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**Original Article**

# Changes in cardiovascular function and related psychophysiological pathways in a rat model of post-traumatic stress disorder

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**Abstract:** This study was aimed to investigate the cardiovascular function in rats with post-traumatic stress disorder (PTSD) and the potential association with the activities of the rostral ventrolateral medulla (RVLM) and the medial habenular nucleus (MHb). Multi-channel *in vivo* recordings were used to simultaneously acquire spontaneous neuronal firing and peripheral physiological indices, and FluoroGold (FG) retrograde tracing technique was used to observe the projections of labeled neurons in the MHb. The results showed that the discharge frequency of RVLM and MHb neurons, the systolic blood pressure (SBP), and the mean arterial pressure (MAP) in the PTSD group were all increased significantly compared with those in control group ($P < 0.05$). MHb neurons were retrogradely labeled by FG through microinjection (4% FG, 0.5 μL) into the RVLM. In the control group, electrical stimulation in the MHb increased heart rate (HR) at 100–300 μA ($P < 0.05$), elevated SBP and MAP at 200–300 μA ($P < 0.05$), and remarkably increased the RVLM neuronal discharge frequency at 100–500 μA ($P < 0.05$ or $P < 0.01$). In the PTSD group, however, only the discharge frequency of RVLM neurons was increased by the electrical stimulation at 100–300 μA ($P < 0.05$). These results suggest that cardiovascular activities of the PTSD model rat are enhanced, and this change may be related to the activity changes of RVLM and MHb and the potential connection between the two nuclei.

**Key words:** post-traumatic stress disorder; cardiovascular physiology; rostral ventrolateral medulla; medial habenular nucleus

# 创伤后应激障碍大鼠心血管功能变化及相关心理生理学通路

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**摘 要**：本研究旨在探究创伤后应激障碍(post-traumatic stress disorder, PTSD)模型大鼠心血管功能以及延髓头端腹外侧(rostral ventrolateral medulla, RVLM)区和内侧缰核(medial habenular nucleus, MHb)神经元活动间的可能性联系。运用在体多通道同步记录技术结合荧光金(FluoroGold, FG)逆向示踪等技术，同步记录神经元自发放电和多项外周生理指标，并观察荧光金逆行标记神经元分布。结果显示，PTSD组大鼠RVLM、MHb神经元放电频率及收缩压和平均动脉压均显著高于正常对照组($P < 0.05$)。微量注射0.5 μL 4% FG至RVLM区，MHb区神经元被逆行标记；电刺激MHb后，正常对照组大鼠RVLM神经元放电频率在100~500 μA下较刺激前明显升高($P < 0.05$或$P < 0.01$)，心率在100~300 μA下较刺激前显著加快($P < 0.05$)，收缩压和平均动脉压在200~300 μA下较刺激前显著上升($P < 0.05$)；相比之下，PTSD组大鼠仅RVLM放电频率在100~300 μA下较刺激前明显升高($P < 0.05$)。以上结果提示，PTSD模型大鼠的心血管功能活动增强，该变化可能与RVLM、MHb神经元活动以及二者之间潜在的神经通路联系有关。

**关键词**：创伤后应激障碍；心血管生理学；延髓头端腹外侧区；内侧缰核
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Post-traumatic stress disorder (PTSD), also termed delayed psychogenic reaction, is a complex mental disorder that can occur following a catastrophic or fatal event [1]. The disorder is typically associated with a delayed onset, with high incidence and prolonged course. Prominent symptoms of PTSD are heightened re-experience and avoidance behavior related to negative emotions, including fear and anxiety [2, 3]. Previous studies have reported that PTSD patients frequently have cardiovascular functional changes, such as elevated heart rate (HR) and higher blood pressure [4, 5], however, the neural connection between the disorder and cardiovascular function remains to be elucidated.

