

# Effects of social hierarchy on innate fear-induced panic responses

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Studies have previously demonstrated a relationship between social status and anxiety disorders such as panic disorder. Repeated episodes of panic attacks do not occur in combination with an actual fear stimulus or stressor. However, social ranking modulates the perception of the social signals of a threat or stressor. The hypothalamic nuclei are well-known for their role in the elaboration of fear-induced reactions. The dorsomedial hypothalamus (DMH) and the ventromedial hypothalamic (VMH) nuclei are hypothalamic subnuclei involved in the processing of threatening stimuli-evoked aversive response and innate fear development. These structures are also located in the medial amygdala-hypothalamus-brainstem circuit that modulates innate fear-induced defensive behaviors. This work aimed to investigate the relationship between social hierarchy and innate fear-induced panic-like responses in male rats. In our study, the dominance tube test was used to determine the social hierarchy. Then, DMH/VMH nuclei were unilaterally implanted with a guide cannula. After intra-DMH/VMH injection of bicuculline (GABA<sub>A</sub> receptor antagonist), both innate fear induction and differences in dominant/subordinate rats were evaluated by the open field test. Intra-DMH/VMH bicuculline increased the frequency of defensive immobility, forward escape movements, and crossing behaviors, as well as the duration of defensive immobility and forward escape movements in dominant rats. Subordinate rats showed a higher frequency of defensive attention, defensive immobility, and crossing than dominant rats. Additionally, dominant rats demonstrated a lower duration of defensive attention and defensive immobility than subordinate rats. Dominant rats seemed to adopt a form of innate-fear characterized by increased proactivity with the environment. In contrast, subordinate rats exhibited a reactive form of innate-fear characterized by passivity and freezing.

**Key words:** social hierarchy, innate fear-induced panic responses, ventromedial hypothalamus, dorsomedial hypothalamus

## INTRODUCTION

Social hierarchy plays a pivotal role in characterizing inter-individual relationships across a wide range of social animals, from insects to primates (Cheney and Seyfarth, 1990; Zhou et al., 2018). Within social dominance relationships, individuals gain asymmetric access to limited resources, such as reproductive partners, territories, and food. Thus, social hierarchy is beneficial to social groups in terms of reproduction, well-being, and health (Bercovitch and Clarke, 1995;

Sapolsky, 2005). However, social hierarchy has been associated with a greater risk of mental disorders (Cheng et al., 2015; Hudson, 2005). Previous studies have shown a relationship between social status and anxiety disorders (Mwinyi et al., 2017). Panic disorder is an intense anxiety disorder diagnosed by unexpected and repeated episodes of panic attacks, accompanied by several physiological symptoms including increased heart rate, blood pressure, and respiration rate. These episodes do not occur in combination with an actual fear stimulus or stressor. However, social ranking modulates the per-

ception of social signals of threat as either reliable or not (Morozov and Ito, 2018).

Several reports describe a vital role for the dorsal column of periaqueductal grey matter (dPAG), the corpora quadrigemina, the amygdala, the anterior hypothalamus, the dorsal pre-mammillary hypothalamic nucleus, and the ventromedial hypothalamic nucleus (VMH) nucleus in panic-induced violence (Canteras, 2002; Osaki et al., 2003; Castellan-Baldan et al., 2006; Coimbra et al., 2006; da Silva et al., 2013). The dorsomedial hypothalamus (DMH) and the VMH are hypothalamic subnuclei involved in the processing of threatening stimuli-evoked aversive response and innate fear development (Biagioni et al., 2013; de Freitas et al., 2014). Furthermore, the compact and reciprocal connections between the VMH and the amygdala and dPAG confirm the central role of the VMH in the amygdala-medial hypothalamus-brainstem pathway. This circuit crucially modulates and organizes innate defensive behaviors (Canteras, 2002). It has been suggested that fear-induced emotional responses, such as panic attack-like behavior, are partially controlled by both the DMH and VMH in laboratory animals (Freitas et al., 2009; Biagioni et al., 2013). Wilent and colleagues (2010) have reported that both the DMH and VMH are also involved in emotional responses in humans.

It has been well documented that gamma-aminobutyric acid (GABA) has a tonic inhibitory effect on the DMH neurons that elicit elaborated defensive behaviors (Shekhar and DiMicco, 1993). Correspondingly, the activation of GABA<sub>A</sub> receptors in the DMH has a pivotal role in the inhibition of panic-like responses. Furthermore, both transient GABA<sub>A</sub> receptor blockade and prolonged reduction of GABA function in the DMH lead to the development elaborated defensive behavioral responses and panic-like disorder in an experimental model of panic attack (Shekhar et al., 1996). Additionally, persistent dysfunction of GABAergic inhibition in the DMH elicits a panic-prone state recognized by increased anxiety, heart rate, blood pressure, and respiration rate (Shekhar et al., 1996; Shekhar and Keim, 1997). Moreover, both the panic-like responses and the elaborated escape behavior of laboratory animals are involve the DMH and VMH. These responses can be considered innate fear physiological reactions followed by a critical innate fear-induced antinociception (Freitas et al., 2009).

As mentioned earlier, blockade of GABA<sub>A</sub> receptors in the VMH/DMH nuclei can be used to induce innate fear in laboratory animals. Thus, the present study was designed to study the relationship between social status and innate fear-induced panic-like responses in the intrinsic neuronal networks of the VMH/DMH nuclei of male rats.

## METHODS

### Animals

Male Wistar rats (n=3 or 4 per colony), weighing 160–200 g, were obtained from the animal facility of the faculty of psychology at the University of Tabriz. Prior to the hierarchy-induction experiments, animals were left undisturbed for at least one week after arrival. All subjects were housed in opaque plastic cages on a 12 h light/12 h dark cycle (lights on at 8:00 a.m.) at a constant temperature of 23–25°C with food and water available ad libitum. The floors of the cages were covered with sawdust that was changed every two days.

### Dominance tube test

In this research, dominant and subordinate rats were first categorized by using the dominance tube test adapted from Wang et al. (2011). Before any surgical procedures, rats were placed in the open field arena to assess their basal innate fear state (pre-test). Afterward, cannulas were implanted and fixed into the VMH/DMH nuclei by stereotaxic surgery. After a recovery period and drug injection, rats were placed in the open field arena again to evaluate the innate fear-induced panic-like responses (post-test).

Since dominant animals win more frequently in competitions, the tube test was developed to mimic this situation in laboratory rodents (Hand, 1986; Lindzey, 1961). To score social dominance in the male rats, a clear Plexiglas custom-made tube (inner diameter, 2.6 cm; length, 30 cm) was used for the dominance tube test. Small acrylic boxes (10 cm × 10 cm × 10 cm) were added to each end of the tube to facilitate entry into the tube. Then, rats were trained to walk through the narrow tube four times from each end of the tube (eight trials per day) for 2 sequential days. A nonviolent conflict situation was created when two rodents with similar body weights entered the tube from opposite ends and met in the middle (testing phase was five days, two trials per day). The subject that consistently forced its opponent to retreat was scored as the most dominant of the pair. Before each trial, the tube was cleaned with 75% ethanol.

