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Research report

Influence of the anteromedial thalamus on social defeat-associated contextual fear memory

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ABSTRACT

The ventral part of the anteromedial thalamic nucleus (AMv) is heavily targeted by the dorsal premammillary nucleus (PMd), which is the main hypothalamic site that is responsive to both predator and conspecific aggressor threats. This PMd-AMv pathway is likely involved in modulating memory processing, and previous findings from our group have shown that cytotoxic lesions or pharmacological inactivation of the AMv drastically reduced contextual fear responses to predator-associated environments. In the present study, we investigated the role of the AMv in both unconditioned (i.e., fear responses during social defeat) and contextual fear responses (i.e., during exposure to a social defeat-associated context). We addressed this question by placing N-methyl-D-aspartate (NMDA) lesions in the AMv and testing unconditioned fear responses during social defeat and contextual fear responses during exposure to a social defeat-associated context. Accordingly, bilateral AMv lesions did not change unconditioned responses, but decreased contextual conditioning related to social defeat. Notably, our bilateral AMv lesions also included, to a certain degree, the nucleus reuniens (RE), but single RE lesions did not affect innate or contextual fear responses. Overall, our results support the idea that the AMv works as a critical hub, receiving massive inputs from a hypothalamic site that is largely responsive to social threats and transferring social threat information to circuits involved in the processing of contextual fear memories.

1. Introduction

Social interactions are essential for defining access to sexual partners, territories and nutritional resources [see 1]. The definition of social hierarchy is established aggressively, with different physiological and behavioral consequences for defeated animals and winners [2].

Defeated animals present clear defensive responses when re-exposed to a potential aggressor or to a context previously associated with a social defeat [3–7]. Studies conducted in our laboratory showed that defeated animals avoid cages in which they had been defeated and performed a careful exploration of the environment through risk assessment behaviors [7]. Importantly, social defeat has been proposed to be an animal model of depression [8], resulting in similar behavioral and neuroendocrine changes to those found in depressed patients [9].

Combining the results of behavioral, neuronal immediate early gene activation, lesion, and neuroanatomical experiments, we have delineated a putative circuit that is involved in both innate and contextual defense responses in a subordinate conspecific [7,10,11]. During social defeat, the dorsal premammillary nucleus (PMd) is the hypothalamic site that presents the most striking activation, which appears to be particularly confined to the dorsomedial part of the nucleus [10,11]. The dorsomedial part of the PMd receives strong inputs from specific regions of the lateral hypothalamic area (i.e., the juxtaparaventricular and juxtadorsomedial regions), which upregulate Fos expression during social defeat and likely convey septo-hippocampal information that encodes the environmental boundary restriction imposed by the presence of a dominant aggressor [11]. Moreover, the dorsomedial part of the PMd is further influenced by the conspecific-responsive circuit of the medial zone of the hypothalamus (including the medial preoptic area, the ventrolateral part of the ventromedial nucleus and the ventral premammillary nucleus), which is also mobilized during social defeat and integrates conspecific cues conveyed by the medial amygdalar nucleus [10]. Interestingly, PMd lesions block the passive components of social defense (i.e., freezing and sustained on the back position), as seen during a confrontation with a dominant aggressor [10]. It is noteworthy that the PMd is believed to influence the mnemonic processes related to contextual defensive responses [12,13] and likely involves the projection branch to the ventral part of the anteromedial nucleus (AMv) [14].

The anterior thalamic nuclei have been shown to support multiple

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and complementary forms of learning. In humans, the anterior thalamic nuclei, along with mammillary body atrophy, have been implicated in the amnesic symptoms of Korsakoff's syndrome and are required for normal episodic memory [15–17]. In rodents, there is considerable evidence from both lesion and electrophysiological studies that the anterior thalamic nuclei are involved in navigation and spatial memory, especially when distal cues are essential for a successful performance [18–21]. Moreover, the anterior thalamic nuclei influence hippocampal-dependent non-spatial tasks, such as those required to solve a temporal order problem and sample a sequence of successive odors [22], as well as the ability to use contextual information to resolve conflicts in an olfactory list learning task [23].

Previous studies from our group have shown that cytotoxic lesions or pharmacological inactivation of the anteromedial thalamic nucleus drastically reduced contextual fear responses to predator-associated environments [24,25]. However, it remains to be determined whether the AMv is involved in contextual fear in socially defeated animals. Thus, as we have previously shown for predator threats, we presently investigated whether the AMv works as an effective hub to convey social threat information from hypothalamic sites to systems involved in the processing of contextual fear memory. In the present investigation, we addressed this question by placing N-methyl-D-aspartate (NMDA) lesions in the AMv and testing both unconditioned fear responses during social defeat and contextual fear responses during exposure to a social defeat-associated context.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (N = 34) weighing approximately 300 g (3–4 months old) were used as intruders; adult Long Evans male rats (N = 5) weighing approximately 600 g (9–12 months old) were used as residents and were housed with Long Evans female rats (N = 5) weighing approximately 350 g (3–5 months old). Both lineages were obtained from local breeding facilities. The animals were housed under a controlled temperature (23 °C) and illumination (12-h cycle) in animal quarters and had free access to water and a standard laboratory diet.

2.2. Ethics

Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996). All experimental procedures had been previously approved by the Committee on the Care and Use of Laboratory Animals of the Institute of Biomedical Sciences – University of São Paulo, Brazil (Protocol number 085/2012). In the present study, the experiments were planned to minimize the number of animals used and their suffering. In addition, all surgical procedures were performed under deep anesthesia, and analgesic and antibiotic medication were given postoperatively.