Ventrolateral medulla serves as the cardiovascular regulation center, comprising of the rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM). The RVLM is a critical pressor area, where the descending neuron axons can innervate the cardiac sympathetic preganglionic and sympathetic vasoconstrictor preganglionic neurons in the intermediolateral column (IML) of spinal cord, regulating cardiovascular activities. Additionally, the RVLM is an indispensable medium for other cardiovascular centers within the brain, which participate in the control of cardiovascular function, mediate cardiovascular reflexes and maintain resting sympathetic tone [6, 7]. We therefore hypothesized that the RVLM could be an important nucleus in the central nervous system involved in the regulation of cardiovascular activity of PTSD patients.

The main symptom of PTSD in patients is the persistent long-term fear memory and inability to forget it. The occurrence of mental disorders based on fear memory involves the medial habenular nucleus (MHb) [8], where the MHb cholinergic system plays an important role in mental disease of emotional-related behavior, which is closely related to stress and fear [9]. Moreover, MHb is the pressor region, which integrates the function of autonomic nervous system, regulating cardiovascular activities. Studies have established that MHb participates in the formation of stress-induced hypertension (SIH) through the release of acetylcholine (ACh) via the locus coeruleus (LC)-RVLM pathway [6]. However, there is a lack of studies that can demonstrate whether changes in cardiovascular function of PTSD patients are associated with these nuclei.

In the present study, we investigated the changes of cardiovascular activities in a rat PTSD model and explored whether these changes were related to the activities of the RVLM and MHb neurons by using a retrograde tracing technique and electrical stimulation of nucleus. This work may provide a theoretical basis for the treatment of cardiovascular dysfunction in clinical PTSD.

## 1 MATERIALS AND METHODS

### 1.1 *Animal preparation*

Adult male Sprague-Dawley (SD) rats, weighing 190–230 g, were provided by Qinglongshan Animal Farm (Nanjing, China). All experimental animals and procedures were approved by the Experimental Animal Ethics Committee of Wannan Medical College. The rats were randomly divided into PTSD group and control group, and ate and drank freely under a temperature of 24–26 °C for 7 days. During this process, the rats were observed daily, such as food intake, water consumption, daily activities, and mental state.

Rat models of PTSD were prepared according to the following protocols. Based on the internationally recognized single prolonged stress (SPS) method, the compound stress (CS) method with an electrical stimulation protocol was used to establish the model [10]. Firstly, the rats were placed into an enclosed hard plastic bottle for 2 h, where only the tail could move. In parallel, the rats' tails were subjected to inescapable constant electrical stimulation (30 V) for 20 s at 10 s intervals for 1 h. The rats were immediately placed into a water container, with a depth of 40 cm at 20 °C, and were forced to swim until exhaustion. The rats returned into the cage for routine feeding following the protocol. Rats in the control group were fed in the same environment without special treatment.

The effectiveness of the 7-day modeling was determined by weight gain and behavioral tests. The open-field (OF) test was used to reflect the activity and anxiety-like behavior, and the elevated plus maze (EPM) was used to evaluate the anxiety-like behavior of the experimental animals.

The test parameters for the OF test included the total distance, the times of crossing, and the stopover time in the central region. The test area and parameters were set in Smart 3.0 system (Panlab, Harvard Apparatus, USA) in advance. Four rats were selected and put into the box of the OF test for detection at the same time, and the exploration time was 5 min. After that, the data were analyzed by computer.

The EPM test parameters included the ratios of open arm entry (OE%) and open arm time (OT%) within 5 min. In Smart 3.0 system, the scope of open arm, closed arm and central area and various parameters were also set in advance. Each time, one rat was placed into the box of the EPM (Zhenghua Biological Instrument Equipment Co., Ltd., Anhui, China) from the central area with its face facing the open arm, and the 5-minute trajectory was recorded.