### Surgery and microinjection

Animals were anesthetized with 92 mg/kg ketamine and 9.2 mg/kg xylazine and fixed in a stereotaxic frame (Stoelting, USA). A stainless steel guide

cannula (23-gauge, outer and inner diameter 0.6 and 0.4 mm, respectively) was implanted in the diencephalon, targeting the DMH and VMH nuclei. The upper incisor bar was set at 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. Stereotaxic coordinates for the guide cannula implantation into the DMH/VMH nuclei were selected from the rat brain atlas of Paxinos and Watson (1997): anteroposterior, -2.76 mm; mediolateral 0.4 mm; and dorsoventral, 9.3 mm. The guide cannula was fixed into the skull using acrylic resin and two stainless steel screws. At the end of the surgery, each guide cannula was sealed with stainless steel wire to protect it from obstruction. Subsequently, the rats were allowed to recover from the surgical procedure for 5–7 days.

On the day of the experiment, the rats were gently wrapped in a cloth and handheld during injection of the GABA<sub>A</sub> receptor antagonist bicuculline (40 ng/0.3 µl, Sigma Chemicals, St. Louis, MO, USA) into the DMH/VMH. To this end, bicuculline was microinjected into the DMH/VMH nuclei by a 30-gauge injection cannula. The injection cannula was attached by a polyethylene tube to a 1 µl Hamilton syringe. Intra-DMH/VMH infusions were carried out in each side over a period of 60 s, followed by an additional 30 s to facilitate drug diffusion from the tip of the injection cannula.

## Open field

During the pre-test experiment, rats were first placed in the open field arena (50 × 50 × 40 cm), with the floor divided into 25 sections, to record their exploratory behaviors and defensive reactions before any surgical procedures. After the recovery period, to assess the innate fear-induced panic-like responses (the post-test experiment), a dose of 40 ng/0.3 µl bicuculline was administered into the DMH/VMH nuclei of rats. Immediately after the drug microinjection, rats were placed in the open field test arena to record the exploratory behaviors and behavioral defensive reactions over 10 min using a camera. The exploratory behavior was measured as the number of crossings (four paws in a given division of the open field floor) as well as frequency and duration of rearing (upright posture) behavior. The behavioral defensive reactions were expressed as frequency and duration of the backward escape attempts (rapid defensive backwards movement), frequency and duration of elaborated forwards escape behavior (running intercalated with exploratory behavior), and frequency and duration of defensive attention, as well as frequency and dura-

tion of defensive immobility (Freitas, 2009). Defensive attention is considered to be a measure of alertness, defined as the interruption of ongoing behavior and alteration of the rats' orientation toward the stimulus accompanied by the evoking of attentive posture with small head movements, rearing, and sniffing of the surrounding air. Defensive immobility or "freezing" is defined as perfect immobility accompanied by at least two of the following autonomic reactions: urination, defecation, piloerection, or exophthalmos (Freitas, 2009).

## Experimental protocols

At first, rats were trained to walk through the narrow tube four times from each end of the tube (eight trials per day) for 2 consecutive days. After training and prior to VMH/DMH nuclei cannulation, the open field test was performed to investigate the basal innate fear state of the animals over a 10 min period (pre-test). After a recovery period following VMH/DMH nuclei cannulation, bicuculline was bilaterally applied into the nuclei. Immediately after the drug microinjection, the innate fear-induced panic-like responses of rats were rechecked in the open field arena (post-test). The experiments were performed between 8:00 a.m. and 2:00 p.m. The doses of drugs were chosen based on both pilot studies and previous investigations (Freitas, 2013).

## Experiment 1

This experiment aimed to evaluate innate fear induction in dominant and subordinate rats. To address this purpose, the basal panic-like responses of dominant and subordinate rats (pre-test) were compared, within-subject, with the panic-like behaviors after innate fear induction by bicuculline treatment (post-test). For this experiment, experimental groups were included in the pre-test and post-test of both dominant and subordinate groups.

## Experiment 2

This experiment examined the basal innate fear state of the dominant and subordinate rats (pre-test). To determine the relationship, the basal panic-like responses of dominant rats were compared with subordinate rats (pre-test). For this experiment, experimental groups were included in the pre-test of both dominant and subordinate groups.

### Experiment 3

This experiment was designed to assess panic-like behavioral responses of both dominant and subordinate rats after innate fear induction by bicuculline treatment (post-test). To determine differences, innate fear-induced panic-like responses of dominant rats were compared with subordinate rat responses (post-test). For this experiment, experimental groups were included in the post-test of both dominant and subordinate groups.

### Histology

Upon completion of the experiments, the brains were removed and coronally cut by a vibroslicer (Axio Imager Z1, Zeiss) in 50  $\mu$ m sections to verify the microinjection placement. The neuroanatomical locations of cannula tips were verified by the Paxinos and Watson rat brain atlas (1997). From a total of 30 animals, there was a single incorrect microinjection placement in one animal, and both microinjection placements were wrong in one other animal. Because of the low number of missed microinjection placements (one or both sides), these rats could not serve as a negative control group. Only the animals with correct cannula placements in DMH/VMH regions were included in the data analysis.

### Statistical analysis

To explore innate fear induction in dominant and subordinate rats, data from the pre-test and post-test experiment of each group were subjected to a paired t-test. Furthermore, an unpaired t-test with Welch's correction was used to determine the role of social dominance state in the innate fear-induced panic-like responses of dominant and subordinate rats. All values are reported as mean  $\pm$  standard error of mean (SEM) at a statistical significance of  $P < 0.05$ .

## RESULTS

### Innate fear induction in dominant and subordinate rats

Intra-DMH/VMH infusion of the GABA<sub>A</sub> receptor antagonist (bicuculline, 40 ng/0.3  $\mu$ l) in the dominant rats significantly increased behavioral defensive reactions and exploratory behaviors of animals in the open field arena. The frequency [paired t (4)=6.462;

$P < 0.01$ ; Fig. 1A] and duration [paired t (4)=4.793;  $P < 0.01$ ; Fig. 1B] of defensive attention was increased by intra-DMH/VMH injection of bicuculline. Similarly, intra-DMH/VMH bicuculline increased the duration of defensive backward movement [paired t (4)=5.571;  $P < 0.01$ ; Fig. 1F], as well as forward escape behavior [paired t (4)=4.793;  $P < 0.01$ ; Fig. 1H]. Furthermore, the frequency of crossing was significantly enhanced by bicuculline administration into the DMH/VMH nuclei of dominant rats [paired t (7)=4.032;  $P < 0.01$ ; Fig. 2]. However, intra-DMH/VMH application of bicuculline had no significant effect on the frequency and duration of defensive immobility and rearing behaviors in the open field test. Likewise, non-significant changes were observed in the duration of defensive backward movement, as well as forward escape behavior, of dominant rats.

Microinjection of bicuculline into the DMH/VMH nuclei of the subordinate rats significantly increased the frequency of crossing in the open field arena [paired t (7)=3.874;  $P < 0.01$ ; Fig. 4A]. Nevertheless, intra-DMH/VMH infusion of bicuculline had no significant effect on the frequency and duration of defensive attention, defensive immobility, defensive backward movement, forward escape behavior, and rearing behaviors in the open field test (Fig. 3).

