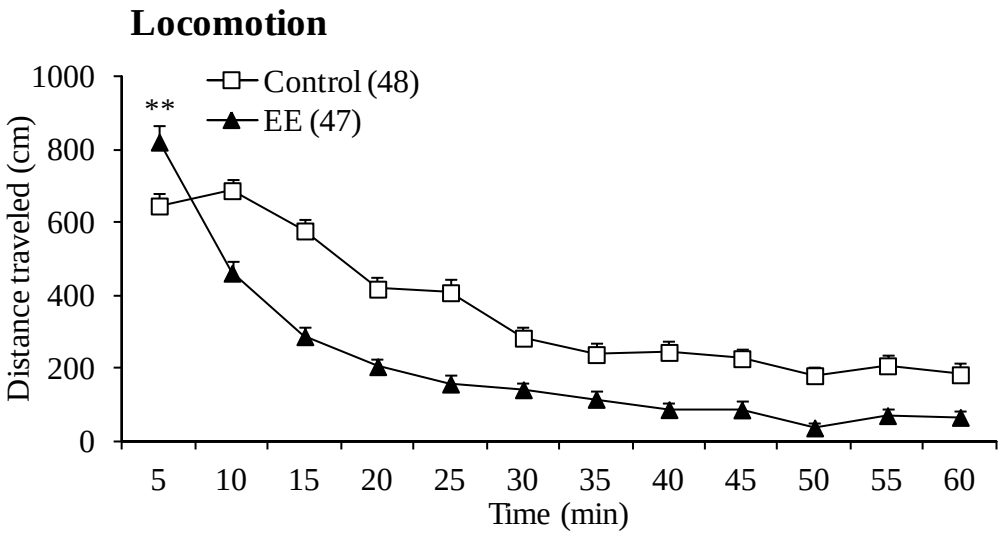

Locomotion
Control (48)
EE (47)
**
Distance traveled (cm)
1000
800
600
400
200
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (min)