1.2 *Multi-channel synchronous in vivo recording*

In accordance with our previous study [11], rats were intraperitoneally injected with mixed anesthetics (14 g urethane, 0.7 g α-chloralose and 0.7 g borax per 100 mL normal saline, 0.6–0.7 mL/100 g) and fixed on a double-arm stereotaxic apparatus (Model 68002, RWD Life Science Co., Ltd., China). A plastic catheter filled with 0.1% heparin sodium saline (Biosharp, China) was carefully inserted into the proximal end through the incision at the distal end of the right femoral artery, and connected to a bridge amplifier (ML 221, AD Instruments, Australia) via a blood pressure transducer. The input signals were sent to the PowerLab multichannel physiological signal acquisition and processing system (PowerLab 8/30, AD Instruments, Australia) and a standard limb II lead electrocardiogram was collected by needle electrodes.

To synchronously record the discharges of neurons in both nuclei, the right RVLM (12.00–12.36 mm posterior to bregma, 1.90–2.40 mm lateral to midline, and 8.00–8.50 mm below the meninges) and the right MHb (2.52–4.20 mm posterior to bregma, 0.15–0.60 mm lateral to midline, 4.45–5.20 mm below the meninges) were respectively located, according to the atlas of Paxinos and Watson [12], and a precise hole was subsequently drilled into them. A glass recording microelectrode (resistance 5–15 MΩ) , which contained 2% Chicago Sky Blue (Sigma, USA) dissolved in 0.5% sodium acetate solution (Sinopharm, China), was fixed above the skull window. The reference electrode was inserted into the parietal edge. The electrode was manually fine-tuned until clear and stable neuronal discharge signals were recorded. Neuronal input signals were acquired by the PowerLab system via an amplifier (ML 408, AD Instruments, Australia).

1.3 *Electrical stimulation of MHb neurons*

Anesthetized rats were fixed with the same method as described above. The concentric stimulation electrode, fixed on the left arm, was positioned and slowly inserted into the MHb. The recording electrode fixed on the right arm was located and slowly inserted into the RVLM. To observe the stimuli-induced activities of RVLM neurons and the changes of peripheral physiological indices, once signals and peripheral indicators were synchronous and stable, the electrical sitmulation (100–500 μA, 0.2 ms, 100 Hz) was applied to the MHb by the pulse stimulator (MODEL 2100, A-M Systems, USA) for 0.2 s.

1.4 *Retrograde tracing with FluoroGold (FG)*

The rats from the control group were anesthetized and fixed by the aforementioned method. In conjunction with our previous study [13], 0.5 μL saline solution containing 4% FG (Fluorochrome Inc., Denver, USA) was slowly injected into the RVLM for 5 min and maintained for 10 min to prevent reflux. After microinjection, the wound was filled with a gelatin sponge and sterilized with 75% alcohol for surgical suturing. Following a 7-day recovery period, the rats were injected with mixed anesthetics (0.6–0.7 mL/100 g) again and perfused through the left ventricular intubation. The heart was perfused with 0.9% sodium chloride injection (Shuanghe Pharmaceutical Co., Ltd, Anhui, China), and 4% paraformaldehyde solution (PFA, Biosharp, China). Brain tissues were obtained and refrigerated in 4% PFA solution for 24 h, and then successively dehydrated with 20% and 30% sucrose solution until sinking. Finally, 40-μm brain slices were prepared in a cryostat box microtome (HM525 NX, Thermo Scientific, Germany). The retrogradely labeled neurons in the target area were observed under a standard fluorescent microscope (BX 53, Olympus, Japan), and identified according to the atlas [12].

1.5 *Data analysis*

LabChart software (AD Instruments, Australia) was used to analyze raw signals. Data were expressed as mean ± SEM. An independent sample *t*-test was used to compare the data between groups, and repeated measures analysis of variance (ANOVA) was used to compare data before and after electrical stimulation. A *P* value of less than 0.05 ($P < 0.05$) was considered to be statistically different.

## 2 RESULTS

### 2.1 Identification of PTSD in rats

#### 2.1.1 Body weight gain

The difference of body weight before and after the

establishment of the model was the body weight gain. After 7 days of modeling, the body weight gain of the PTSD group was significantly lower than that of the control group ($P < 0.05$, Fig. 1).

