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NEW INSIGHTS ON AMYGDALA: BASOMEDIAL AMYGDALA REGULATES THE PHYSIOLOGICAL RESPONSE TO SOCIAL NOVELTY

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Abstract—The amygdala has been associated with a variety of functions linked to physiological, behavioral and endocrine responses during emotional situations. This brain region is comprised of multiple sub-nuclei. These sub-nuclei belong to the same structure, but may be involved in different functions, thereby making the study of each sub-nuclei important. Yet, the involvement of the basomedial amygdala (BMA) in the regulation of emotional states has yet to be defined. Therefore, the aim of our study was to investigate the regulatory role of the BMA on the responses evoked during a social novelty model and whether the regulatory role depended on an interaction with the dorsomedial hypothalamus (DMH). Our results showed that the chemical inhibition of the BMA by the microinjection of muscimol (γ-aminobutyric acid ($GABA_A$) agonist) promoted increases in mean arterial pressure (MAP) and heart rate (HR), whereas the chemical inhibition of regions near the BMA did not induce such cardiovascular changes. In contrast, the BMA chemical activation by the bilateral microinjection of *bicuculline methiodide* (BMI; $GABA_A$ antagonist), blocked the increases in MAP and HR observed when an intruder rat was suddenly introduced into the cage of a resident rat, and confined to the small cage for 15 min. Additionally, the increase in HR and MAP induced by BMA inhibition were eliminated by DMH chemical inhibition. Thus, our data reveal that the BMA is under continuous GABAergic influence, and that its hyperactivation can reduce the physiological response induced by a social novelty condition, possibly by inhibiting DMH neurons.

Key words: social novelty basomedial amygdala, dorsomedial hypothalamus, intruder rat.

INTRODUCTION

Specific brain nuclei are recruited during emotional and environmental stress, leading to several autonomic, endocrine and behavioral responses (Blanchard and Blanchard, 1989a,b; DiMicco et al., 2006; Szczepanska-Sadowska, 2008) that improve chances of survival. However, heightened stress promotes the continuous activation of neuronal pathways for compensatory adaptation, which compromises the organism's physiological integrity and the pattern of the aforementioned responses (Szczepanska-Sadowska, 2008; Chrousos, 2009). The neuronal circuitry involved in the stress response is not yet completely understood, specifically, the regions that can inhibit the stress response.

The amygdaloid complex and the dorsomedial hypothalamus are recognized today as important nuclei involved in the regulation of cardiovascular and behavioral responses evoked by emotional stress (Petrovich et al., 1996; Sah et al., 2003). However, the basomedial amygdala's (BMA) role in emotional regulation has been mostly neglected, even though there is evidence that this region could be important in influencing several emotional states. For instance, studies have shown that there is an increase in BMA neuronal activity in rats submitted to the inhibitory avoidance test (Silveira et al., 2001; de Andrade et al., 2012), suggesting that this region could be involved in controlling the animal's anxiety state. Moreover, matched lesions in the BMA and basolateral amygdala (BLA) reduced the conditioned fear responses, suggesting that the BMA could be involved in the neural pathway of fear control (Anglada-Figueroa and Quirk, 2005).

The BMA sends excitatory projections to the central amygdala (CeA) and the bed nucleus of stria terminalis (BNST) (Petrovich et al., 1996), both of which control the inhibitory tonus to the dorsomedial hypothalamus (DMH), an integratory region of the cardiovascular responses evoked by stressful situations (DiMicco et al., 1996; de Menezes et al., 2009; Fontes et al., 2011; Abreu et al., 2014). These observations support

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Abbreviations: BLA, basolateral amygdala; BMA, basomedial amygdala; BMI, *bicuculline methiodide*; BNST, bed nucleus of stria terminalis; CeA, central amygdala; DMH, dorsomedial hyphothalamus; $GABA_A$, γ-aminobutyric acid; HR, heart rate; MAP, mean arterial pressure; mPOA, medial preoptic area.

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a possible role of the BMA in the brain circuitry activated by stress.

To our knowledge, only one study has attempted to investigate the BMA's role in cardiovascular control. The study showed that while the injection of *bicuculline methiodide* (BMI; γ-aminobutyric acid ($GABA_A$) receptor antagonist) in the BMA of anesthetized rats increased arterial pressure and heart rate, the injection of muscimol ($GABA_A$ receptor agonist) did not alter those parameters (Yoshida et al., 2002). However, because anesthesia itself is known to affect cardiovascular parameters (Shimokawa et al., 1998), it remains unclear whether or not the BMA has a role in the brain related control of cardiovascular responsiveness in conscious animals.

In this study, we investigated the regulatory role of the BMA in the cardiovascular response evoked by social novelty and whether that regulatory role was influenced by an interaction with the DMH. To that end, we examined the influence of acute inhibition of BMA neurons by injecting muscimol on basal cardiovascular parameters. We have also evaluated the effect of BMA disinhibition by injecting BMI bilaterally on the cardiovascular parameters of an intruder Wistar rat, when it was suddenly introduced into the home cage of a resident rat. Furthermore, we assessed the functional connection between the BMA and the DMH, by examining the influence of acute inhibition of the DMH on responses evoked by BMA acute inhibition.

EXPERIMENTAL PROCEDURES

Ethical approval

All procedures were previously approved by the ethics committee for animal research of the Federal University of Ouro Preto (CEUA-UFOP; #2012/51) and were performed according to the regulations set forth by the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (8th edition; 2011) in accordance with the rules and regulations of articles focused on animal experimentation. All investigators understand the ethical principles under which Neuroscience operates, and that our work complies with this journal's animal ethics checklist. Care was taken to minimize the number of animals used and to avoid their unnecessary suffering.

Animals

The experiments were carried out on 25 male Wistar rats (220-240 g) from the University Centre of Animal Science. The animals were housed collectively in cages with dimensions of 41 × 34 × 17 cm (three animals per cage) that were maintained at an average temperature of 23 ± 1 °C in a light/dark cycle of 12 h, and were allowed free access to water and food (commercial feed Nuvilab®, Brazil). After the completion of the first surgical procedure (described below), the animals were housed individually in cages with dimensions of 30 × 19 × 13 cm.