### Comparison of basal innate fear state in dominant and subordinate rats (pre-test)

In the open field arena before DMH/VMH nuclei cannulation, subordinate rats demonstrated more defensive behavior reactions and exploratory behaviors than dominant rats. The frequency [Welch corrected t (4.156)=3.602;  $P < 0.05$ ; Fig. 5A] and duration [Welch corrected t (7.312)=3.211;  $P < 0.05$ ; Fig. 5B] of defensive attention in subordinate rats were significantly higher than in dominant rats. Similarly, subordinate rats showed a higher frequency [Welch corrected t (5.969)=5.021;  $P < 0.01$ ; Fig. 5C] and duration [Welch corrected t (4.038)=3.554;  $P < 0.05$ ; Fig. 5D] for defensive immobility than dominant rats. Non-significant differences were observed between frequency (n=5, Fig. 5E) and duration (n=5, Fig. 5F) of defensive backward movement, as well as frequency (n=5, Fig. 5G) and duration (n=5, Fig. 5H) of forward escape behavior of dominant and subordinate rats. The greater defensive responses of the subordinate rats were accompanied by augmented exploratory behavior characterized by a higher crossing frequency [Welch corrected t (2.462)=13.21;  $P < 0.05$ ; Fig. 6A] in the open field test arena. However, there were no significant differences between frequency (n=5, Fig. 6B) and duration (n=5,

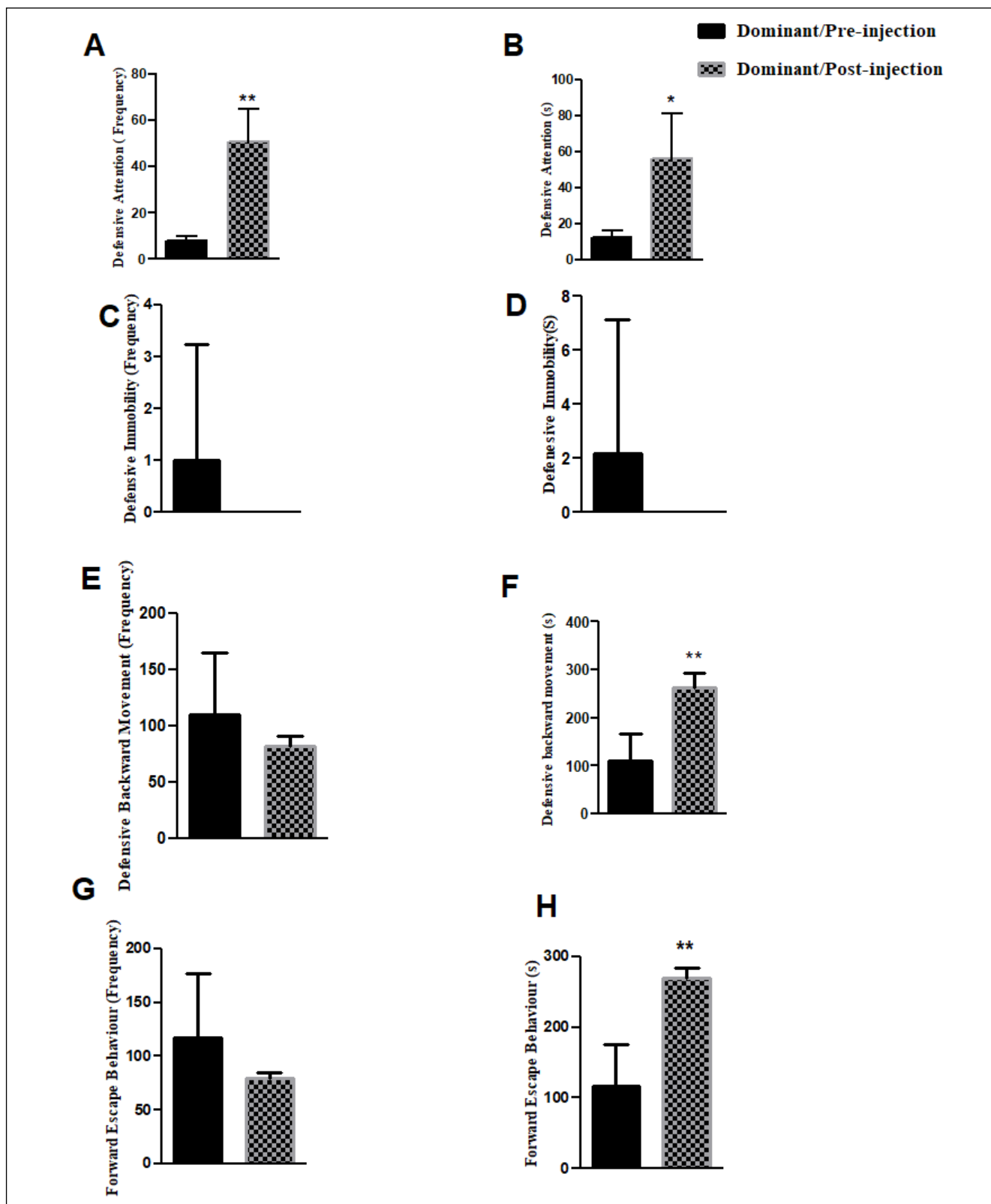


Fig. 1. Intra-DMH/VMH injection of the GABA<sub>A</sub> receptor antagonist (bicuculline, 40 ng/0.3 μl) in the dominant rats (n=5) increased the defensive behavior reactions of rats in the open field arena. Defensive behavior reactions included the frequency and duration of defensive attention (A and B), defensive immobility (C and D), defensive backward movement (E and F), and forward escape behavior (G and H). The columns and bars represent the mean and the SEM, respectively. \*P<0.05 according to a paired t-test.