Figure 4

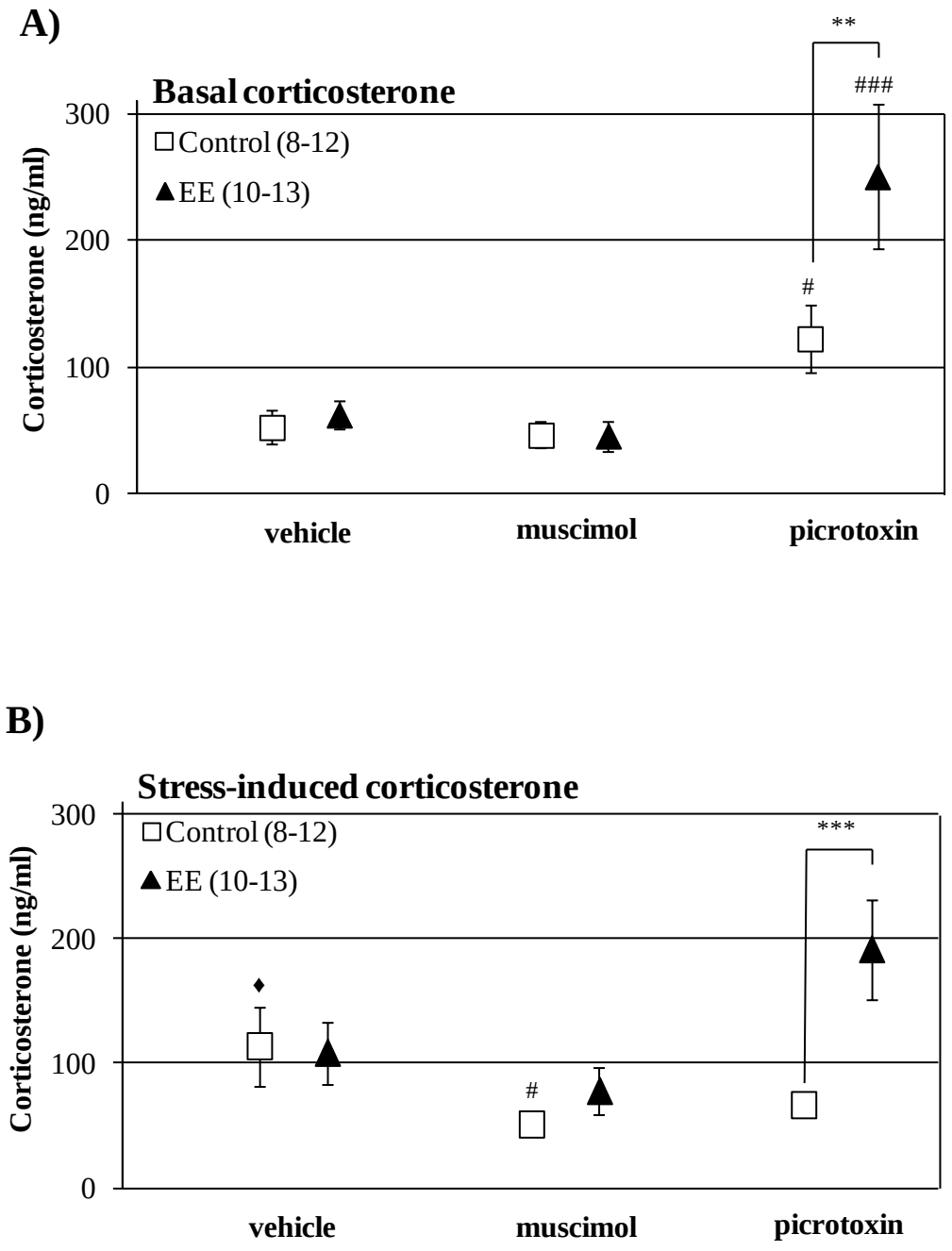
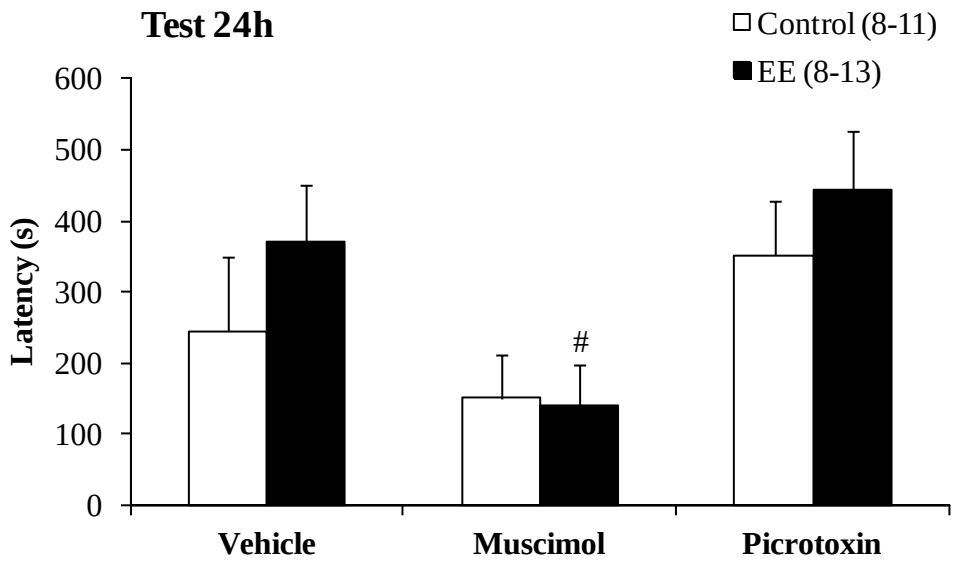

A)
Basal corticosterone
Control (8-12)
EE (10-13)
Corticosterone (ng/ml)
300
200
100
0
**
###
#
vehicle
muscimol
picrotoxin
B)
Stress-induced corticosterone
Control (8-12)
EE (10-13)
Corticosterone (ng/ml)
300
200
100
0
***
♦
#
vehicle
muscimol
picrotoxin

Figure 5


Test 24h
Control (8-11)
EE (8-13)
Latency (s)
600
500
400
300
200
100
0
#
Vehicle
Muscimol
Picrotoxin