Surgical procedures

Surgical procedures started when the rats reached a weight of 300 ± 20 g. The animals were anesthetized (80 mg kg^{-1} ketamine and 11.5 mg kg^{-1} xylazine, ip, supplemented when necessary) to perform the implant guide cannula (23G) for drug injection in the BMA unilaterally, the BMA bilaterally, the DMH bilaterally, and in a region near the BMA, as previously described (de Menezes et al., 2006, 2008, 2009). For this procedure, the rats were placed in a stereotaxic apparatus with the tooth bar fixed at −3.3 mm below the interaural line. After dissecting and cleaning the skull region, we made a 1-mm incision for stainless steel screws and guide cannula implants. Guide cannulas were positioned according to the coordinates of the Paxinos and Watson atlas (Paxinos and Watson, 2007) using the bregma as a reference point. Coordinates for the BMA: 2.3 mm posterior, 4.1 mm lateral, 7.3 mm ventral; and for the DMH: 3.2 mm posterior, 0.6 mm lateral, 7.5 mm ventral. Screws and dental acrylic secured the guide cannulas. After 7 days of recovery, the animals were placed under the anesthetic isoflurane (2.5% isoflurane in 3 L/min O_2; Cristalia, Brazil), and a polyethylene catheter was inserted into the femoral artery for later measurement of cardiovascular parameters. Briefly, an incision was made in the inguinal region of the rat, and the catheter was inserted into the femoral until it reached the aorta (about 4 cm). The catheter was tunneled subcutaneously and exteriorized on the back of the neck. Analgesic (ketoflex 4 mg/kg, 0.1 ml/300 g s.c., Mundo Animal, Brazil) and antibiotics (0.2 ml/100 g, s.c., Fort Dodge Animal Health, Brazil) were administered after both surgical procedures. We initiated the experimental procedures 48 h after the last surgery, during which the catheter was connected to a data acquisition system for obtaining cardiovascular parameters.

Cardiovascular measurements

The catheter, connected to the data acquisition system (PowerLab/400, ADInstruments, NSW, Sydney, Australia), allowed for the measurement of the pulsatile blood pressure of the animals. The pressure oscillations that were captured were amplified and converted into signals that were sent to a data acquisition board by an analogical-to-digital converter. The software Chart 7.0 for Windows (ADI Instruments, NSW, Sydney, Australia) held a continuous collection of pulsatile blood pressure, and then calculated mean arterial pressure (MAP) and heart rate (HR).

Experimental design

On the day of the experiment, the animals were brought to the experimental room in their home cages. The experiment began after the stabilization of physiological parameters (MAP and HR) for at least 30 min. For injecting the drugs, injection cannulas (30 gauge, 1 mm longer than the guide cannula) were connected to a Hamilton syringe (5 μL), filled with the compound to be microinjected, using a Teflon tubing (ID 0:12 mm; OD

0.65 mm; Bioanalytical Systems, West Lafayette, IN, USA). The contents (100 nl) were administered over 10 s, approximately. Each rat was subjected to two different series, each lasting two days, in random order, in which either vehicle (phosphate-buffered saline (PBS) – 100 nL) or the drug was injected into the region of interest.

Our first series of experiments aimed to verify the influence of BMA chemical inhibition on cardiovascular parameters of conscious rats. Therefore, we examined the effect of unilateral injection of muscimol (100 pmol/100 nL), a $GABA_A$ agonist, or vehicle (100 nL) into the BMA, on baseline levels of MAP and HR. Additional experiments were made to test the specificity of the response-induced BMA inhibition. For those experiments, we examined the effect of injection of muscimol (100 pmol/100 nL) or vehicle (100 nL) into the BLA on basal MAP and HR.

The muscimol dose (100pml/100 nl) we used in our experiments was based on previous experiments, where its injection in the DMH was able to reduce the cardiovascular response induced by air jet stress (Abreu et al., 2014).

Our second experimental series aimed to verify the influence of BMA chemical disinhibition in cardiovascular control of animals exposed to social novelty by intruder rat model. Therefore, we examined the effect of bilateral injection of BMI (10 pmol/100 nL), a $GABA_A$ antagonist, or vehicle (100 nL) into the BMA 3 min prior to the social novelty model. This model consisted of the insertion of the experimental animal into the home cage of an unfamiliar animal (resident), thereby making the experimental animal the "intruder" in the situation (Fig. 1). We used different resident animals each experimental day in order to rule out habituation. The rats do not have direct contact with each other since the resident animal is inside a grid cage. The animals remained in this condition for 15 min. This social novelty model has been adapted from two other models of social stress using intruder rats (Keeney et al., 2006; Mohammed et al., 2014).

The BMI dose (10pml/100 nl) used in our experiments was based on previous experiments, where its injection in the DMH was able to disinhibit this region (Abreu et al., 2014).

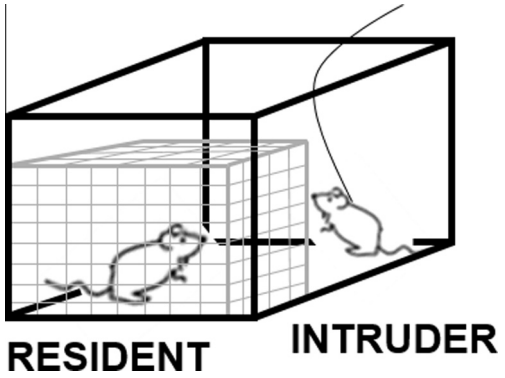

Fig. 1. Illustration of the social novelty by intruder rat model. This model consists of the insertion of the experimental animal analyzed in the cage of an unfamiliar animal (resident – in grid cage), making the experimental animal an "intruder" in the situation.

Additional experiments were made to test if a new environment, without a social component, would be sufficient stimulus to trigger the cardiovascular changes obtained in our model. For this experiment, the experimental animal was inserted into a new empty cage, remaining in the new environment for 15 min.

