

# The alteration of neuronal activities of the cuneiform nucleus in non-hypovolemic and hypovolemic hypotensive conditions

Alterações da atividade neuronal no núcleo cuneiforme em condições hipovolêmicas e não hipovolêmicas

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## ABSTRACT

**Background:** The cuneiform nucleus is located in the center of the circuit that mediates autonomic responses to stress. Hemorrhagic hypotension leads to chemoreceptor anoxia, which consequently results in the reduction of baroreceptor discharge and stimulation of the chemoreceptor. **Objective:** Using the single-unit recording technique, the neuronal activities of the cuneiform nucleus were investigated in hypotensive states induced by hemorrhage and administration of an anti-hypertensive drug (hydralazine). **Methods:** Thirty male rats were divided into the control, hemorrhage, and hydralazine groups. The femoral artery was cannulated for the recording of cardiovascular responses, including systolic blood pressure, mean arterial pressure, and heart rate. Hydralazine was administered via tail vein. The single-unit recording was performed from the cuneiform nucleus. **Results:** The maximal systolic blood pressure and the mean arterial pressure significantly decreased and heart rate significantly increased after the application of hydralazine as well as the following hemorrhage compared to the control group. Hypotension significantly increased the firing rate of the cuneiform nucleus in both the hemorrhage and hydralazine groups compared to the control group. **Conclusions:** The present data indicate that the cuneiform nucleus activities following hypotension may play a crucial role in blood vessels and vasomotor tone.

**Keywords:** Hemorrhage; Blood Pressure; Cardiovascular System; Hydralazine; Electrophysiology.

## RESUMO

**Antecedentes:** O núcleo cuneiforme está localizado no centro do circuito que media as respostas autonômicas ao estresse. A hipotensão hemorrágica leva à anóxia dos quimiorreceptores, que, conseqüentemente, resulta na redução da descarga dos barorreceptores e estimulação do quimiorreceptor. **Objetivo:** Utilizando a técnica de registro em unidade única, as atividades neuronais do núcleo cuneiforme foram investigadas em estados de hipotensão induzida por hemorragia e administração de um anti-hipertensivo (hidralazina). **Métodos:** Trinta ratos machos foram divididos nos grupos controle, hemorragia e hidralazina. A artéria femoral foi canulada, para o registro de respostas cardiovasculares, incluindo pressão arterial sistólica, pressão arterial média e frequência cardíaca. A hidralazina foi administrada na veia da cauda. O registro de unidade única foi realizado a partir do núcleo cuneiforme. **Resultados:** A pressão arterial sistólica máxima e a pressão arterial média diminuíram significativamente, e a frequência cardíaca aumentou significativamente após a aplicação de hidralazina, bem como a hemorragia seguinte, em comparação com o grupo controle. A hipotensão aumentou significativamente a taxa de disparo da população do núcleo cuneiforme em ambos os grupos de hemorragia e hidralazina, em comparação com o grupo de controle. **Conclusões:** Os presentes dados indicam que as atividades do núcleo cuneiforme após hipotensão podem desempenhar um papel crucial nos vasos sanguíneos e no tônus vasomotor.

**Palavras-chave:** Hemorragia; Pressão Sanguínea; Sistema Cardiovascular; Hidralazina; Eletrofisiologia.

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**Conflict of interest:** There is no conflict of interest to declare.

**Authors' contributions:** RM: conceptualization, data curation, investigation, methodology, project management, software and writing of the first draft; HA: investigation, methodology, software and validation; MNS: formal analysis, methodology, visualization and writing of the first draft; AG: funding acquisition, project management, supervision, validation, writing, reviewing and editing; SSN: conceptualization, data curation, investigation, supervision and writing, reviewing and editing.

Received on December 03, 2020; Received in its final form on February 02, 2021; Accepted March 05, 2021.