#### 2.1.2 Behavioral changes

The stopover time in the central region and the total distance of the PTSD model group in the OF test were less than those of the control group ($P < 0.05$, Table 1). This indicated that the range of activity was reduced and level of anxiety was increased in the PTSD model group. For the EPM test, OE% and OT% decreased distinctly in the PTSD model group compared with the control group ($P < 0.05$, Table 1). This suggested that the rats in the model group were more anxious and fearful.

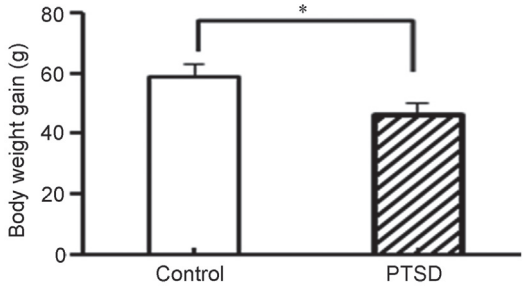


Fig. 1. Comparison of body weight gain between the control group ($n = 14$) and PTSD group ($n = 14$) at the end of the 7-day modeling. Data represent mean ± SEM, independent sample *t*-test. $^{*}P < 0.05$.

### 2.2 Changes in cardiovascular activities and neuronal discharges of MHb and RVLM after PTSD

Neuronal discharges of the two nuclei and multiple peripheral physiological indices were recorded simultaneously in the PTSD group ($n = 14$) and the control group ($n = 14$), respectively. Discharge frequency of RVLM and MHb neurons, systolic blood pressure (SBP) and mean arterial pressure (MAP) in the PTSD group were all significantly higher than those in the control group ($P < 0.05$, Table 2). An accelerated trend in HR was observed in the PTSD group, but was not significantly different compared with the control group.

### 2.3 Connection between MHb and RVLM in PTSD

#### 2.3.1 Morphological connection between MHb and RVLM

FG, a retrograde tracer, was used to investigate whether there was a neural potential connection between neurons. Brain tissue slices were placed under the fluorescent microscope following FG microinjection into the RVLM to observe the distribution of neurons. We observed that the soma of neurons in MHb area were marked retrogradely (Fig. 2*A*, *B*, *C*); In addition, the descending fibers of the hypothalamic paraventricular nucleus (PVN) neurons were known to project to RVLM, and we observed that the soma of PVN were marked retrogradely as well (Fig. 2*D*, *E*, *F*).

#### 2.3.2 Functional connection between MHb and RVLM

Rats from the control group ($n = 14$) and PTSD group

Table 1. Data comparison of control and PTSD groups in OF and EPM tests

| Group | OF | | | EPM | |
|---|---|---|---|---|---|
| | Total distance (cm) | Times of crossing | Stopover time in the central region (s) | OE% | OT% |
| Control | 2 167.83 ± 307.24 | 50.71 ± 3.00 | 14.12 ± 3.17 | 27.27 ± 2.77 | 21.17 ± 3.94 |
| PTSD | 1 286.61 ± 120.27$^{*}$ | 43.86 ± 3.26 | 6.35 ± 1.57$^{*}$ | 20.18 ± 1.84$^{*}$ | 11.10 ± 1.84$^{*}$ |

Data represent mean ± SEM, $n = 14$. Control group and PTSD group data were compared using an independent sample *t*-test. $^{*}P < 0.05$ *vs* control. OF: open-field; EPM: elevated plus maze; OE%: the ratio of open arm entry; OT%: the ratio of open arm time.

Table 2. Comparison of cardiovascular parameters and discharge frequency of RVLM and MHb neurons in control and PTSD groups

| | Control ($n = 14$) | PTSD ($n = 14$) |
|---|---|---|
| RVLM discharge frequency (Hz) | 14.73 ± 0.84 | 18.51 ± 1.10$^{*}$ |
| MHb discharge frequency (Hz) | 14.06 ± 0.90 | 17.17 ± 1.06$^{*}$ |
| HR (beats/min) | 390.39 ± 13.81 | 403.90 ± 16.14 |
| SBP (mmHg) | 101.31 ± 3.63 | 112.05 ± 3.52$^{*}$ |
| DBP (mmHg) | 81.50 ± 4.20 | 92.30 ± 4.64 |
| MAP (mmHg) | 89.01 ± 4.36 | 103.64 ± 4.04$^{*}$ |

Data represent mean ± SEM. Control group and PTSD group data were compared using an independent sample *t*-test. $^{*}P < 0.05$ *vs* control. HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure.