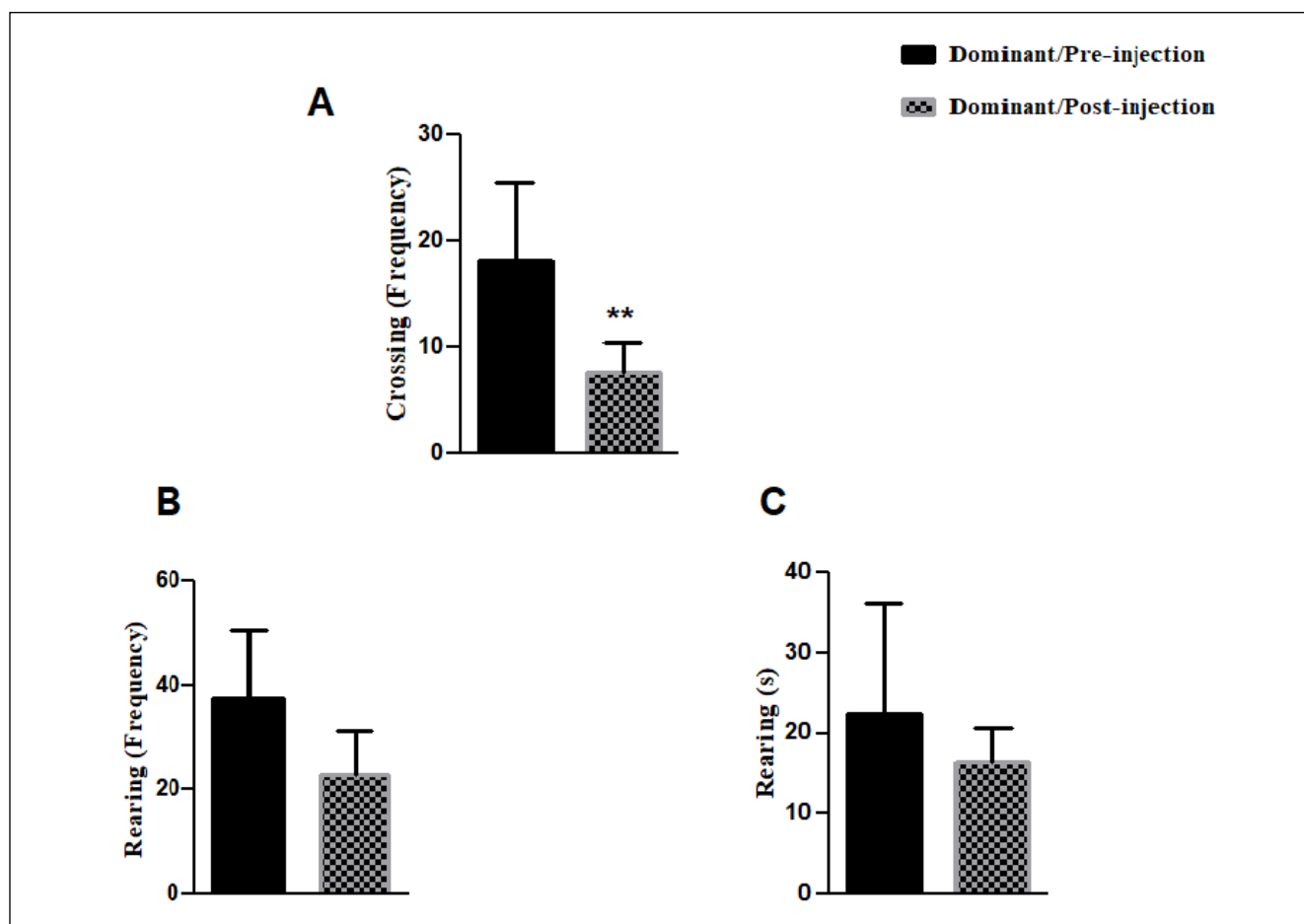


Fig. 2. Injection of bicuculline (40 ng/0.3  $\mu$ l) into the DMH/VMH nuclei of the dominant rats increased the exploratory behaviors of rats in the open field arena. Exploratory behaviors included the frequency of crossings [n=8; (A)] and the frequency and duration of rearing [n=5; B, and C] during elaborated escape behavior. The columns and bars represent the mean and the SEM, respectively. \* $P < 0.05$  according to a paired t-test.

Fig. 6C) of rearing behavior in the dominant and subordinate rats.

### Comparison of innate fear-induced panic-like responses in dominant and subordinate rats (post-test)

The transient reduction of GABAergic neurotransmission in both the DMH and VMH nuclei was followed by defensive behaviors characterized by defensive immobility and more elaborated forwards escape behaviors in both dominant and subordinate rats, but the subordinate rats showed augmented behavioral defensive reactions and exploratory behaviors.

The transitory blockade of GABA<sub>A</sub> receptors in both the DMH and VMH nuclei by bicuculline microinjection evoked a higher frequency [Welch corrected  $t$  (4.000)=11.31;  $P < 0.001$ ; Fig. 7C] and duration [Welch cor-

rected  $t$  (4.000)=6.514;  $P < 0.01$ ; Fig. 7D] of defensive immobility in the subordinate rats than in the dominant rats. Moreover, the bicuculline-induced enhancement in frequency [Welch corrected  $t$  (4.119)=2.842;  $P < 0.05$ ; Fig. 7G] and duration [Welch corrected  $t$  (4.697)=4.788;  $P < 0.01$ ; Fig. 7H] of forward escape behavior for subordinate rats was significantly greater than in dominant rats. Further, a greater number of exploratory behaviors were observed in subordinate rats during hypothalamic GABA<sub>A</sub> receptor blockade. Likewise, a higher crossing frequency [Welch corrected  $t$  (10.40)=3.377;  $P < 0.01$ ; Fig. 8A] was observed after bicuculline treatment of the DMH and VMH nuclei for the subordinate rats in the open field test arena. In contrast, microinjections of bicuculline into the DMH and VMH nuclei did not lead to significant differences between the frequency (n=5, Fig. 8A) and duration (n=5, Fig. 8B) of defensive attention, defensive backwards movement, and rearing behavior for the dominant or subordinate rats.