In our third experimental series, we evaluated whether the cardiovascular responses generated from the inhibition of the BMA depended on DMH neurons. We examined the effect of a bilateral muscimol injection (100 pmol/100 nL) or vehicle (100 nL) into the DMH five minutes prior to muscimol injection into the BMA.

At the end of the experiments, the rats were deeply anesthetized (80 mg kg^{-1} ketamine and 11.5 mg kg^{-1} xylazine, ip) and submitted to transcardiac perfusion with 60 mL of saline followed by 120 mL of a 4% buffered paraformaldehyde in 0.1 M phosphate-buffered saline. After that, the brains were removed and stored in 4% buffered paraformaldehyde overnight and then transferred to a 20% sucrose solution until saturation. The sites of injection were confirmed by coronal sections at the level of BMA and DMH by the use of a cryostat. The slides were mounted and examined with a Leica DM LB microscope (Diagnostics Instruments Inc., Starling Heights, MI, USA), and the sites were confirmed with the support of the Paxinos and Watson atlas (Paxinos and Watson, 2007).

Data analysis

Data for MAP and HR values were recorded continuously. For statistical analysis, we averaged MAP and HR on a minute-to-minute basis during the observation period. The normal distribution of each variable was evaluated using the Kolmogorov–Smirnov test. In the BMA and BLA chemical inhibition protocols ($n = 7/n = 5$, respectively), the cardiovascular changes were calculated considering 5 min prior to microinjection and 15 min after microinjection. In the BMA chemical activation protocols ($n = 6$) followed by the social novelty by intruder rat model, we performed the analysis considering 3 min before, 15 min during, and 10 min after the social novelty. In the DMH chemical inhibition protocol ($n = 7$) followed by chemical inhibition of the BMA, we performed the analysis considering 4 min before microinjection in BMA and 15 min after microinjection in BMA. The baseline values for MAP and HR were obtained by averaging the values of the 5 min-period that preceded the drug injections. Maximal changes (as mean ± standard error of the mean) were calculated differently, depending on the protocol. For the BMA and BLA chemical inhibition protocols, we used 1 min averaged after the muscimol injection, starting from 3 min after the injection. For the social novelty protocols, we averaged 3 min of the peak response. In the DMH chemical inhibition protocol followed by chemical inhibition of the BMA, we used 1-min average response, starting from 3 min after the injection in the BMA.

All of the data analyzed were obtained from animals that had the injection sites confirmed by histology. Prism 6.0 (GraphPad Software, La Jolla, CA, USA) was used to analyze all data. The data were expressed as mean ± standard error of the mean (SEM). Two-way (treatment and time as factors) repeated measures analysis of variance (ANOVA) with post hoc comparison with Bonferroni post-test was used to analyze differences between treatments. Student's Paired *t*-test was used to analyze maximal changes within groups, and Student's unpaired *t*-test was used to analyze maximal changes between groups. The significance threshold level was set at 0.05.

RESULTS

Muscimol injection into the BMA increases MAP and HR in conscious rats

The muscimol injection (100 pmol/100 nL) into the BMA evoked increases in MAP and HR when compared to vehicle treatment (Fig. 2). A repeated measures two-way ANOVA revealed a significant effect of treatment [$F(1,6) = 23.78$; $p = 0.0028$] and time by treatment interaction [$F(19,114) = 3.414$; $p < 0.0001$] on MAP (Fig. 2-D). Student's Paired *t*-test revealed an increase in the maximum change in MAP after the muscimol injection (10 ± 2 vs. −2 ± 1 mmHg; $p = 0.0037$) when compared to the vehicle (Fig. 2-E). We did not find a significant different effect on treatment [$F(1,6) = 4.48$; $p = 0.0788$], but there was a significant interaction effect on time by treatment [$F(19,114) = 3.67$; $p < 0.0001$] on HR (Fig. 2-F). Student's Paired *t*-test revealed an increase in the maximum change in HR after the muscimol injection (84 ± 10 vs. −5 ± 5 bpm; $p = 0.0003$) when compared to the vehicle injection (Fig. 2-G).

In contrast, the injection of muscimol into regions adjacent to the BMA, specifically in the BLA, did not affect MAP and HR (Fig. 3). A repeated measures two-way ANOVA revealed no significant effect of treatment [$F(1,4) = 0.01$; $p = 0.9368$] and time by treatment interaction [$F(15,60) = 1.60$; $p = 0.1003$] on MAP. Also, there was no significant effect of treatment [$F(1,4) = 0.27$; $p = 0.6282$] and time by treatment interaction [$F(19,76) = 0.88$; $p = 0.6030$] on HR.

BMI injection into the BMA reduces the cardiovascular response induced by a social novelty

During our social novelty model, we observed the intruder rat increases in the HR and MAP (Fig. 4-A). Bilateral injection of BMI (10 pmol/100 nL) into the BMA, reduced the increases in HR and MAP observed during the animal's exposure to the social novelty, compared to vehicle treatment (Fig. 4-B). A repeated measures two-way ANOVA did not reveal a significant effect of treatment [$F(1,5) = 1.75$; $p = 0.2425$], but there was a significant interaction effect on time by treatment [$F(26,130) = 3.24$; $p = 0.0016$] on MAP (Fig. 4-D). Student's Paired *t*-test revealed a decrease in the maximum change in the MAP during the social novelty model after BMI injection (4 ± 1 vs. 18 ± 4 mmHg; $p = 0.0211$), compared to the control (Fig. 4-E). Likewise we did not find a significant different effect on treatment [$F(1,5) = 4.49$; $p = 0.0762$], but there was a significant interaction effect on time by treatment [$F(26,130) = 1.93$; $p = 0.0086$] on HR (Fig. 4-F). Student's Paired *t*-test revealed a decrease in the maximum change in the HR during the social novelty model after BMI injection (22 ± 14 vs. 116 ± 10 bpm; $p = 0.0117$) (Fig. 4-G).