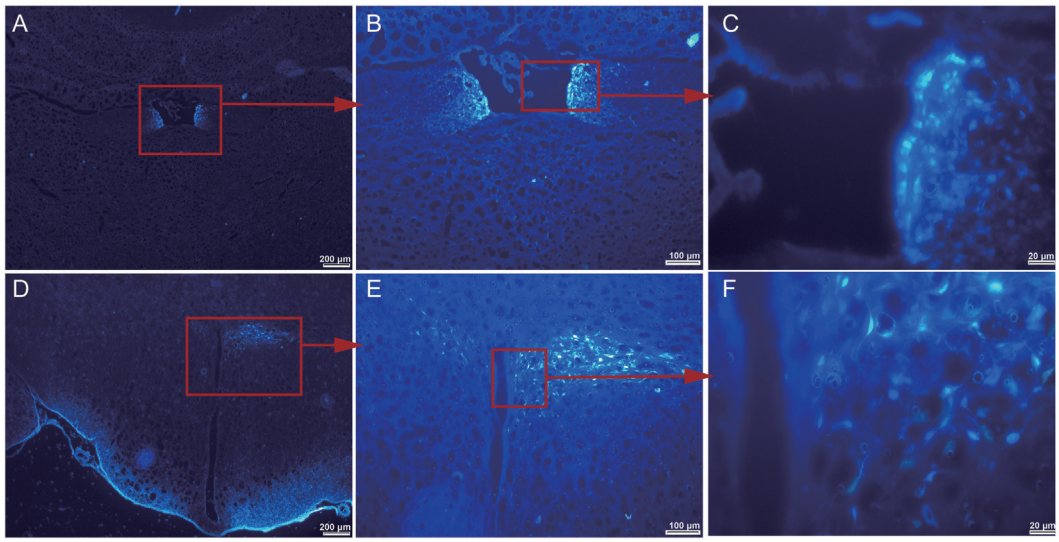


Fig. 2. Original images of MHb (*A*, *B*, *C*) and PVN (*D*, *E*, *F*) neurons retrogradely labeled after FluoroGold (FG) microinjection in the RVLM. Scale bar: 200 μm (*A*, *D*), 100 μm (*B*, *E*), or 20 μm (*C*, *F*). The fluorescent area in the red box is the distribution site of fluorescent retrogradely labeled neurons. RVLM: rostral ventrolateral medulla; MHb: medial habenular nucleus; PVN: hypothalamic paraventricular nucleus.

($n$ = 13) were subjected to MHb electrical stimulation. The changes of RVLM neuronal activity and peripheral physiological indices were depicted in Fig. 3. In the control group, rat MHb was electrically stimulated (100–500 μA), resulting in elevated HR at 100–300 μA ($P < 0.05$). Additionally, SBP and MAP were significantly increased at 200–300 μA ($P < 0.05$) and were accompanied by a notable increase in the RVLM neuronal discharge frequency at 100–500 μA ($P < 0.05$ or $P < 0.01$) (Table 3). In contrast, in the PTSD rat group, HR showed an upward trend at 100–300 μA, but a downward trend at 400–500 μA, and only the RVLM neuronal discharge frequency increased significantly at 100–300 μA ($P < 0.05$) (Table 3).