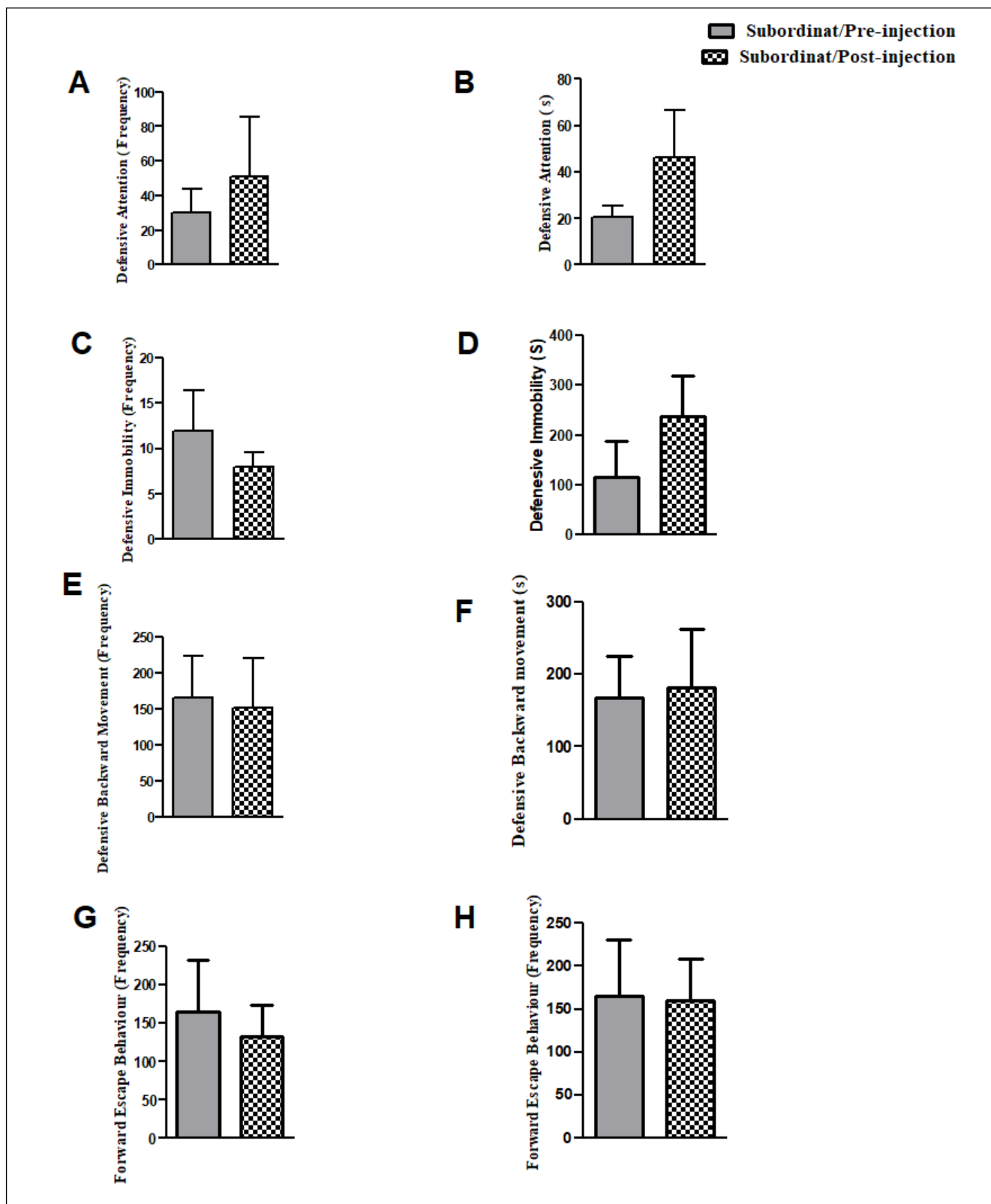


Fig. 3. Intra-DMH/VMH injection of bicuculline, 40 ng/0.3  $\mu$ l in the subordinate rats ( $n=5$ ) increased the defensive behavior reactions of rats in the open field arena. The defensive behavior reactions included the frequency and duration of defensive attention (A and B), defensive immobility (C and D), defensive backward movement (E and F), and forward escape behavior (G and H). The columns and bars represent the mean and the SEM, respectively. \* $P<0.05$  according to a paired t-test.

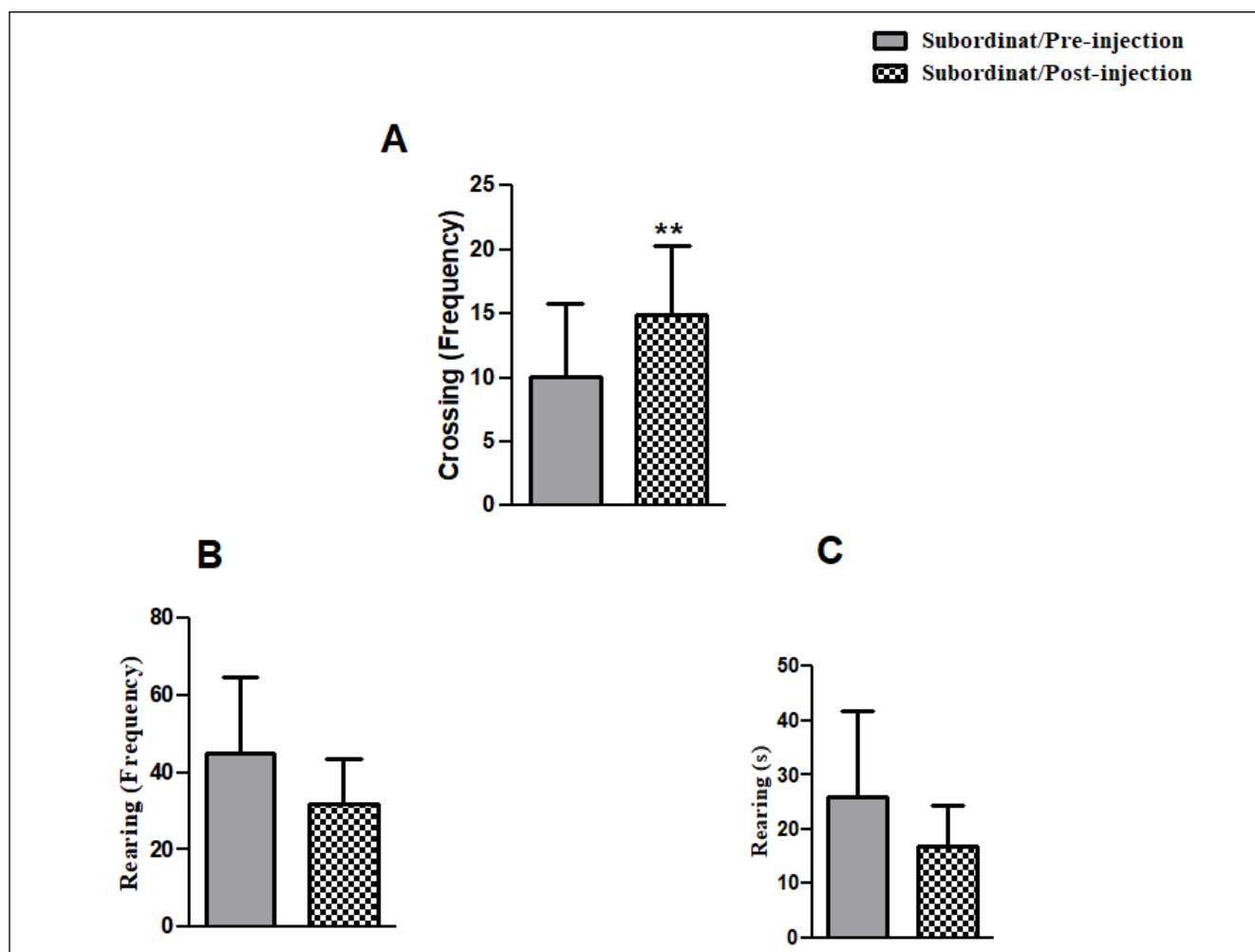


Fig. 4. Injection of bicuculline (40 ng/0.3  $\mu$ l) into the DMH/VMH nuclei of subordinate rats increased the exploratory behaviors of rats in the open field arena. Exploratory behaviors included the frequency of crossings [(n=8); (A)] and frequency and duration of rearing [(n=5); (B, and C)] during elaborated escape behavior. The columns and bars represent the mean and the SEM, respectively. \* $P < 0.05$  according to a paired t-test.

## DISCUSSION

The results of the present study indicate that subordinate rats showed more defensive and exploratory behaviors than dominant rats in the pre-test and post-tests experiments. The defensive behavioral reactions, evoked by transient blockade of GABAergic inhibition in the DMH/VMH, were characterized by defensive attention, defensive immobility, risk assessment, defensive backward movement and forward escape behavior. Similarly, the bicuculline-induced exploratory behaviors are demonstrated by crossings and rearing. Consistent with our results, Morrison and colleagues (2011) have suggested that subordinate Syrian hamsters show more defensive and submissive responses than dominants in the conditioned defeat test. Our findings are also in line with substantial evidence that

subordinate individuals have higher plasma corticosterone levels (a stress indicator in rats) than dominants (Haller et al., 1996; Akers et al., 2008; Michopoulos et al., 2012; Haller, 2014; Williamson et al., 2017; Sherman and Mehta, 2020). Given the relationship between social hierarchy and stress levels in rodents, it is reasonable to expect that this social factor may also be related to innate fear-induced panic-like responses, including behavioral defensive reactions and exploratory responses in dominant and subordinate rats.