Importantly, just moving the animal to a new cage (new environment), without the social component, induced a less prominent cardiovascular response when compared to the response evoked by social novelty (MAP: 8 ± 2 vs. 18 ± 3; $p = 0.0315$ and HR: 42 ± 12 vs. 120 ± 10; $p = 0.0006$ – Student's unpaired *t*-test).

Muscimol injection into the DMH suppresses the cardiovascular response evoked by muscimol injection into the BMA

Our results showed that the bilateral injection of muscimol (100 pmol/100 nL) into the DMH suppressed the cardiovascular changes promoted by muscimol injection into the BMA, compared to vehicle treatment (Fig. 5). Repeated measures two-way ANOVA revealed a significant effect of treatment [$F(1,6) = 8.62$; $p = 0.0261$] and time by treatment interaction [$F(19,114) = 2.99$; $p = 0.0002$] on MAP (Fig. 5-D). Student's Paired *t*-test revealed a decrease in the maximum change in the MAP evoked by muscimol injection into the DMH before muscimol injection into the BMA (−5 ± 1 vs. 5 ± 1 mmHg; $p = 0.0006$) (Fig. 5-E). Similarly, we found a significantly different effect on treatment [$F(1,6 = 15.32$; $p = 0.0079$] and time by treatment interaction [$F(19,114) = 3.10$; $p < 0.0001$] on HR (Fig. 5-F). Student's Paired *t*-test revealed a decrease in the maximum change in the HR evoked by muscimol injection into the DMH before muscimol injection into the BMA (13 ± 6 vs. 49 ± 7 bpm; $p = 0.0005$) (Fig. 5-G).

Post-mortem histology confirmed that the injection sites were located in the BMA unilaterally, BLA bilaterally, and DMH bilaterally (Fig. 6-A, B and C).

DISCUSSION

In this study, we showed that BMA chemical inhibition by muscimol increased basal MAP and HR. These changes were suppressed by DMH neuronal inhibition. Moreover, BMA activation, by injecting BMI bilaterally, blocked the MAP and HR increases evoked by social novelty in an intruder rat model. Considering that the DMH is tonically inhibited (DiMicco et al., 2002), our results suggest that the BMA has an important role in controlling the DMH's neuroactivity. Moreover, the BMA sends excitatory projections to the BNST, which in turn sends inhibitory projections to the DMH (Myers et al., 2014), thus disinhibiting the BMA could be suppressing the cardiovascular response, observed during the social novelty, by inhibiting the DMH. Our data reveal that the BMA is under continuous GABAergic influence, and that its activation