## INTRODUCTION

The central nervous system plays a central role in regulating hypotension during hypovolemia and non-hypovolemic hypotension. Baroreceptors and chemoreceptors are activated by low blood pressure<sup>1</sup>. This afferent information is transmitted by carotid receptors (chemo- and baroreceptors) to the nucleus of the solitary tract (NTS)<sup>2</sup>. Thereafter, signals from the nucleus tractus solitarius (NTS) project to several brain areas that are involved in cardiovascular regulation, such as the caudal ventrolateral medulla (CVLM), paraventricular nucleus (PVN), and the rostral ventrolateral medulla (RVLM)<sup>2</sup>, to restore blood pressure to normal range. Despite intensive investigations, the exact modulatory mechanism of different brain regions during hypotension needs to be elucidated.

The cuneiform nucleus (CnF), a reticular nucleus of the midbrain, is a mesencephalic area implicated in several functions, including regulation of the cardiovascular system, pain modulation, sleep, and movement<sup>3</sup>. The CnF is reciprocally connected to different brain areas involved in the regulation of the cardiovascular system, such as the Kolliker-Fuse nucleus (KF), the periaqueductal gray matter (PAG), the NTS, the locus coeruleus, the raphe nucleus, the lateral hypothalamus, PVN, and the RVLM<sup>4</sup>. The CnF is located in the center of a circuit that mediates autonomic responses related to stress. This circuit has two parts: a sympathetic part that increases the blood pressure and a parasympathetic part that decreases the heart rate<sup>5</sup>. However, it is reported that the most effective area of the CnF is the sympathoexcitatory area, through which electrical or chemical stimulation increases the arterial blood pressure<sup>6</sup>.

Hypotension disrupts blood supply to the tissues, which may lead to tissue hypoxia<sup>7</sup>. Hypovolemic (hemorrhage) and non-hypovolemic (heart failure, sepsis, and drug-induced vasodilation) factors are common causes of severe hypotension<sup>8</sup>. Several brain areas such as the preoptic area, PVN, and RVLM are involved in the regulation of the cardiovascular system during hypotension. In addition, the CnF might also be involved in this process. It has been reported that hemorrhage increases expression of C-fos in the CnF, as well as the functional interconnection between the CnF and PVN and NTS areas that are involved in hypotension<sup>9</sup>. Furthermore, the glutamatergic, cholinergic, serotonergic, and opioidergic pathways that are involved in hypotension are found in the CnF<sup>4,10,11,12,13,14</sup>. However, the exact mechanism of the CnF action during hypotension and whether this nucleus is involved in hypovolemic (induced by hemorrhage) or non-hypovolemic (induced by hydralazine, a vasodilator drug) hypotension is still not elucidated. Therefore, the present study aimed to investigate blood pressure response and neuronal activities of the CnF following hypovolemic and non-hypovolemic hypotension in rats.

## METHODS

### Animals and surgery

Thirty Wistar rats (220–250 g) were provided from the animal house of the Faculty of Medicine of Mashhad. Animals were kept in a 12-h light-dark cycle, constant temperature (22±2°C), and were allowed free access to standard laboratory diet and water. Experiments were approved by the Ethics Committee of Mashhad University of Medical Sciences. Animals were anesthetized with urethane (1.5 g/kg, i.p.; Sigma, USA) and supplementary doses (0.7 g/kg) were given if necessary<sup>15</sup>. The body temperature of the animal was maintained at 37°C with a thermostatically controlled heating lamp. A polyethylene catheter (PE-50) was inserted into the femoral artery for blood pressure recording by the Power Lab instrument<sup>15</sup>. Animals were secured on stereotaxic apparatus and a hole was drilled above the CnF region according to the coordinates of the Paxinos atlas (Anterior-posterior; -7.6 to -8.5, Lateral; 1.7 to 2.2 and Height; -5.5 to -6.2<sup>16</sup>).