The present findings show a robust difference between subordinate and dominant rats in defensive immobility or “freezing”, a response to potential or distal aversive stimuli. Consistent with our results, de Freitas and colleagues (2013) reported that microinjection of bicuculline into the DMH and VMH nuclei augments the frequency and duration of defensive attention,



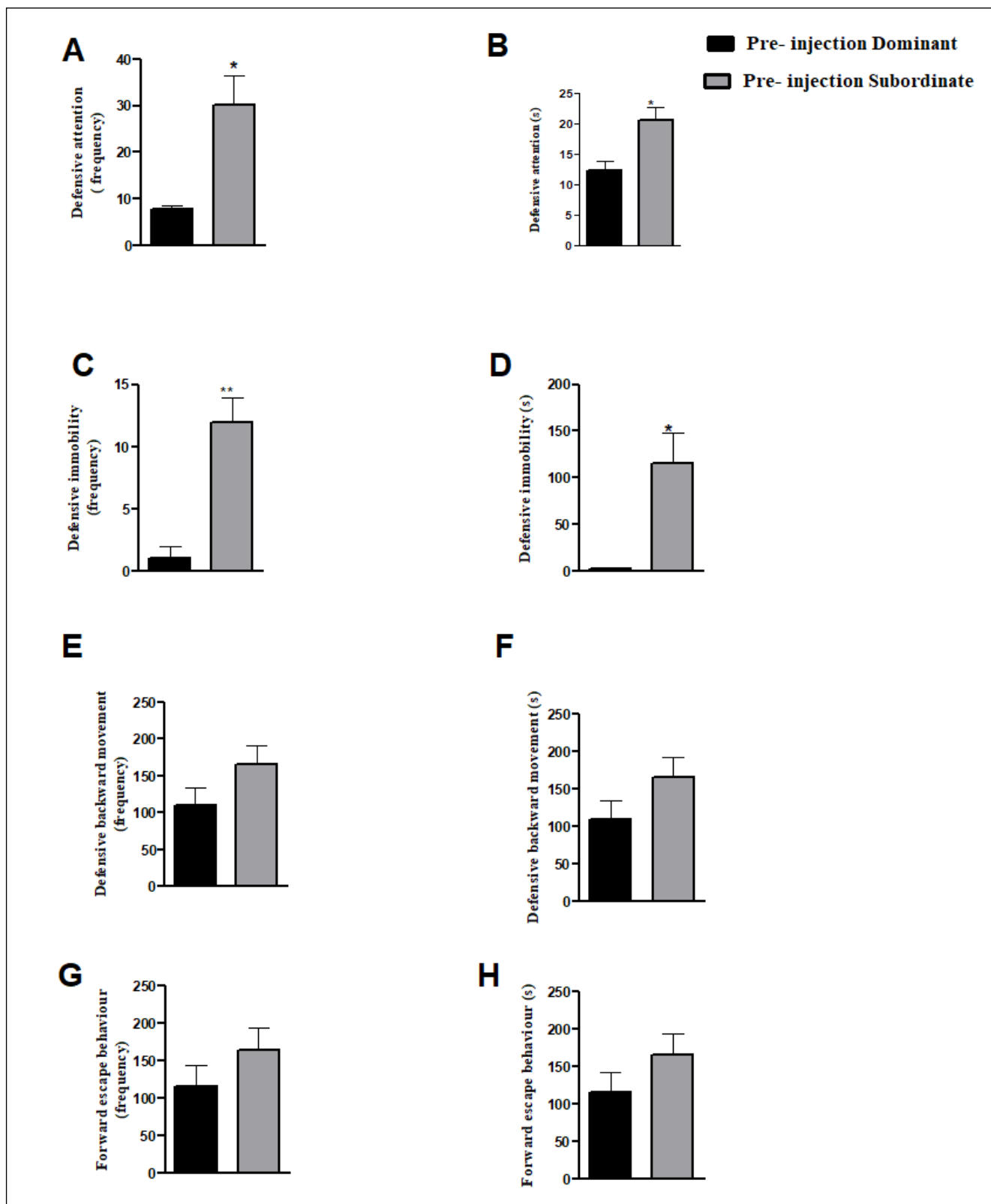


Fig. 5. Comparison of the defensive behavior reactions of the dominant and subordinate rats [pre-test;  $n=5$ ] in the open field arena. The frequency and duration of defensive attention (A and B) and defensive immobility (C and D) in subordinate rats were higher than in dominant rats. There were no significant differences in defensive backward movement (E and F) and forward escape behavior (G and H) between dominant and subordinate rats. The columns and bars represent the mean and the SEM, respectively. \* $P < 0.05$  according to an unpaired t-test with Welch's correction.