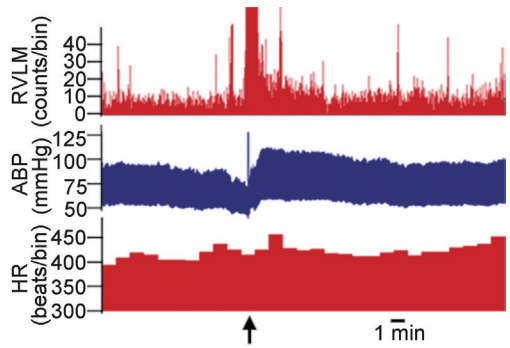


Fig. 3. Histograms of RVLM neuron firing (bin = 1 s), HR (bin = 60 s) and the tracing of ABP before and after MHb electrical stimulation. RVLM: rostral ventrolateral medulla; MHb: medial habenular nucleus; HR: heart rate; ABP: arterial blood pressure. The time of electrical stimulation was indicated by the black arrow.

### 2.4 Identification of MHb and RVLM

Following morphological and functional analysis, rat brain tissue was obtained to determine the accuracy of the sites according to the atlas [12]. The tracer injection site and the diffusion maps closely matched the coordinates of the RVLM (Fig. 4*A*). A typical correct position of nuclei in multi-channel synchronous *in vivo* recording was shown as Fig. 4*C*, *E*. The representative site of MHb under electrical stimulation was also retained (Fig. 4*G*).

## 3 DISCUSSION

PTSD is characterized by an imbalance of mental state. Humans and other organisms experience negative emotions such as anxiety, abnormal alertness, and fear when confronting catastrophic or life-threatening situations, which can lead to the occurrence of PTSD. The neurophysiology link between this mental disorder and clinical changes in cardiovascular function however remains unknown.

The CS method was adopted to establish a PTSD rat model to gain crucial insights into the neurophysiology connection between PTSD and cardiovascular function. This method is more practical and animal behavior responses are more sensitive compared with the recog-

Table 3. Comparison of cardiovascular parameters and RVLM neuronal discharge frequency before and after MHb electrical stimulation in control and PTSD groups

| | Group | Before stimulation | Electrical stimulation intensity (μA) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 100 | 200 | 300 | 400 | 500 |
| RVLM discharge frequency (Hz) | Control | 15.27 ± 0.52 | 16.42 ± 0.65$^{\#}$ | 17.07 ± 0.63$^{\#}$ | 16.99 ± 0.46$^{\#\#}$ | 17.16 ± 0.51$^{\#\#}$ | 16.44 ± 0.51$^{\#}$ |
| | PTSD | 17.22 ± 0.77 | 18.03 ± 0.62$^{\#}$ | 19.01 ± 0.51$^{\#}$ | 18.79 ± 0.59$^{\#}$ | 18.43 ± 0.49 | 17.53 ± 0.45 |
| Heart rate (beats/min) | Control | 412.86 ± 16.61 | 426.68 ± 16.23$^{\#}$ | 437.54 ± 15.40$^{\#}$ | 445.17 ± 11.46$^{\#}$ | 440.24 ± 10.28 | 427.44 ± 10.03 |
| | PTSD | 442.62 ± 10.57 | 443.91 ± 11.99 | 446.05 ± 9.68 | 440.57 ± 10.37 | 424.44 ± 12.01 | 401.85 ± 15.01 |
| SBP (mmHg) | Control | 104.15 ± 5.01 | 106.95 ± 6.42 | 113.15 ± 4.80$^{\#}$ | 113.42 ± 4.57$^{\#}$ | 109.69 ± 4.70 | 104.73 ± 4.63 |
| | PTSD | 109.25 ± 4.98 | 105.23 ± 5.85 | 109.37 ± 5.52 | 108.37 ± 6.97 | 105.50 ± 7.74 | 99.40 ± 8.41 |
| DBP (mmHg) | Control | 70.09 ± 2.73 | 71.83 ± 3.97 | 74.20 ± 3.55 | 75.27 ± 3.54 | 69.97 ± 3.37 | 66.72 ± 3.45 |
| | PTSD | 81.56 ± 5.59 | 81.57 ± 4.26 | 84.50 ± 4.14 | 83.18 ± 3.19 | 77.57 ± 2.88 | 73.48 ± 3.55 |
| MAP (mmHg) | Control | 82.70 ± 2.76 | 84.41 ± 4.23 | 88.51 ± 3.50$^{\#}$ | 89.21 ± 3.19$^{\#}$ | 84.21 ± 3.23 | 75.61 ± 6.51 |
| | PTSD | 93.34 ± 4.50 | 90.74 ± 4.40 | 94.14 ± 3.81 | 92.79 ± 3.32 | 88.65 ± 3.59 | 83.62 ± 4.58 |