2.3. Surgery

For the lesion procedure, rats were deeply anesthetized with sodium pentobarbital (Cristália; Itapira, SP, Brazil; 40 mg/kg, i.p.) and were placed in a stereotaxic apparatus. Bilateral iontophoretic deposits of a 0.15 M solution of *N*-methyl-D-aspartate (NMDA, Sigma, St. Louis, MO, USA) were bilaterally centered in the ventral part of the anteromedial thalamic nucleus (n = 10; coordinates: anteroposterior 1.40 mm from bregma; laterolateral, ± 1.0 mm from the midline of the sagittal sinus; dorsoventral 6.1 mm from the surface of the brain) or in the rostral half of the nucleus reuniens (n = 10; coordinates: anteroposterior, 1.40 mm from bregma; laterolateral, 0.0 mm from the midline of the sagittal sinus; dorsoventral 6.30 mm from the surface of the brain) using the stereotaxic coordinates from *The Brain Maps: structure of the rat brain* [26]. In addition, in 7 other animals, control saline injections were performed bilaterally at the same coordinates used for the ventral part of the anteromedial thalamic nucleus. NMDA deposits were performed over a 15-min period through a glass micropipette (30 μm tip diameter) using a constant-current device (model CS3, Midgard Electronics, Canton, MA, USA) set to deliver −10 μA, with 7-s pulse and interpulse durations (for NMDA lesion protocol see [27]). Animals received postoperative analgesics (Ibuprofen; Medley; Campinas, SP, Brazil; 30 mg/kg in drinking water) and antibiotics (Pentabiótico©; Zoetis; Campinas, SP, Brazil; 0.1 ml/100 g, i.p.). After a 1-week post-surgical period, the animals were placed in the experimental apparatus.

2.4. Experimental apparatus and procedure

The experimental protocol presently used to investigate innate and contextual fear related to social defeat followed Faturi and Rangel et al. [7]. The experimental apparatus was made of clear Plexiglas and consisted of a 25 × 25 × 25 cm home cage connected to another 25 × 25 × 25 cm chamber (the food compartment) by a hallway that was 12.5 cm wide and 100 cm long, with 25-cm high walls. Between the home cage and hallway, there was a sliding door (12.5 cm wide and 26 cm high) that remained closed most of the time, except for when animals were allowed to explore the remainder of the apparatus. For 10 days before the testing procedures (habituation period), the intruders were isolated and lived in the home cage, and then at the beginning of the dark phase, the home cage door was opened and animals were allowed to explore the remainder of the apparatus and obtain food pellets that were stored in the food compartment. To maintain the animals in an active state without eating, during all of the habituation and testing periods of the intruders, all pellets in the home cage were removed 3 h before the test. After the testing procedures, the food pellets were returned to the home cage. The resident male, an adult Long Evans rat, had been housed in a 25 × 25 × 25 cm cage together with a female Long Evans rat for at least three weeks prior to the social encounter with the intruder. To prevent pregnancy and discharge of pups, the females housed with the resident males had been previously hysterectomized under deep anesthesia (mixture of ketamine and xylazine; 1:2 v/v; 1 ml/kg body weight) by severing the uterine horns at the tubo-vaginal junction and at the anterior end of the cervix, but their ovaries remained intact. After a 2-week recovery period, the sterilized females were housed with the resident males.

The testing procedure consisted of three phases. Phases 1 and 3 consisted of a 5-min observation period during the last day of habituation (Phase 1) and context exposure (Phase 3). Phase 2 consisted of a 10-min observation period during the social defeat procedure. During the tests, animals were recorded with a horizontally mounted video camera.

2.4.1. Phase 1 – last day of habituation

On the last day of habituation (day 10), we observed the intru'ior in a familiar environment during the beginning of the dark period. To maintain the animals in an active state without eating, no food pellets were offered.

2.4.2. Phase 2 – social defeat

On the 11th day, the food compartment had been replaced by the home cage of a resident male. On the defeat day, the female was removed, and once the Wistar male intruder had entered the resident's cage, the cage's door was closed and the resident started attacking the intruder in less than 1 min. Only experienced resident males were used in the present study. After the first resident attack with a painful experience (i.e., a bite), residents and intruders were left together for a 10-min period. Only intruders that had suffered a clear social defeat were used for further analysis.

2.4.3. Phase 3 – context exposure test

On the day after defeat, defeated animals were observed for 5 min while they explored the apparatus linked to the resident cage, which contained the resident's soiled bedding. Similar to other phases, no food pellet was offered during the test period.

2.5. Behavior analysis

Behaviors were scored by a trained observer using the ethological analysis software 'The Observer' (version XT, Noldus Information Technology, Wageningen, Netherlands). The analysis consisted of spatiotemporal and behavioral measurements. The spatiotemporal measurements were the time spent in the home cage, hallway, or food compartment (or resident cage). The behavioral data were processed in terms of duration (total duration per session). The behavioral categories followed Grant and Mackintosh [28], Blanchard et al. [29] and Ursin and Olff [30].

The following behavioral items were encoded on the last day of habituation and the social defeat context:

Exploration (fearless exploration), including fearless locomotion (locomotion with an arched back) and upright position (animals actively exploring the environment, standing on the rear paws and leaning on the walls with their forepaws).

Risk assessment behaviors, including crouch-sniff (animal immobile with their back arched but actively sniffing and scanning the environment) and stretch postures (the body was stretched forward either motionless or moving slowly toward the cage of the resident).

Freezing, including cessation of all movements, except for those associated with breathing.

Grooming or self-cleaning behavior.

Rearing, where the animal stands over its rear paws without wall contact. All behavioral scoring was conducted by an observer who was blind with respect to the rat's condition.