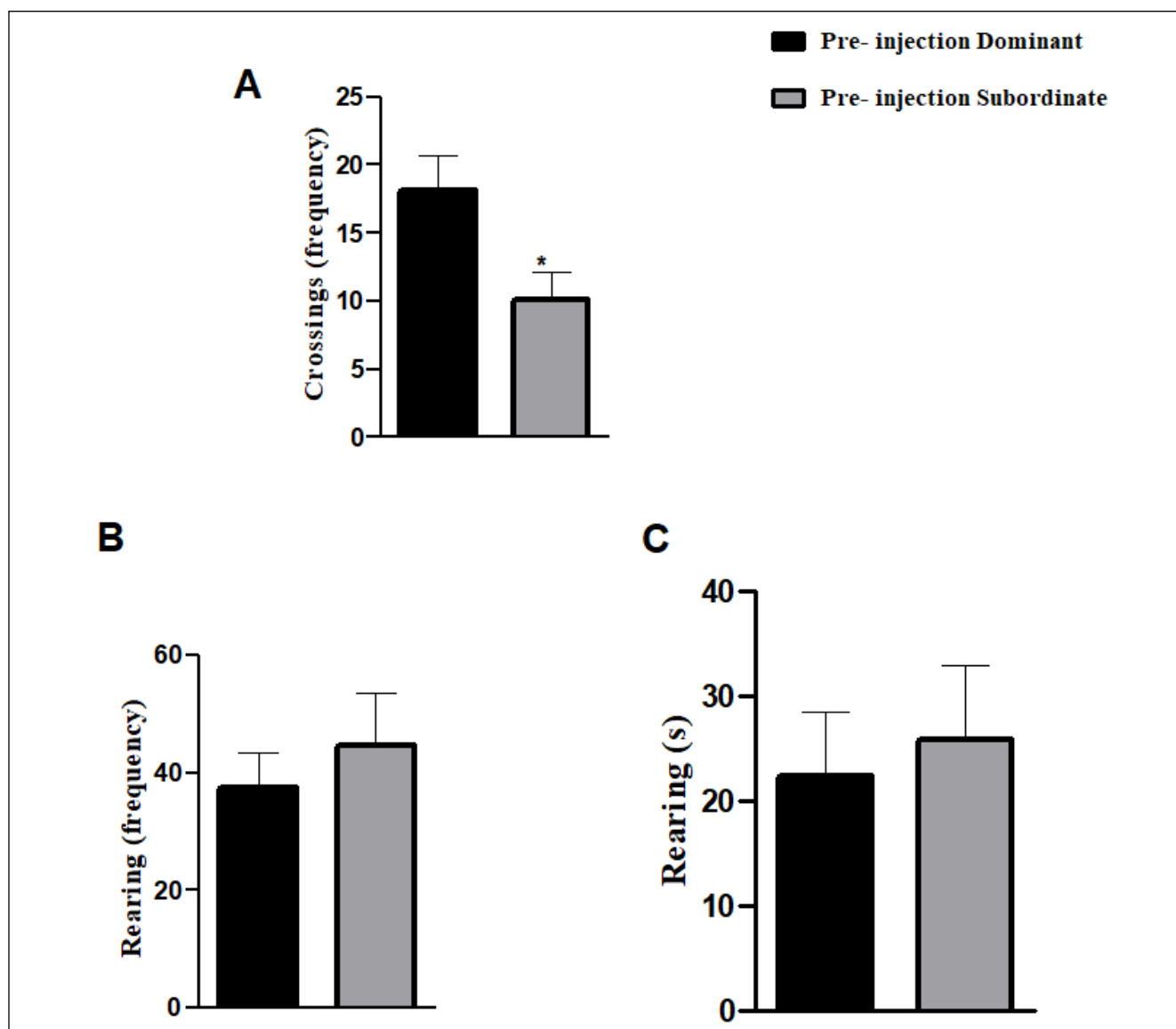


Fig. 6. Comparison of the exploratory behaviors of the dominant and subordinate rats [pre-test;  $n=5$ ] in the open field arena. The frequency of [ $n=8$ ; (A)] crossing of subordinate rats was greater than for dominant rats. Nonetheless, there were no significant differences in frequency and duration of rearing [ $n=5$ ; B, and C] between dominant and subordinate rats. The columns and bars represent the mean and the SEM, respectively. \* $P<0.05$  according to an unpaired t-test with Welch's correction.

defensive immobility, defensive backward movement, and forward escape behavior. It has been reported that both hypothalamic and mesencephalic regions are involved in the elaboration of this defensive reaction (Ferreira-Netto et al., 2007). The activation of DMH and VMH evoked less intense and more oriented defensive reactions than mesencephalic structures (Brandao et al., 1986; Freitas et al., 2009). Interestingly, research using immunohistochemical techniques have shown that an encounter between rodents and natural predators enhances the Fos protein level in the medial hypothalamic defensive system. This defensive system is

composed of the dorsal pre-mammillary hypothalamic nuclei, anterior hypothalamic nucleus, and dorsomedial division of the VMH (Canteras et al., 1997; Canteras, 2002; Martinez et al., 2008; Paschoalin-Maurin et al., 2018). Additionally, it has been reported that the medial hypothalamic defensive system is connected to hypothalamic nuclei, such as the dorsomedial rostral perifornical region, the posterior hypothalamic nuclei, and the DMH (Canteras, 2002). These data demonstrate that the medial hypothalamus plays a key role in controlling defensive behavior. The reciprocal connection between the DMH/VMH and cortex, as well as mesen-

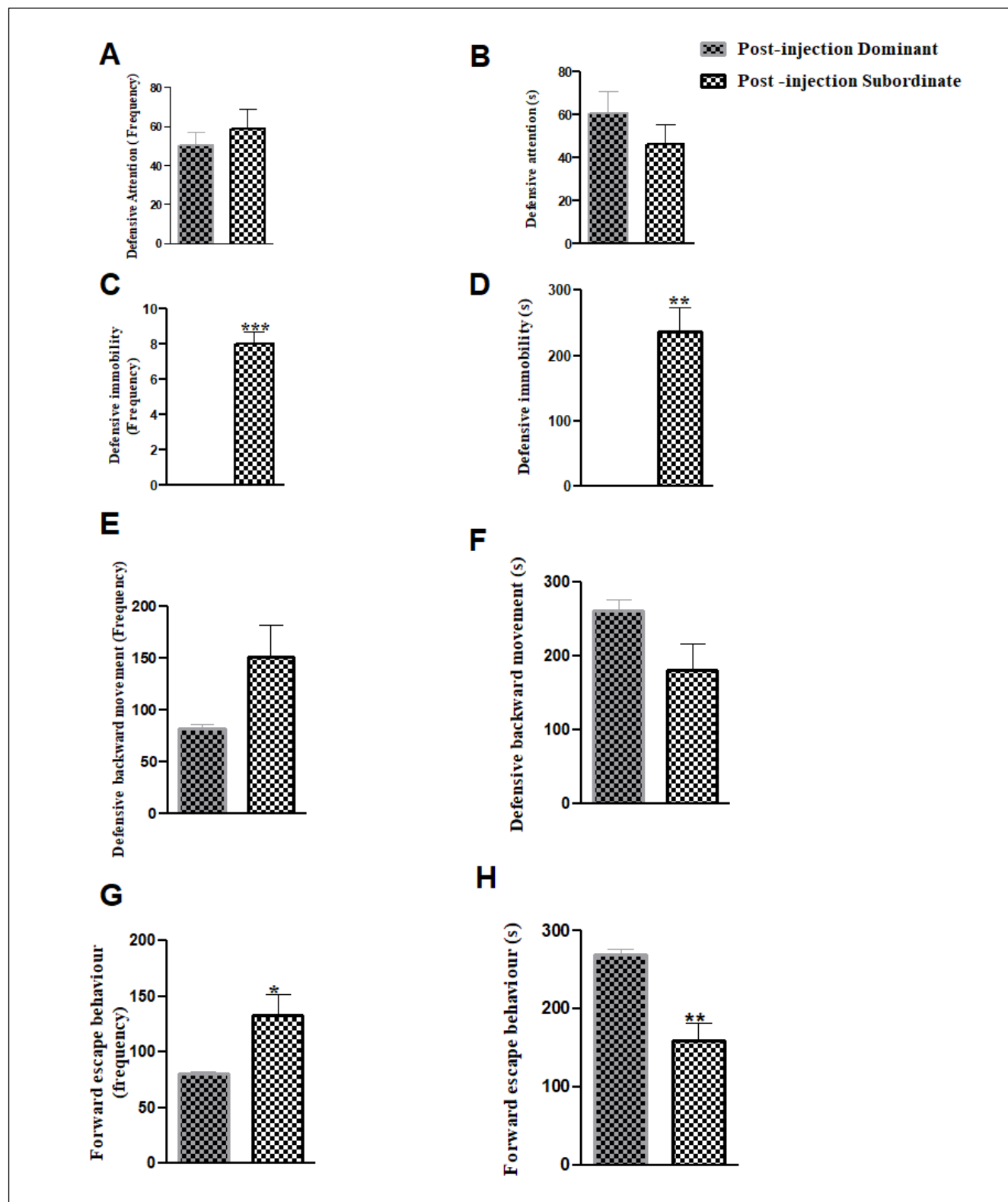


Fig. 7. Comparison of the innate fear-induced defensive behavior reactions of the dominant and subordinate rats [post-test;  $n=5$ ] in the open field arena. Intra-DMH/VMH injection of bicuculline (40 ng/0.3  $\mu$ l) evoked more defensive immobility [C and D;  $n=5$ ] and frequency of forward escape behavior [G;  $n=5$ ] in subordinate rats than dominant rats. Yet, there were no significant differences in defensive attention (A and B) and defensive backward movement (E and F) between dominant and subordinate rats. The columns and bars represent the mean and the SEM, respectively. \* $P < 0.05$  according to an unpaired t-test with Welch's correction.