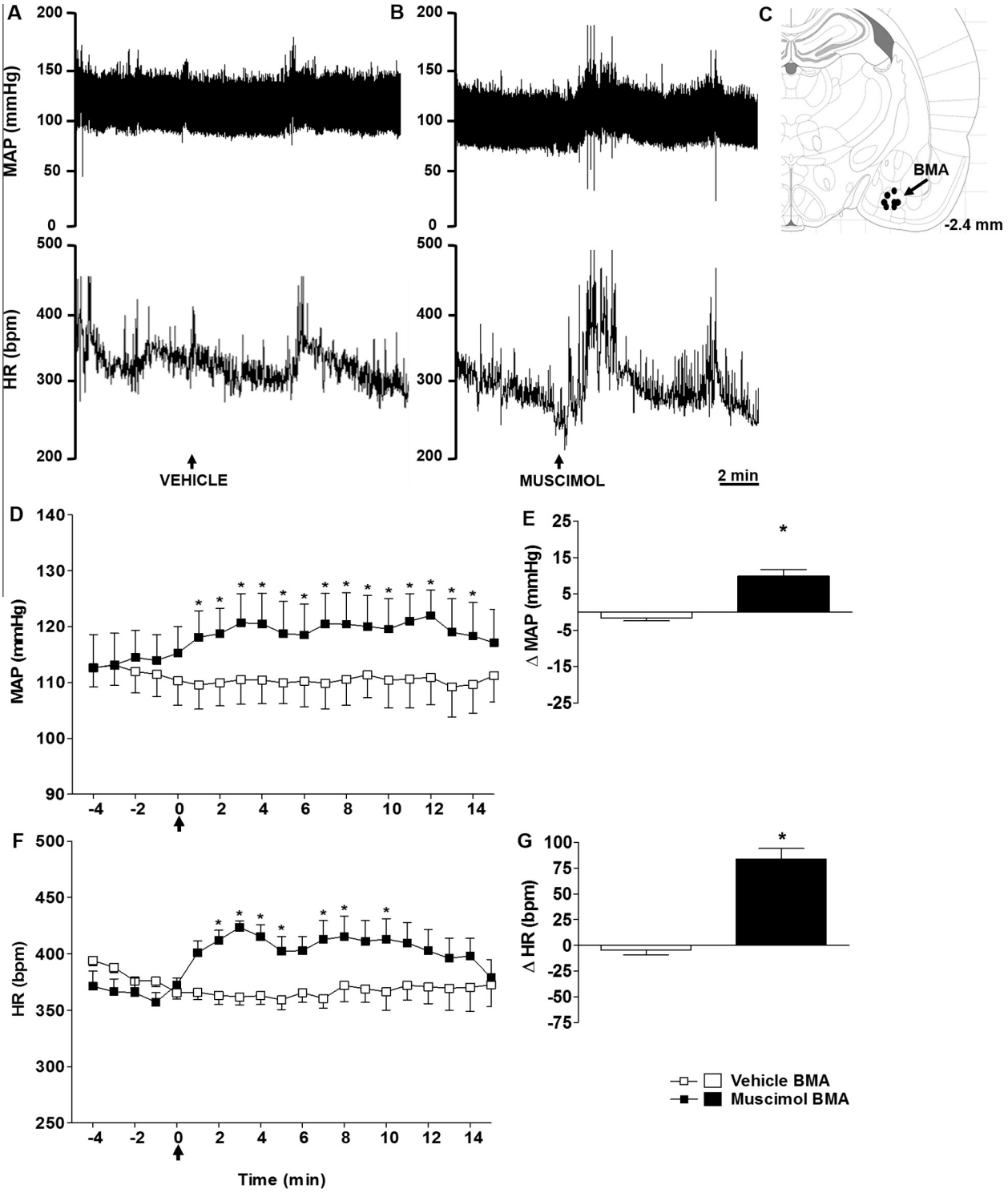

Fig. 2. Muscimol injection into the BMA increases basal MAP and HR in rats. A and B – Original recordings showing the effects of vehicle (100 nL) (left panel – A) or muscimol (100 pmol/100 nL) (right panel – B) injections into the BMA on the cardiovascular parameters. Recordings of pulsatile arterial pressure (MAP; top trace) and heart rate (HR; bottom trace) are shown. C – Sites of injection in the BMA. Schematic coronal sections of the rat brain adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 2007) illustrating approximate sites of injections into the BMA for all experiments for which data are reported. Numbers indicate distance from the bregma in millimeter. Filled circles represent injections of muscimol or vehicle into the BMA. D and F – Mean changes in MAP (D) and HR (F) as a result of muscimol (filled circles) versus vehicle (open squares) injection into the BMA ($n = 7$). $^{*}p < 0.05$ by two-way (treatment and time as factors) repeated measures analysis of variance (ANOVA) with post hoc comparison with Bonferroni post-test. E and G – Maximum changes in MAP (E) and HR (G) induced by injection of vehicle (black) or muscimol (white) into the BMA. Comparison within groups by paired Student *t*-test ($^{*}p < 0.05$). All of the values are expressed as mean ± SEM. Arrows indicate muscimol or vehicle injection. Abbreviations: BMA (basomedial amygdala); HR (heart rate); MAP (mean arterial pressure).

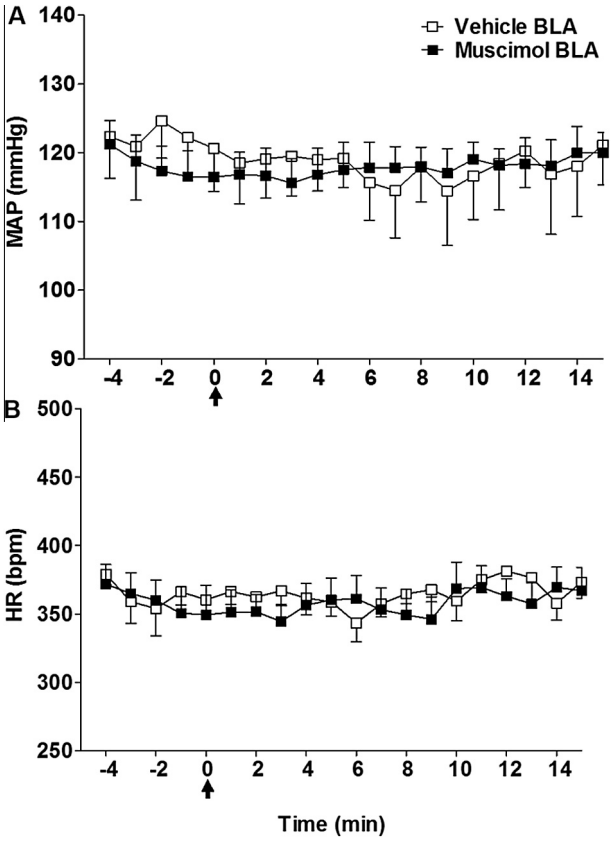

Fig. 3. Effect of injection of muscimol into the BLA on MAP and HR in rats. A and B – Mean changes in MAP (left panel – A) and HR (right panel – B) as a result of muscimol (100 pmol/100 nL, filled circles) versus vehicle (100 nL open squares) injection into the BLA (n = 5). Values are expressed as mean ± SEM. $^{*}p < 0.05$ by two-way (treatment and time as factors) repeated measures analysis of variance (ANOVA) with post hoc comparison with Bonferroni post-test. Arrows indicate muscimol or vehicle injection. Abbreviations: BLA (basolateral amygdala); HR (heart rate); MAP (mean arterial pressure).

can reduce the physiological response induced by a social novelty condition, possibly by inhibiting DMH neurons.

Although increases in MAP and HR were observed following BMA inhibition, other amygdaloid subnuclei, such as BLA e CeA, have to be activated in order to obtain these same responses (Sanders and Shekhar, 1991; Soltis et al., 1998; de Menezes et al., 2006). One could argue that changes in MAP and HR evoked by injecting muscimol into the BMA could be caused by the spread to neighboring areas, such as the BLA. However, when we injected muscimol directly into the BLA, MAP and HR remained unchanged (see Fig. 3). Moreover, previous studies have already reported that BLA neuronal inhibition does not change resting MAP and HR (Salome et al., 2007; de Abreu et al., 2015), although it does blunt the increases in cardiovascular parameters induced by aversive states (Salome et al., 2007). Corroborating these data, it has been shown that disinhibiting BLA neurons leads to increases in MAP and HR (Soltis et al., 1997, 1998; de Abreu et al., 2015). Thus, our results, in conjunction with previous data, indicate that the increase in MAP an HR induced by a muscimol injection into the BMA, is derived from the inhibition of BMA neurons and not by BLA neurons. Furthermore, an anterograde tracer study revealed BMA glutamatergic projections arising from the lateral amygdala subnuclei (Pitkanen et al., 1997). Taken together, the data support our hypothesis that the BMA is activated under physiological conditions and could influence other encephalic nuclei involved in cardiovascular control.

In anesthetized rats, disinhibition of the BMA with BMI promoted a dose-dependent increase in MAP and HR, which was eliminated by muscimol (Yoshida et al., 2002). These findings differ from ours, performed in conscious rats, and may have been influenced by the anesthesia, which is known to compromise autonomic and cardiovascular functions (Menezes and Fontes, 2007; Chiou et al., 2009). However, it is important to point out that in their work, Yoshida and colleagues used a much larger volume (200 nL) than we used in the present study (100 nL), which could have spread to the BLA, where bicuculline injection is known to induce tachycardia and hypertension (de Abreu et al., 2015). Interestingly, the smaller bicuculline dose (10 pmol), the same dose we used in our experiment, did not induce increases in basal HR. On the other hand, injection of 50 pmol or 100 pmol increased HR in their study. We could speculate that a bicuculline injection into the BMA, when performed in a larger volume combined with a larger dose, could spread to the BLA and effectively activate its neurons, leading to increases in HR and MAP. We believe that our results provide a more accurate understanding about the BMA's role, since it was performed using lower volumes and doses, and was performed in conscious animals.