For the social defeat encounter, the following behaviors were counted:

Passive defense, including freezing (animal completely crouched and immobile) and on the back posture (animal laid down with their belly turned up).

Active defense, including upright position with sparse boxing and dashing away from the resident and flight.

Locomotion, such as the exploratory behaviors described during the other phases.

Grooming or self-cleaning behavior.

Social investigation, including approach, contact with the resident, sniffing, and anogenital sniffing.

2.6. Histology

Upon completion of the behavioral testing, all rats were injected with sodium pentobarbital (Cristália; 40 mg/kg, i.p.) and perfused transcardially with a solution of 4.0% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4; the brains were removed and placed overnight in a solution of 20% sucrose in 0.1 M phosphate buffer at 4 °C. The brains were then frozen and four series of 30 μm sections were cut with a sliding microtome in the frontal plane. One series of sections was mounted on gelatin-coated slides and stained with thionin to serve as the reference series for cytoarchitectonic purposes.

The sections were examined with the 10X objective of a Nikon Eclipse 80i (Nikon Corporation, Chiyoda-Ku, Tokyo-To, Japan) microscope equipped with a Nikon digital camera DXM1200F (Nikon Corporation). For each thalamic nucleus targeted by NMDA lesions (i.e., the rostral half of the nucleus reuniens or the ventral part of the anteromedial thalamic nucleus), the lesion size was estimated by measuring, in each section, the non-lesioned area of the targeted nucleus and then subtracting these values from the total area of the nucleus, which was obtained from a non-lesioned animal reference series of sections. The area measurements were made with the aid of a computer program (Image-Pro Plus, version 4.5.1; Media Cybernetics, Silver Spring, MD, USA). Parcellation of the thalamic regions examined in the present investigation followed *The Brain Maps: structure of the rat brain* [26].

2.7. Statistical analysis

After testing for homogeneity of the variance with the Levene's test, our behavioral data (spatiotemporal and behavioral parameters) were logarithmically transformed and initially analyzed via multivariate analysis of variance (MANOVA). After obtaining a significant result via the omnibus MANOVA, univariate analysis of variance (ANOVA) was performed for each dependent variable, followed by a Newman-Keuls post hoc analysis when the result was statistically significant. To maintain the overall type I error at 5%, the significance level employed in the ANOVA was adjusted downward (Bonferroni's correction) according to the respective number of variables in each experiment ($\alpha = 0.007$ for the last day of habituation and the context exposure test, and $\alpha = 0.01$ for exposure to the dominant male). The average results are expressed as the mean ± SEM throughout the text.

3. Results

The parameters described above for the NMDA iontophoretic injections resulted in significant thalamic lesions that were characterized by neuronal cell loss filled with gliosis (Fig. 1B and C). From the 10 bilateral NMDA AMv injections, we obtained good AMv bilateral lesions in seven injected animals, and these lesions also included the adjacent RE to a variable degree; in the remaining three bilateral AMv injected rats, we did not observe good lesions. As schematically shown in Fig. 2, the bilateral lesions of the AMv extended throughout the entire rostrocaudal extent, comprising 81.9 ± 3.5% of the nucleus, and expanded to the dorsal part to the anteromedial nucleus (AMd) and nucleus reuniens (RE). Only one out of the seven AMv lesions extended more severely in the AMd, comprising close to 40% of the nucleus, and the lesions of the other cases spread to include less than 20% of the AMd. On the other hand, the AMv lesions largely included the RE, comprising 75.7 ± 5.4% of the RE rostral half (Fig. 2). To control for the RE damage, six out of the ten NMDA injected animals in the RE presented with sizable and restricted lesions to the nucleus, including 76.5 ± 5.1% of the rostral half (Fig. 2).

3.1. Phase 1 – last day of habituation

During the habituation days, there was a clear decline of risk assessment responses (mostly seen in the first three to five days of the habituation period) to a more active and fearless exploration of the environment. During the habituation period, there was no obvious difference among the groups. By the 10th day, the animals were fully adapted to the apparatus, showed minimal defensive responses (i.e., freezing and risk assessment), and presented a great deal of fearless locomotion (exploration), as well as an upright position leaning on the apparatus walls while actively investigating the environment (Table 1). A one-way MANOVA did not reveal any significant difference among the experimental groups in this phase (Wilks lambda = 0.3105, $F(21, 49.36) = 1.18$, $p = 0.3071$). In addition, no significant effect was revealed by the one-way ANOVA for the variables analyzed (Table 1).

3.2. Phase 2 – defeat

During the defeat, all experimental groups exhibited intense defensive responses (e.g., animals showed passive defense responses, such as freezing and on the back postures, as well as active defense responses, such as flight and boxing). A one-way MANOVA revealed a significant difference among the experimental groups (Wilks