### Recordings of cardiovascular and neuronal activities

Blood pressure (BP) and heart rate (HR) were continuously recorded by an MLT844 pressure transducer coupled to a pre-amplifier (FE221 Bridge amplifier, AD instruments, AU) connected to a Power Lab 4/35 data acquisition system (model PL3504 AD instruments, AU). The animals were mounted on the stereotaxic apparatus and a microdrive was used for positioning and lowering the electrode into the CnF. The electrode slowly lowered (2 µm/sec) under continuous recording of neuronal signals until an active neuron was isolated. Administration of hydralazine or the induction of hemorrhage was done for a 15-min session and the spike firing rate was recorded for a subsequent period of 30 min. The recordings were filtered between 300 and 3k Hz with a resolution of 24 bits/SPS and analyzed in offline mode. Spike sorting and clustering was performed using the e-lab software. Spike sorting and peri-stimulus time histograms (the number of spikes per discrete time; PSTH) were built using compatible E sorter software. After determining the appropriate gain in raw data in the offline sorter, the number of clusters between 4 to 8 in the whole session (1800 seconds) was measured.

### Experimental design

The male Wistar rats (n=30) were randomly divided into three groups; (i) control group that received saline (i.v); (ii) hydralazine group that received hydralazine (200 µg/kg, i.v; Sobhan Daru, Iran), and (iii) hemorrhage group, which bleeding was performed from the tail vein. The application of saline or hydralazine, as well as the bleeding from the tail vein, was performed after stable recordings of both blood pressure and spike activity of the CnF. Hydralazine and saline were injected into the systemic blood flow via tail vein. Hypotension was induced by bleeding from the tail vein to reach a 40-50 mmHg decrease in mean arterial pressure (MAP). Evaluation of cardiovascular parameters and single unite recordings were performed for 1,800 seconds (Figure 1).

At the end of each experiment, the animal was sacrificed by a high dose of urethane. The brain was then removed and stored in 10% formalin for 24 h. The electrode sites were determined according to a rat brain atlas under a light microscope<sup>16</sup>.

### Data analysis

MAP and HR values were expressed as mean  $\pm$  SEM. The cardiovascular response and the neuronal firing patterns for each injection were aligned and compared with the control. The maximum changes of systolic blood pressure (SBP), MAP, and HR and firing rate of neurons were compared with those of the pre-injection and the control values by one way ANOVA followed by Tukey post-hoc test.  $P < 0.05$  was used to indicate statistical significance. Correlation between variables in each group was assessed by the Pearson test in Statistical Package for the Social Sciences (SPSS) v11.5.

## RESULTS

### Cardiovascular parameters

A significant difference was observed in HR ( $p < 0.001$ ), SBP ( $p < 0.001$ ), and MAP ( $p < 0.001$ ) between the control, hydralazine, and hemorrhage groups. The results showed that in the hydralazine group, the maximal changes of SBP ( $-35.5 \pm 1.8$  vs  $-1.5 \pm 2.2$ ;  $p < 0.001$ ) and MAP ( $-28.2 \pm 1.7$  vs  $-1.1 \pm 1.9$ ;  $p < 0.001$ ) were significantly lower and the alteration of HR ( $58.3 \pm 10.5$  vs  $3.2 \pm 5.6$ ;  $p < 0.01$ ) was significantly higher compared with the control group. Furthermore, the changes of SBP ( $-40.6 \pm 3.7$  vs  $-1.5 \pm 2.2$  in the control group;  $p < 0.001$ ) and MAP ( $-30.5 \pm 3.3$  vs  $-1.1 \pm 1.9$ ;  $p < 0.001$ ) in the hemorrhage group were significantly lower, and the alterations of HR were significantly higher ( $65.4 \pm 17.4$  vs  $3.2 \pm 5.6$ ;  $p < 0.01$ ) compared with the control group (Figures 2 and 3).