Data represent mean ± SEM. In control group, $n$ = 14; In PTSD group, $n$ = 13. Differences between the indices before and after electrical stimulation were compared by repeated measures ANOVA. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* before stimulation. The abbreviations are identical to those listed in Table 2.

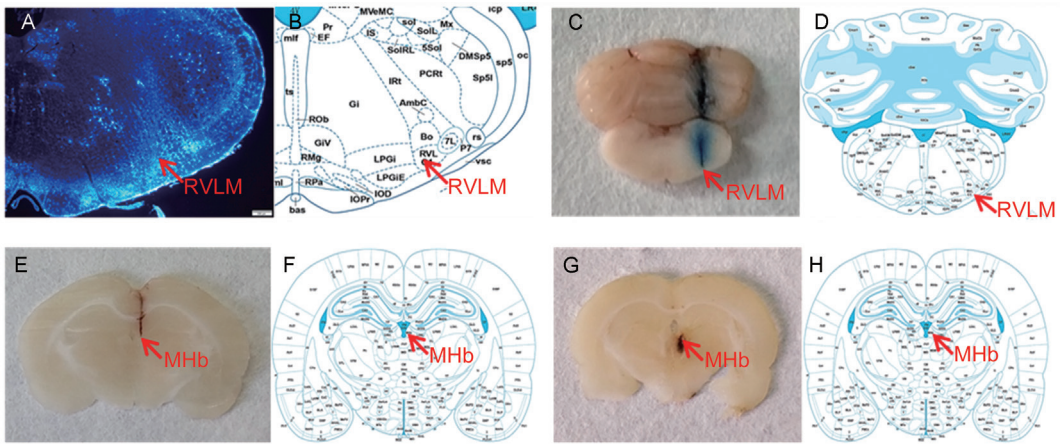


Fig. 4. Coronal sections of rat brain tissue. *A*: FluoroGold (FG) injection site in the RVLM. Scale bar, 200 μm. *C*, *E*: The RVLM and MHb sites for synchronous recording. *G*: Electrical stimulation site of the MHb. *B*, *D*, *F*, and *H* are coronal maps of RVLM and MHb selected from the brain atlas. RVLM: rostral ventrolateral medulla; MHb: medial habenular nucleus.

nized SPS method, and additionally it better reflects the occurrence of human fear-related PTSD, eliminating the effects of anesthetics on the CNS [10]. The success of using this method to replicate a PTSD model was reinforced by the EPM and OF test results, showing a decrease in rat activity and a noticeable state of anxiety in the PTSD model group.

Analogous to clinical findings of cardiovascular function between PTSD patients and non-PTSD controls [5], the SBP and MAP of PTSD rats were significantly higher compared with those of the control group, and had an accelerated trend in HR. The RVLM discharge frequency was also notably higher in the PTSD model group. Neurons regulating cardiovascular activity are widely distributed within the brain. The function of the cardiovascular system is coordinated by closely related neurons, performing distinctive functions [6]. It is believed that RVLM neurons have spontaneous electrophysiological activities, receive information from other peripheral and central sources, and control vasoconstrictive activity and arterial pressure [14], and additionally may participate in visceral sympathetic reflex [15]. When neurons in the RVLM are excited, they positively drive sympathetic nerve activity, leading to increased blood pressure.

We speculate that the changes in cardiovascular

activity in PTSD patients may be related to abnormality of autonomic nervous function, which could indeed be an underlying mechanism for anxiety and fear-related behavior.