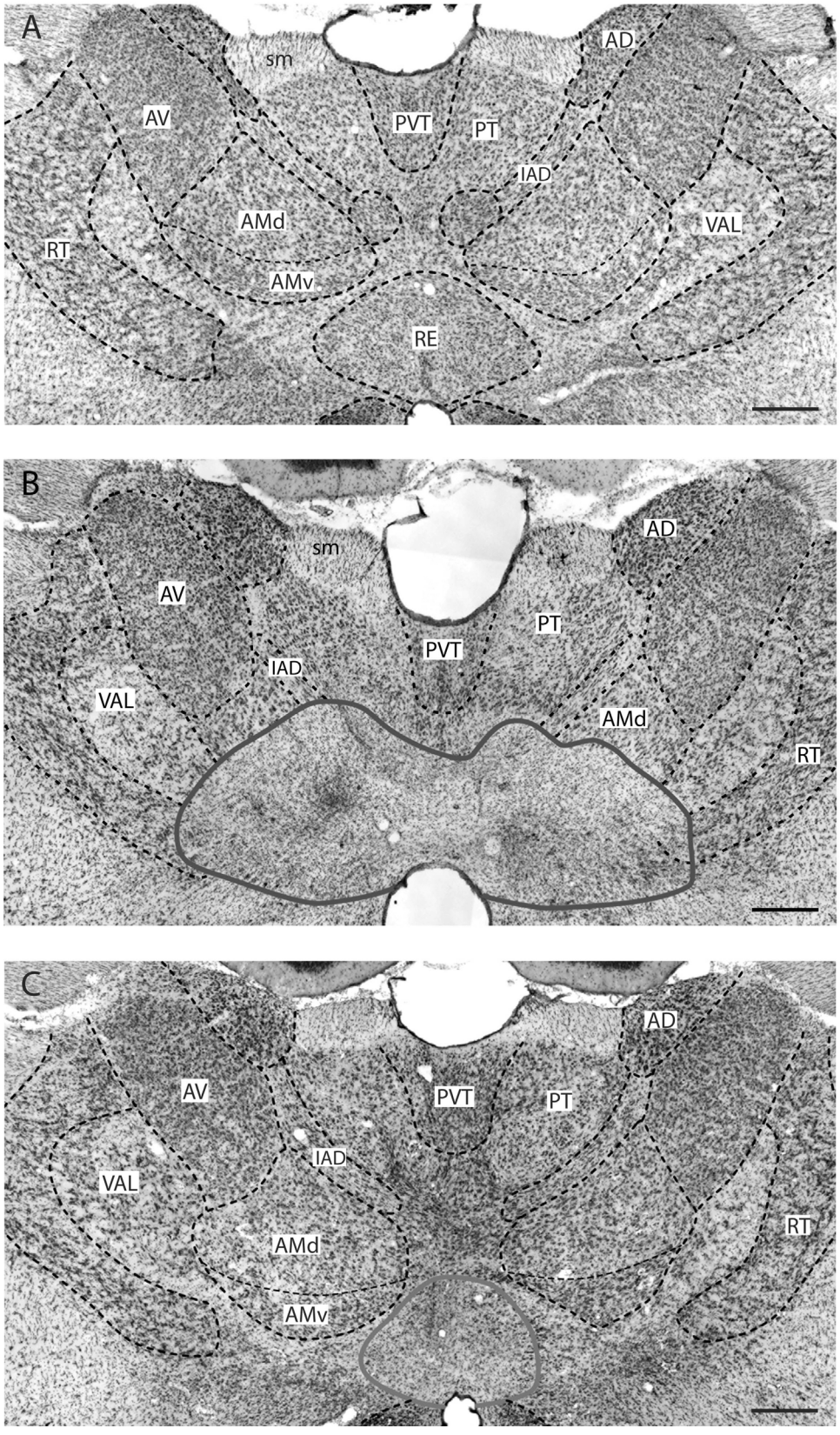

Fig. 1. (A) Photomicrograph of a transverse Nissl-stained section from an intact animal, that served as a reference depicting the thalamic field at the level of the lesions shown in B and C. (B-C) Photomicrographs of transverse Nissl-stained sections from representative cases, illustrating the extent and appearance of a bilateral lesion including the anteromedial and reuniens nuclei (AMv group, B), and a single nucleus reuniens lesion (RE group, C). The dark gray lines delineate the lesion extents. Abbreviations: AD – anterodorsal thalamic nucleus; AMd, v – anteromedial thalamic nucleus, dorsal and ventral parts; AV – anteroventral thalamic nucleus; IAD – intereanterodorsal thalamic nucleus; PT – paratenial nucleus; PVT – paraventricular thalamic nucleus; RE – nucleus reuniens; RT – reticular nucleus thalamus; sm – stria medularis; VAL – ventral anterior-lateral thalamic nucleus. Scale bars = 500 μm.

lambda = 0.2720, F(15, 52.85) = 2.12, p = 0.02289). Although an apparent increase in the active defense responses was seen in the RE lesioned group, alpha-adjusted univariate ANOVAs did not reveal a significant group effect for any behavioral dependent variable (Table 2).

3.3. Phase 3 – context exposure test

During exposure to the social defeat context, a one-way MANOVA revealed a significant effect for the factor group (Wilks lambda = 0.0739, F(21, 49.36) = 3.47, p = 0.00016). For the