Considering all the aforementioned information, we conjecture that the BMA regulates the physiological adaptations facing social novelty. To investigate this hypothesis, we tested the effects of BMA activation on cardiovascular changes, using a social novelty model adapted from the standard intruder rat model. BMA activation blocked the increase of MAP and HR evoked by social novelty. This result suggests that the BMA modulates the animal's reactions to adverse contexts. The activation of the BMA could trigger a specific circuit, blunting the neuroactivity of brain nuclei accounting for the increased sympathetic activity during emotional stress, such as the DMH (Fontes et al., 2011). BMA activation would control the response, thereby avoiding an excessive cardiovascular response that could ultimately overload the cardiovascular system.

In order to evaluate the importance of the social component in our experiments, isolating the novelty component during the social novelty paradigm, we performed a separate set of experiments to evaluate the cardiovascular response induced by a new environment without the presence of a conspecific. Taking the animal from its home cage and placing it in a new cage (i.e. without a resident rat) led to increase in HR and MAP, as expected (Beerling et al., 2011). Nevertheless, these changes were much smaller than the ones observed after the exposure to the social novelty. These data indicate

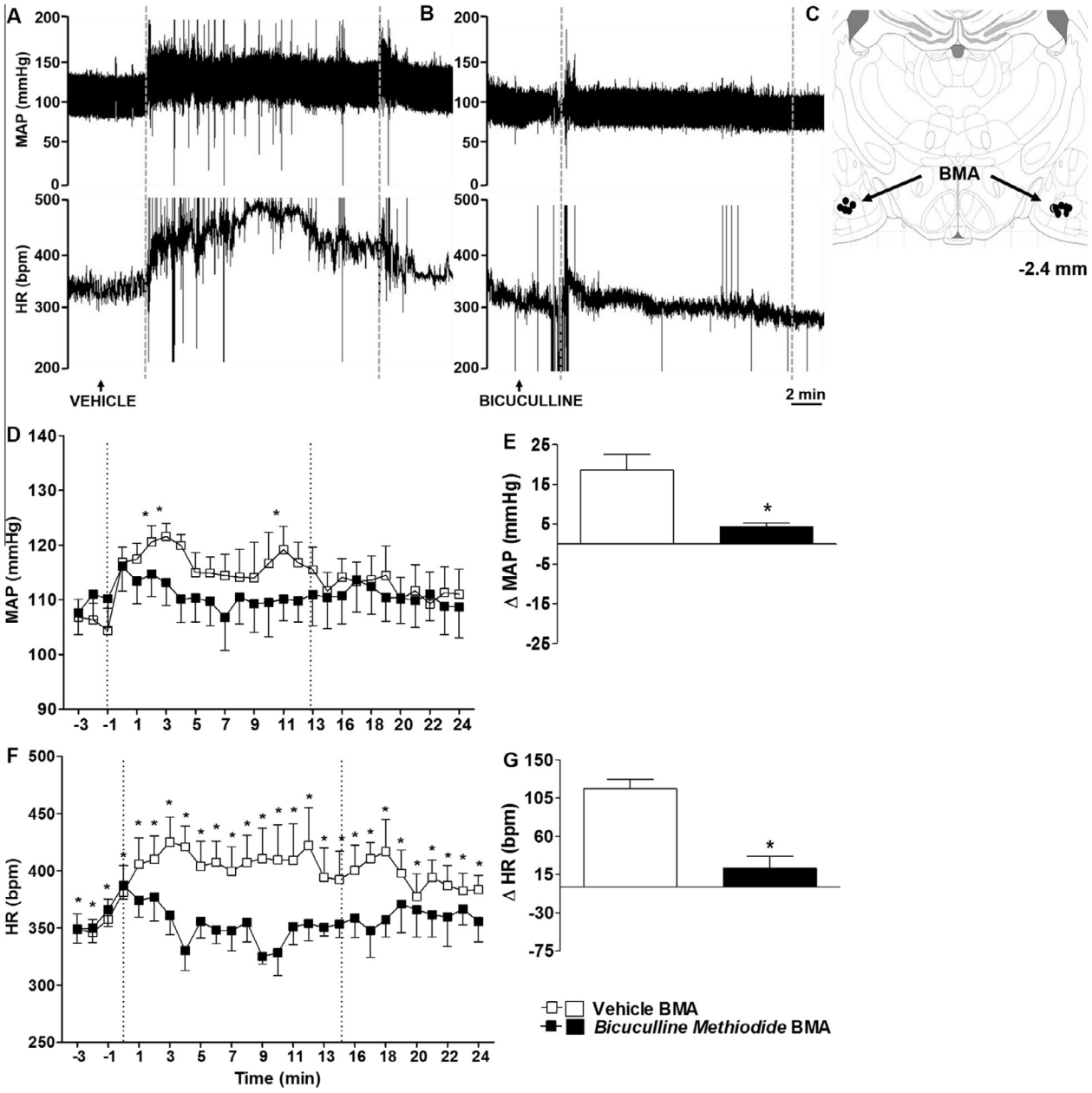

Fig. 4. BMI injection into the BMA reduces the changes in MAP and HR in rats exposed to social novelty by intruder rat model. A and B – Original recordings showing the effects of vehicle (100 nL) (left panel – A) or BMI (10 pmol/100 nL) (right panel – B) injections into the BMA bilaterally on the cardiovascular parameters 5 min before social novelty (between dotted lines). Recordings of pulsatile arterial pressure (MAP; top trace) and heart rate (HR; bottom trace) are shown. C – Sites of injection into the BMA bilaterally. Schematic coronal sections of the rat brain adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 2007) illustrating approximate sites of injections into the BMA bilaterally for all experiments for which data are reported. Numbers indicate distance from bregma in millimeter. Filled circles represent injections of BMI or vehicle into the BMA. D and F – Mean changes in MAP (D) and HR (F) as a result of BMI (filled circles) versus vehicle (open squares) injection into the BMA ($n = 7$). $^{*}p < 0.05$ by two-way (treatment and time as factors) repeated measures analysis of variance (ANOVA) with post hoc comparison with Bonferroni post-test. E and G – Maximum changes in MAP (E) and HR (G) induced by social novelty, after injection of vehicle (black) or BMI (white) into the BMA. Comparison within groups by paired Student *t*-test ($^{*}p < 0.05$). All of the values are expressed as mean ± SEM. Arrows indicate BMI or vehicle injection. Abbreviations: BMA (basomedial amygdala); BMI (*bicuculline methiodide*); HR (heart rate); MAP (mean arterial pressure).

that the social component in our model is very strong, leading to the prominent cardiovascular response observed in our experiments.

To test whether the changes evoked by BMA activation depended on DMH neurons, we looked at whether DMH inhibition could reduce the cardiovascular response evoked by BMA inhibition. Increases in MAP and HR induced by BMA inhibition were suppressed by the injection of muscimol into the DMH, a brain nucleus recognized to integrate the response to emotional stress in rats (Stotz-Potter et al., 1996) Several studies have demonstrated that the neuronal activity within the DMH is essential to regulate the autonomic, neuroendocrine and behavioral responses mediated by emotional stress