There are two possible reasons for the significant increase in the discharge frequency of MHb neurons in the PTSD model group. Firstly, the habenular nucleus could be activated by negative emotions. Interestingly, a recent study reported that the expression of fear memory was reduced when MHb cholinergic neurons were activated, and when inhibited, the reduction of fear memory expression was hindered [8]. This could offer a new approach for the treatment of PTSD besides the traditional amygdala. Secondly, the angiotensin converting enzyme (ACE) in the habenular nucleus is in high density [16], and angiotensin II (Ang II) in MHb can increase blood pressure [17]. Our results suggest that activities of RVLM and MHb neurons may both be involved in the regulation of cardiovascular function in the PTSD rat model.

Electrophysiological *in vivo* recording showed that the discharge frequency of the two nuclei neurons increased. We therefore speculate that there might be a direct or indirect relationship between the RVLM and MHb. As a relay nucleus of the limbic system and brainstem structure, the habenular nucleus receives ascending fiber projections of various neurotransmitters, and also converges multiple afferent fibers of limbic forebrain structure [18]. It has been reported that the efferent neurons of MHb release glutamate (Glu) and ACh [19], and there are a variety of neurotransmitter receptors, including cholinergic receptors and glutamate receptors, in the RVLM.

Retrograde tracing is a method used to explore fiber connections between neurons by injecting a tracer at the end of an axon and retrogradely transporting it into the soma along the axon [20]. FG was selected to be the retrograde tracer in the morphological observations due to its enhanced stable chemical properties and luminosity, and favorable cellular features including the resistance to extravagate and quench after cellular filling [21]. FG can indicate a possible neural fiber connection between MHb and RVLM. Moreover, it has been shown that the axons of neurons distributed in the PVN can descend directly to the RVLM area [22]. In accordance with our previous study [13], the present study showed that the ipsilateral PVN neurons were retrogradely labeled with the tracer injected in the RVLM area, verifying the accuracy of the injection site.

Electrical stimulation of MHb in the control group increased arterial blood pressure (ABP) and HR at 100–300 μA. This was in agreement with previous reports indicating the increase of blood pressure and HR following stimulation of the habenular in rats [23, 24] and was spatially dependent, i.e., when the stimulation was closer to the medial, the more obvious the pressor effect was [24]. MHb integrates the received excitement and transmits it down to the medulla oblongata [25], causing sympathetic excitation to alter peripheral activity. We observed an evident increase of the RVLM firing frequency during the synchronous recording; however, the specific neurotransmitter and receptor pathway connections between MHb and RVLM still remain to be confirmed.

The RVLM neuronal discharge frequency in the PTSD model group increased significantly by electrical stimulation at 100–300 μA in MHb, but no clear changes were observed in other indices. At 400–500 μA, each index showed a downward trend. We suggest that this might be due to the fact that the MHb neurons of PTSD model rats were already in an excited state. High-current stimulation induces a reduction of the excitatory glutamatergic output from the MHb, which results in the deactivation of neurons, thus inhibiting firing frequency [26, 27]. Moreover, MHb coordinates with the hypothalamus to modulate cardiovascular activities. During stressful situations when high-current stimulation is applied, the MHb may become damaged and the hypothalamus will be decompensated. The synergistic effect of the hypothalamus and MHb on cardiovascular regulation could therefore be weakened, leading to the decrease of heart rate and blood pressure [18]. Overall, we speculate that the MHb and RVLM participate in the regulation of cardiovascular function in the rats from the PTSD group.

In this study, the cardiovascular activities of rats from the PTSD group were enhanced, which could be related to the neuronal discharge of RVLM and MHb, and the possible psychophysiological pathway between them. However, there are limitations in the present study. For example, this study preliminarily observed the discharge activity of neurons, which may not definitely explain the psychophysiological problems. The cardiovascular and psychological effects in PTSD patients are very complex, and various mechanisms are involved. Altogether, this study demonstrated neuronal activities of MHb and RVLM, providing insightful knowledge to decipher central mechanisms.

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