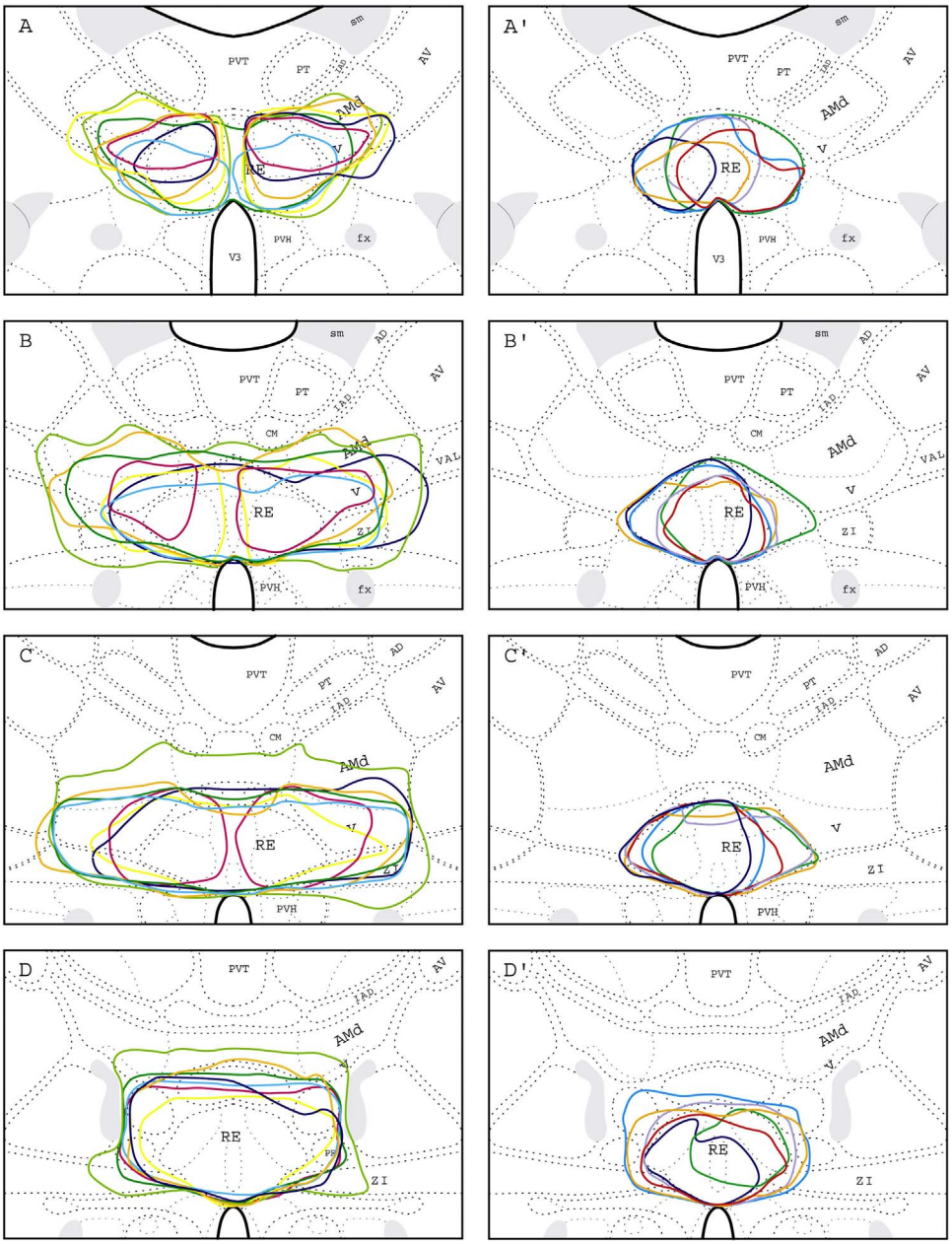

Fig. 2. Location and extent of bilateral *N*-Methyl-D-aspartate (NMDA) lesions including the anteromedial thalamic and reuniens nuclei (AMv group, seven cases, A–D) and the nucleus reuniens (RE group, six cases, A'–D') in socially defeated rats that were used for behavioral analysis. The approximate location and extent of each lesion was determined by analysis of Nissl-stained cytoarchitecture, and for comparison, the data are plotted on a reference rat brain atlas [26]. Abbreviations: AD – anterodorsal thalamic nucleus; AMd, v – anteromedial thalamic nucleus, dorsal and ventral parts; AV – anteroventral thalamic nucleus; CM – central medial thalamic nucleus; fx – fornix; IAD – intereanterodorsal thalamic nucleus; PT– paratenial nucleus; PVH – paraventricular hypothalamic nucleus; PVT – paraventricular thalamic nucleus; RE – nucleus reuniens; RT – reticular nucleus thalamus; sm – stria medularis; VAL – ventral anterior-lateral thalamic nucleus. ZI – zona incerta.

Table 1
Spatiotemporal and behavioral measurements during the last day of habituation (10th day).

	Experimental groups				
	Intact (n = 7)	RE (n = 6)	AMv (n = 7)	Sham (n = 7)	Statistics $F_{(3,23)}$; p
Spatio-temporal measurements					
Home cage	54.8 ± 15.0	39.6 ± 3.8	67.8 ± 14.4	54.5 ± 12.7	0.46; = 0.70
Corridor	90.6 ± 10.9	111,7 ± 10.6	97.3 ± 10.7	121.4 ± 10.7	1.43; = 0.45
Resident cage	154.3 ± 12.6	148.6 ± 9.4	134.7 ± 17.9	124.1 ± 10.7	0.98; = 0.41
Behavioral itens					
Risk assessment	3.5 ± 1.6	10.0 ± 3.9	4.8 ± 0.9	11.5 ± 3.9	2.99; = 0.05
Exploration	276.2 ± 10.1	285.5 ± 4.7	288.6 ± 1.9	278.5 ± 4.0	0.44; = 0.37
Rearing	2.5 ± 0.7	0.25 ± 0.25	3.0 ± 1.3	3.8 ± 1.9	2.05; = 0.13
Grooming	18.1 ± 10.6	3.8 ± 1.8	3.7 ± 1.5	6.1 ± 2.5	0.39; = 0.75

Values are mean ± SEM of the time in seconds during a 5-min observation period. ANOVA test adjusted by Bonferroni's correction, $\alpha = 0.007$.

Table 2
Behavioral measurements during encounter with the conspecific aggressor (11th day).