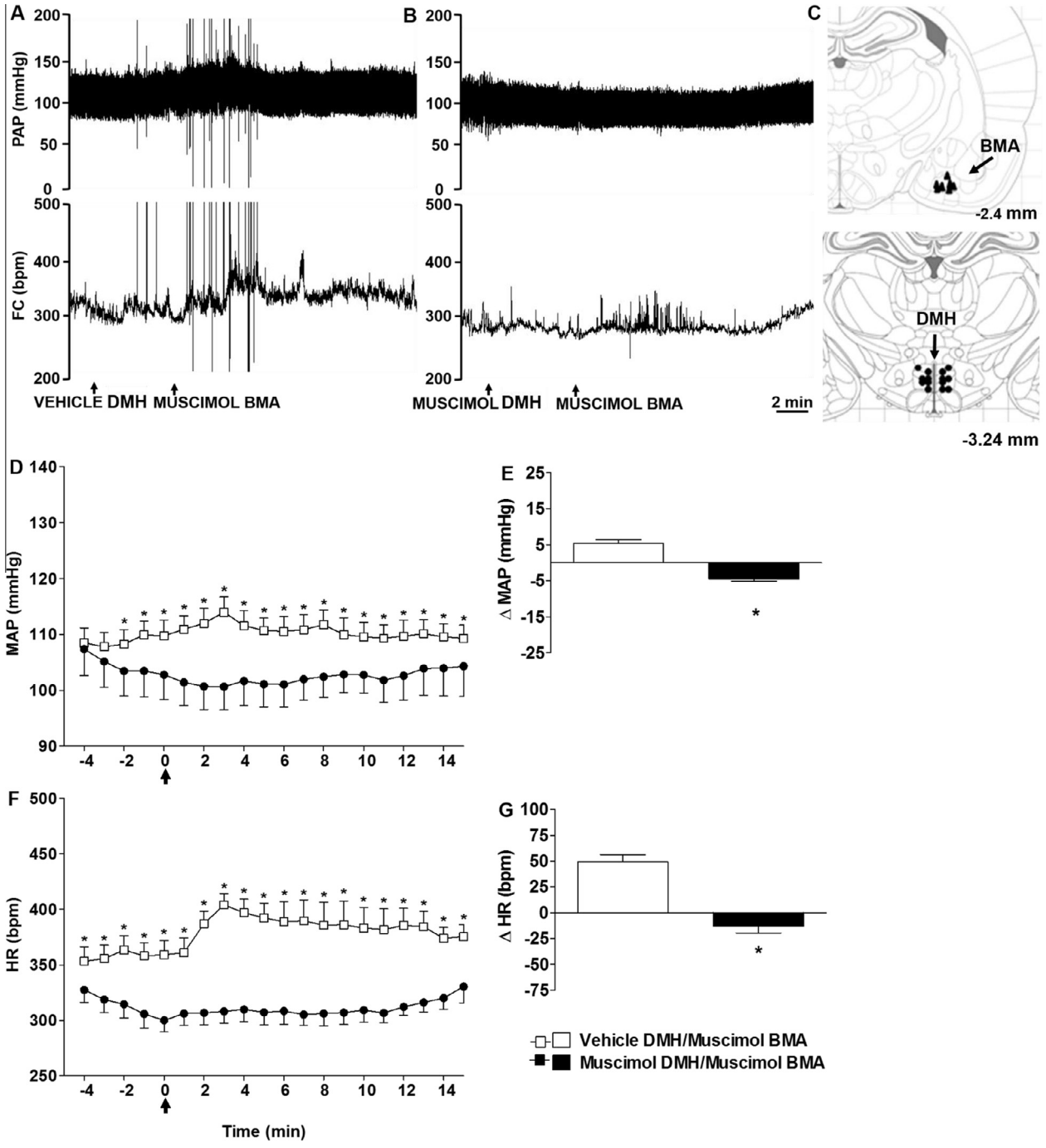

Fig. 5. Muscimol injection into the DMH reduced the changes in MAP and HR evoked by muscimol injection into the BMA. A and B – Original recordings showing the effects of vehicle (100 nL) (left panel – A) or muscimol (100 pmol/100 nL) (right panel – B) injections into the DMH bilaterally on the cardiovascular changes evoked by the injection of muscimol into the BMA. Recordings of pulsatile arterial pressure (MAP; top trace) and heart rate (HR; bottom trace) are shown. C – Sites of injection in the BMA and the DMH bilaterally in all experiments. Schematic coronal sections of the rat brain adapted from the atlas of Paxinos and Watson (Paxinos and Watson, 2007) illustrating approximate sites of injections into the BMA and DMH for all experiments for which data are reported. Numbers indicate distance from bregma in millimeter. Filled triangle represents injections of muscimol into the BMA and filled circle represent injections of vehicle or muscimol into the DMH bilaterally. D and F – Mean changes in MAP (D) and HR (F) as a result of muscimol injection into the BMA followed by vehicle into the DMH (open squares) versus muscimol injection into the BMA followed by muscimol into the DMH (filled circles) ($n = 7$). $^{*}p < 0.05$ by two-way (treatment and time as factors) repeated measures analysis of variance (ANOVA) with post hoc comparison with Bonferroni post-test. E and G – Maximum changes in MAP (E) and HR (G) induced by injection of vehicle (black) or muscimol (white) into the DMH before inhibition of the BMA. Comparison within groups by paired Student *t*-test ($^{*}p < 0.05$). All of the values are expressed as mean ± SEM. Arrows indicate muscimol or vehicle injection into the BMA. Abbreviations: BMA (basomedial amygdala); DMH (dorsomedial hypothalamus); HR (heart rate); MAP (mean arterial pressure).

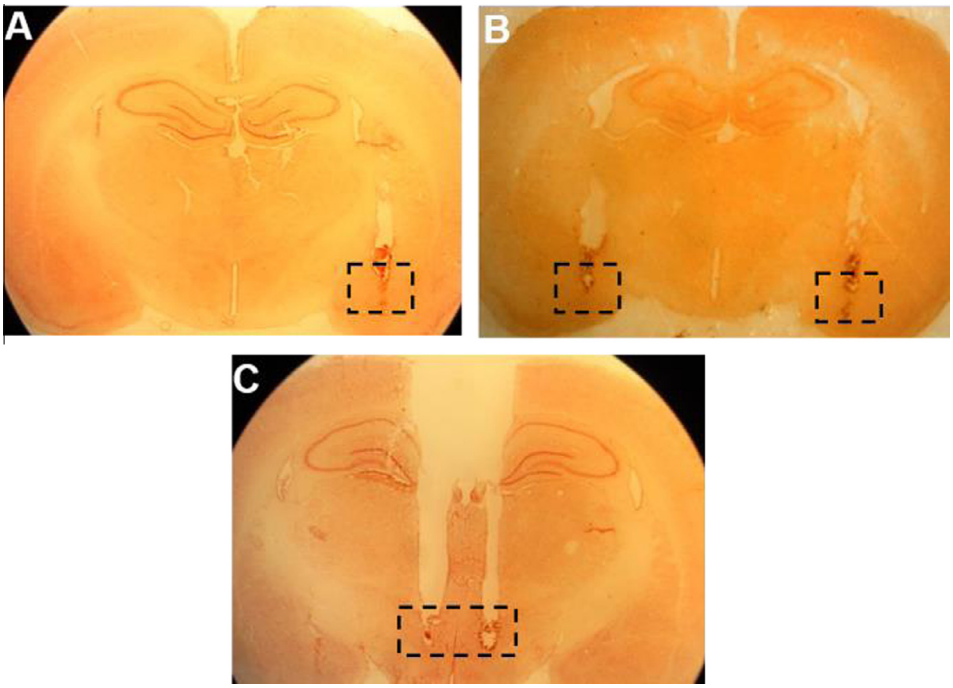

Fig. 6. Examples of typical sites of injections. Photomicrography of typical sites of injection in the BMA unilaterally (A), bilaterally (B) and DMH bilaterally (C).

in rats. In fact, inhibition of this area reduces the increase in MAP and HR induced by air jet stress (Abreu et al., 2014), in the same way that stimulating the BMA can inhibit the cardiovascular response induced during our social novelty paradigm. Moreover, bilateral muscimol injections into the DMH are able to reduce the increase in ACTH, and therefore in corticosterone induced by air jet stress. Therefore, we can infer that direct inhibition of DMH neurons would conceivably inhibit the increases in MAP and HR caused by the social novelty, in a similar manner as activating the BMA did in our present study.