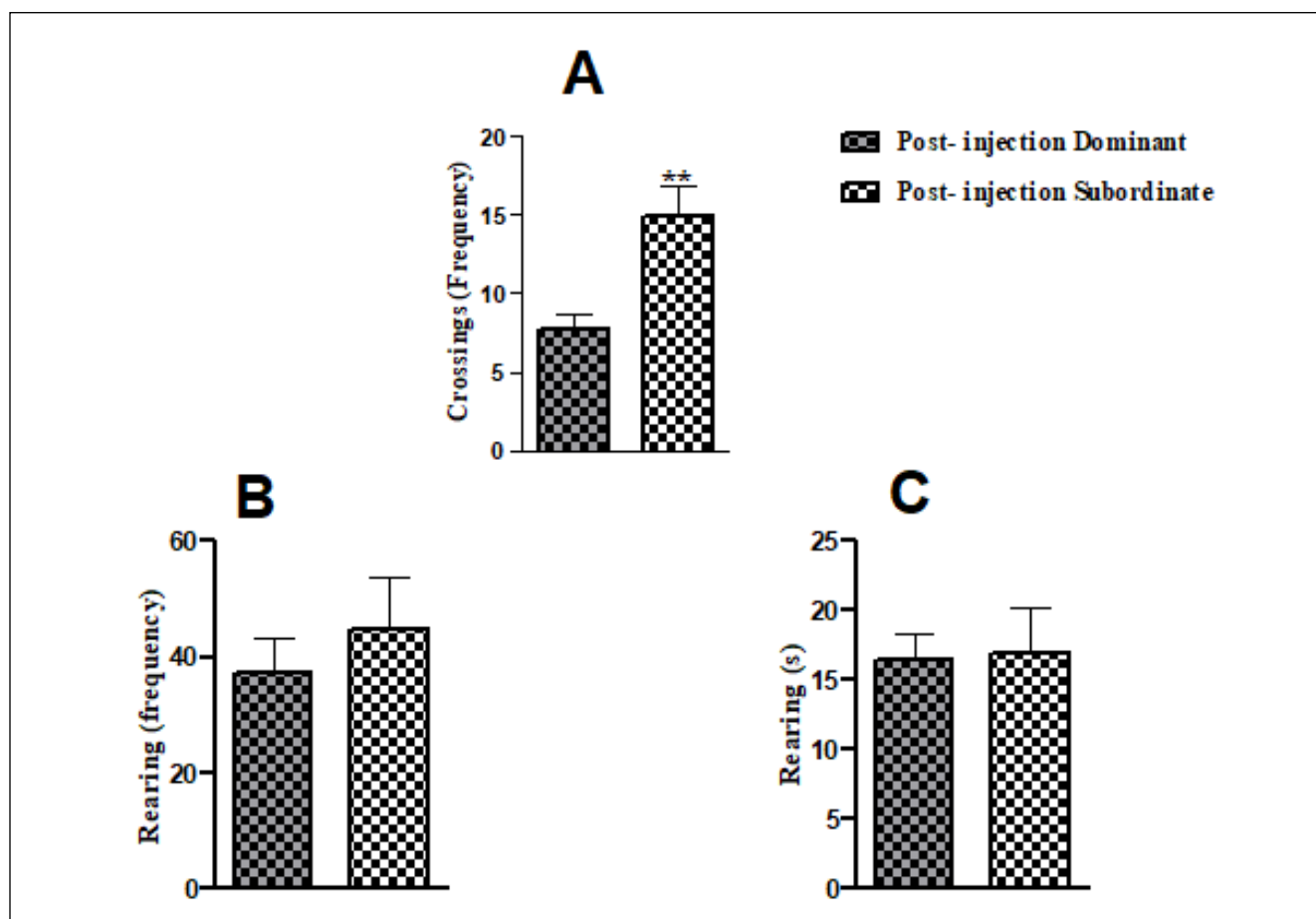


Fig. 8. Comparison of the innate fear-induced the exploratory behaviors of the dominant and subordinate rats [post-test;  $n=5$ ] in the open field arena. Injection of bicuculline (40 ng/0.3  $\mu$ l) into the DMH/VMH nuclei of the dominant and subordinate rats elicited increased crossing [ $n=8$ ; (A)] in the subordinate rats compared to the dominant rats. However, there were no significant differences in rearing (B and C) behavior between dominant and subordinate rats. The columns and bars represent the mean and the SEM, respectively. \* $P<0.05$  according to an unpaired t-test with Welch's correction.

cephalic structures, could possibly exert panicogenic influences. These connections may be associated with the control of fear-induced defensive and exploratory behaviors elicited by GABA<sub>A</sub> receptor blockage in the DMH/VMH (de Freitas et al., 2013). It has been reported that the medial hypothalamus sends its outputs to a mesencephalic structure, the periaqueductal grey matter (PAG), which is involved in organizing various freezing responses (Vianna et al., 2001; Brandão et al., 2008). However, some researchers have suggested that the PAG might be the main structure controlling defensive reactions because electrolytic lesion of PAG could entirely abolish the defensive responses elicited by the stimulation of either the medial or posterior nucleus of the hypothalamus. On the other hand, lesion of the diencephalic structure did not impair the defensive reactions elicited by PAG (Hunsperger, 1956). Despite these findings, it has been broadly suggested that, as an integrated neural network, the PAG and the hypothala-

mus control innate fear-induced behavioral responses (Blanchard and Blanchard, 1988; Canteras et al., 1997; Canteras, 2002). The PAG includes the dorsolateral and dorsomedial columns and the lateral column that separates them from the ventrolateral column (vPAG) (Bandler et al., 1991). The PAG has various functions, such as regulating cardiovascular function, nociception, and vocalizations, while the dorsal and ventral columns appear to be organized for oppositional defensive reactions: escape and freezing, respectively (Bandler and Depaulis, 1991). Based on the aforementioned studies, variations in these mesencephalic and diencephalic structures may underlie the differences observed between dominant and subordinate rats in freezing and forward escape responses.

As demonstrated in the present work, the difference between dominant and subordinate rats in freezing response increased even more after receptor blockade in the DMH and VMH. A plausible explana-

tion for our findings is that transitory dysfunctions of GABAergic inputs to DMH and VMH nuclei evoke flight behavior in laboratory animals (Schmitt et al., 1986; Freitas et al., 2009; Biagioni et al., 2012, 2013; de Freitas et al., 2014). Moreover, following the GABAergic dysfunction of the PAG's dorsolateral columns, Fos protein-positive neurons in ventrolateral PAG during freezing, as well as greater Fos-labeled neurons in the DMH and VMH, were observed after escape behavior induction (Borelli et al., 2005). Therefore, it seems that at least part of the medial hypothalamus GABA<sub>A</sub> receptor blockade-evoked freezing response in subordinate rats recruits the ventrolateral columns of the PAG.

Compared to our pre-test findings, the post-test results revealed that crossing, a defensiveness-based exploration, increased in subordinates. Dysfunction of DMH and VMH GABAergic neurons might explain this exploratory behavior (Duva et al., 2002), which was followed by an enhancement in escape and freezing responses in subordinate rats compared to dominants.

In conclusion, the present study adds to current knowledge of the relationship between social hierarchy and innate fear-induced panic-like responses, demonstrating a negative association between social status and some defensive and exploratory behaviors. It is important to note that cellular investigations are needed in order to analyze and further interpret the role of differential cell expression in dominant and subordinate rats in fear-induced panic-like behaviors.

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