	Experimental groups				
	Intact (n = 7)	RE (n = 6)	AMv (n = 7)	Sham (n = 7)	Statistics $F_{(3,23)}$; p
Behavioral itens					
Passive defense	480.9 ± 42.7	351.5 ± 36.8	413.8 ± 67.5	436.2 ± 52.9	0.71; = 0.55
Active defense	66.8 ± 14.2	168.16 ± 24.8	97.0 ± 14.8	76.1 ± 30.2	3.18; = 0.04
Locomotion	31.4 ± 21.6	23.5 ± 13.2	51.3 ± 33.2	64.7 ± 35.5	0.44; = 0.72
Grooming	2.0 ± 2.0	14.8 ± 6.5	5.3 ± 5.2	2.2 ± 2.1	2.06; = 0.13
Social investigation	18.8 ± 14.8	32.3 ± 14.2	32.7 ± 29.6	20.8 ± 9.5	1.4; = 0.26

Values are mean ± SEM of the time in seconds during a 10-min observation period. ANOVA test adjusted by Bonferroni's correction, $\alpha = 0.01$.

Table 3
Spatiotemporal and behavioral measurements during context exposure (12th day).

	Experimental groups				
	Intact (n = 7)	RE (n = 6)	AMv (n = 7)	Sham (n = 7)	Statistics $F_{(3,23)}$; p
Spatio-temporal measurements					
Home cage	69.0 ± 16.6	108.5 ± 41.4	27.8 ± 2.6*	123.5 ± 28.6	5.28; = 0.006
Corridor	176.0 ± 23.6	95.1 ± 22.4	95.3 ± 16.1	111.4 ± 18.2	1.55; = 0.22
Resident cage	55.3 ± 24.8	95.6 ± 23.0	176.8 ± 41.1	65.0 ± 15.7	2.44; = 0.08
Behavioral itens					
Risk assessment	174.3 ± 13.1	141.5 ± 33.1	29.3 ± 8.1*	125.1 ± 22.0	16.21; = 0.000007
Exploration	112.0 ± 10.4	154.8 ± 32.7	264.6 ± 22.7	177.7 ± 25.3	1.69; = 0.19
Rearing	0.9 ± 0.3	1.5 ± 0.6	1.6 ± 0.8	1.1 ± 0.3	0.10; = 0.95
Grooming	11.2 ± 6.7	1.5 ± 0.8	4.6 ± 2.6	6.2 ± 2.0	1.31; = 0.29
Flight	1.3 ± 0.7	0.5 ± 0.3	0.0 ± 0.0	2.2 ± 1.2	1.76; = 0.18

Values are mean ± SEM of the time in seconds during a 5-min observation period. ANOVA test adjusted by Bonferroni's correction, $\alpha = 0.007$.
* $p < 0.05$ vs. all other groups (Newman-Keuls post hoc analysis).

spatiotemporal variables, univariate ANOVAs revealed a significant main effect for the time spent in the home cage but not for the other spatiotemporal measurements (Table 3). Post hoc pairwise comparisons (Newman-Keuls) revealed that the AMv lesion group spent significantly less time in the home cage compared to the other groups ($p < 0.046$), which exhibited no differences ($p > 0.26$) (Fig. 3). For the behavioral parameters, ANOVAs revealed a significant main effect for the time spent on risk assessment behaviors (i.e., crouch-sniff and stretch postures) but not for the other behavioral variables (Table 3). Compared to the other groups, post hoc pairwise comparisons (Newman-Keuls) revealed that the AMv lesion group exhibited significantly fewer risk assessment behaviors ($p < 0.001$) (Fig. 3).

A subsidiary analysis was carried out in which the spatiotemporal and behavioral dependent variables from phase 1 (last day of habituation) and phase 3 (context exposure test) were included in a single 2 × 4 MANOVA, with the two testing phases and four experimental groups as within-subjects and between-subjects factors, respectively. Following a significant multivariate interaction between the factors phase and group (Wilks lambda = 0.0613, F(21, 49.36) = 3.87, p = 0.000047), significant univariate interactions (adjusted $\alpha = 0.007$) were observed, as before, only for the time spent in the home cage (F(3, 23) = 5.29, p = 0.0063) and risk assessment behaviors (F(3, 23) = 7.53, p = 0.0011). For all of the other spatiotemporal and behavioral variables, no significant interaction between phase and group was observed (F(3, 23) < 2.74 and $p > 0.066$).

4. Discussion

Previous studies from our group have shown that the AMv has a role in contextual, but not innate, defensive responses to a predatory threat [24,25]. In this study, we expanded these findings by showing that an NMDA lesion in the AMv reduced contextual, but not innate, defensive responses to social defeat.

Considering that our AMv lesions included the RE, we further tested single lesions in the RE and found that they did not affect contextual fear responses to social threats. In line with this view, we obtained similar results with contextual fear to a predatory threat, in which cytotoxic lesions that circumscribed to the nucleus reuniens did not affect the contextual defensive responses to predatory threats [24]. In fact, other studies have indicated that the RE is part of the circuitry that controls fear memory generalization; direct silencing or activation of RE projections has been shown to enhance or decrease fear memory generalization, respectively [31]. In addition, AMv lesions extended to a small degree to the dorsal part of the nucleus, in which only one out of seven cases comprised close to 40% of the nucleus, whereas in the other cases, less than 20% of the AMd was included. Although we cannot entirely rule out the participation of the dorsal part of the AM, it appears clear that the AMv can account for the behavioral effects currently reported.