Considering the role of the DMH in the regulation of physiological adaptations when facing acute stress (Fontes et al., 2011; Abreu et al., 2014), the relationship between the BMA and the DMH could be crucial to the fine-tuning of cardiovascular homeostasis. In fact, the DMH receives GABAergic inhibitory projections from medial preoptic area (mPOA), BNST, CeA, and medial amygdala (MeA). Additionally, the BMA sends excitatory projections to the BNST, which in turn sends inhibitory projections to the DMH (Myers et al., 2014). Thus, we suggest a downstream pathway that establishes an excitatory connection from the BMA to the BNST and an inhibitory connection of the latter to the DMH, which in turn regulates the arterial pressure and chronotropic responses to emotional stressors. Thus, we propose that the BMA has an essential regulatory role on DMH responsiveness to social novelty.

Aversive states, cardiovascular, neuroendocrine and behavioral adaptation, are essential to physiological active or passive emotional coping strategies that promote the survival of the species (Amat et al., 2008). It is important to point out, however, that the response to an aversive environment must be fine-tuned, avoiding excessive responses to minimal stress conditions., At the end of threatening stimulus, homeostasis must to be restored, with the cardiovascular parameters returning to the pre stress condition.

CONCLUSION

In this study, we showed that BMA chemical inhibition increased basal MAP and HR, which could be prevented by DMH inhibition. Importantly, BMA disinhibition blocked the cardiovascular responses evoked by a social novelty in an intruder rat model. Our data reveal that the BMA is under continuous GABAergic influence and possibly exerts direct or indirect inhibitory control over the DMH during social novelty conditions. Studies on the function of the BMA nucleus are scarce in the literature. Our work provides new insights about BMA function in the cardiovascular control pathway recruited during aversive situations. Understanding the circuit responsible for fine-tuning the cardiovascular response during defense reactions, and the mechanisms involved in the restoration of conditions after the end of threatening stimulus, could be the key to comprehending the pathophysiology of stress-related disorders.

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