At this point, it cannot be determined from the present set of lesion experiments whether the AMv is involved in the acquisition and/or subsequent expression of contextual fear. However, some insight can be gained by examining the results of pharmacological inactivation of the AMv during a predator threat, where inactivation prior to cat exposure drastically reduced contextual conditioning to the predator-associated environment, but inactivation prior to exposure to the environment associated with the predator threat did not affect contextual fear [25]. In addition, the preliminary results from our laboratory on pharmacogenetic inactivation of the AMv during social defeat and exposure to the social defeat-associated environments also suggest a role in acquisition, but not in expression or recall, of contextual fear memory (M.J. Rangel Jr, I. Araújo and N.S. Canteras, personal observation).

In the present study, we used an experimental protocol that was previously developed by our group, where we observed, in the absence of a conspecific aggressor, robust contextual defensive responses to an environment associated with a single social defeat [17]. This protocol contrasts with previous studies on conditioned fear to social threat in rats and mice that required multiple social defeats and the presence of

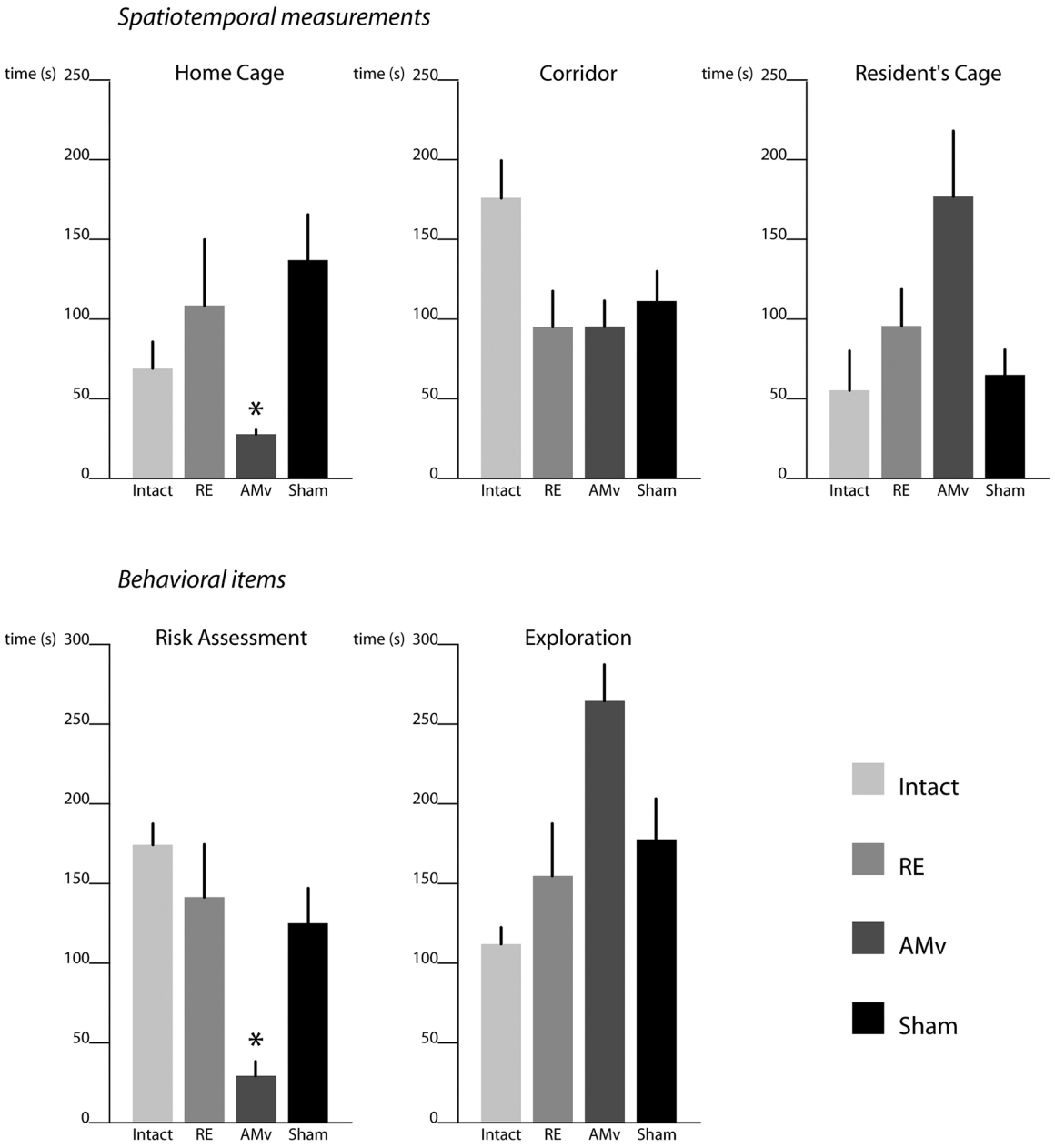

Fig. 3. Histograms representing the spatiotemporal and behavioral measurements during the context exposure test; for the intact animals (Intact; n = 7), and animals with reuniens lesions (RE; n = 6), bilateral AMv lesions (AMv; n = 7) and sham lesions (Sham; n = 7). Values are mean ± SEM of the time in s during a 10-min observation period. *p < 0.05 vs. all other groups (Newman-Keuls post hoc analysis).

the dominant aggressor [32,33]. However, in the present protocol, the socially defeated animals needed to be tested in the presence of sawdust from the resident's soiled bedding, which seemed to be critical for evoking contextual fear responses [7]. Notably, we have previously shown that defeated animals exposed to fresh bedding presented no conditioned responses and that the resident's soiled bedding itself did not induce aversive responses in non-defeated animals [7]. Therefore, the smell of the resident's soiled bedding becomes aversive after social defeat and seems to be necessary to evoke contextual responses.

Studies in hamsters on conditioned responses to social threats suggested that the medial amygdalar nucleus and ventral hippocampus were necessary for the acquisition and expression of conditioned defeat [34,35]. In line with this view, we found with the same experimental protocol from the present study that contextual fear responses in socially defeated animals appear to rely on pathways that originate from the ventral subiculum and medial amygdalar nucleus [7]. As schematically shown in Fig. 4, the ventral hippocampus, along with the lateral septum, likely process contextual cues from the environment associated with social defeat [35,36], and the medial amygdalar nucleus is known to process conspecific olfactory cues [37]. These paths project to the hypothalamus, where the septo-hippocampal branch provides strong inputs to the juxtadorsomedial region of the lateral hypothalamic area (LHAjd) [38]. Both the septo-hippocampal and amygdalar paths target elements of the conspecific-responsive medial hypothalamic circuit, comprising the medial preoptic nucleus, the ventrolateral part of the ventromedial nucleus, the tuberal nucleus and the ventral premammillary nucleus [39,40], which are highly interconnected and form a hypothalamic circuit that is responsive to social cues [see 41]. In the hypothalamus, both the LHAjd and the conspecific-responsive medial hypothalamic circuit influence the dorsal premammillary nucleus, which presents one of the most conspicuous states of activation in response to the social defeat-associated context and, in

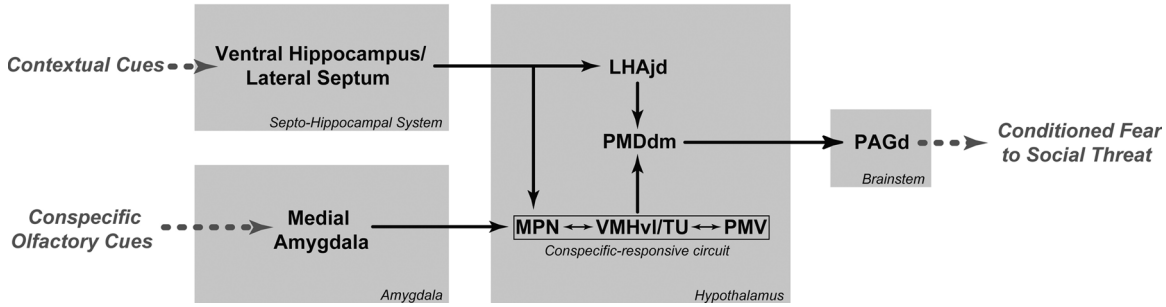

Fig. 4. Schematic diagram showing the putative brain circuits involved in processing contextual fear responses in socially defeated animals. Abbreviations: LHAjd – lateral hypothalamic area, juxtadorsomedial region; MPN – medial preoptic nucleus; PAGd periaqueductal gray, dorsal part; PMDdm – dorsal premammillary nucleus, dorsomedial part; PMV – ventral premammillary nucleus; TU – tuberal nucleus; VMHvl – ventromedial hypothalamic nucleus, ventrolateral part. Modified from Faturi and Rangel [7].

turn, projects to the dorsal periaqueductal gray, where pharmacological inactivation drastically reduces contextual fear response to a social threat [7].

According to the present findings, AMv lesions significantly reduced the amount of risk assessment and time spent in the home cage, but other parameters did not significantly change. However, it is noteworthy that, compared to the other groups, AMv-lesioned rats displayed a clear increase in the time spent in the resident cage and exploring the social defeat-associated environment (see Fig. 3). This partial effect appears to indicate that the AMv influences only part of the neural system that mediates contextual fear responses in socially defeated animals. Considering the two branches (i.e., the hippocampal and amygdalar branches) involved in mediating contextual fear responses in socially defeated animals (Fig. 4), the AMv should have a much larger impact on the hippocampal path. It is noteworthy that lesions of elements related to the hippocampal branch of the social-related contextual fear circuit, such as the juxtadorsomedial region of the lateral hypothalamic area (LHAjd), also yielded only a partial reduction of contextual fear responses to social threats, particularly in the time spent assessing risk but did not significantly change other behavioral or spatiotemporal measurements [42].

The AMv has a strong relationship with the hippocampal formation. We have recently revisited AMv projections and found that, apart from providing moderate projections to the ventral subiculum and CA1 field, the AMv may influence both the medial perforant path (via a pathway involving the presubiculum and superficial layers of the medial entorhinal area) and the lateral perforant path through substantial projections to the superficial layers of the lateral entorhinal area [25]. The evidence suggests that the AMv occupies a strategic position to convey social threat information to hippocampal circuitry, and hence influence contextual fear memory.

Previous studies have shown that anterior thalamic lesions may also affect contextual fear conditioning to footshock, but in a manner that differs from what has been found for predatory and social threats. Thus, anterior thalamic lesions encompassing the anteromedial, anteroventral and anterodorsal thalamic nuclei have been shown to slow the acquisition of contextual, but not cued, fear conditioning [43,44]. However, in contrast to what we have shown for predatory threats, and presently, for social defeat, contextual fear memory for footshock appears to be mostly unaffected in the short term, and anterior thalamic-lesioned animals presented clear freezing behavior when re-exposed to the conditioning context 24 h later [44]. Taken together, the evidence supports the idea that neural systems related to contextual fear responses to ethologically-based threats, such as predators and conspecific aggressors, contrast with the neural processing of contextual fear to physically aversive stimuli, such as footshock. In the case of ethologically-based threats, the AMv appears to be a critical hub, receiving massive inputs from the dorsal premammillary nucleus [14], that is largely responsive to both predatory and social threats [10,27], and the transfer of predatory and social threat information to circuits involved in the processing of contextual fear memories.

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