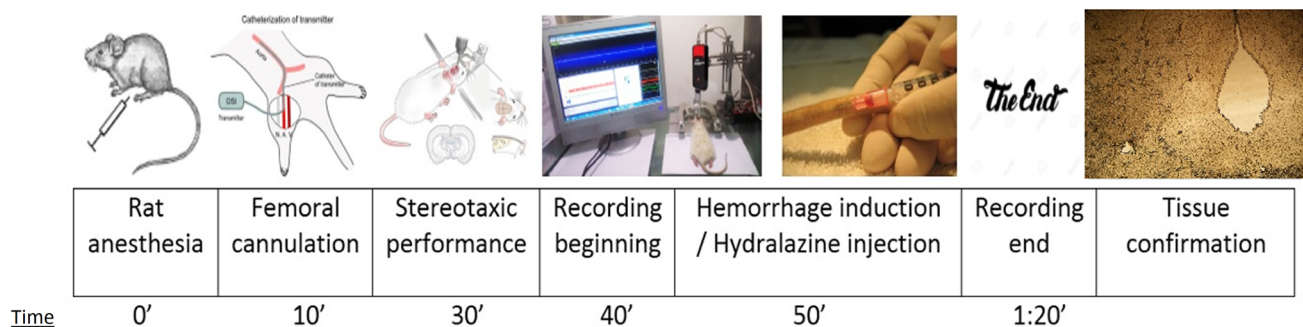


Figure 1. Diagram of the experimental design.

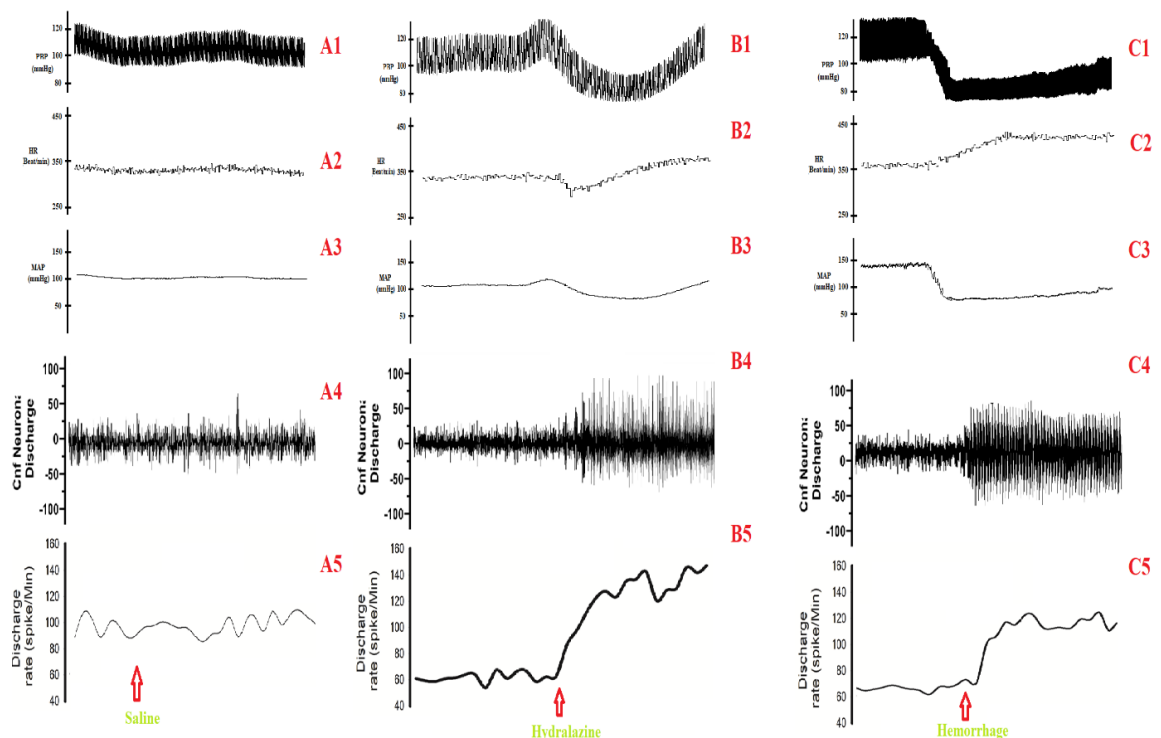
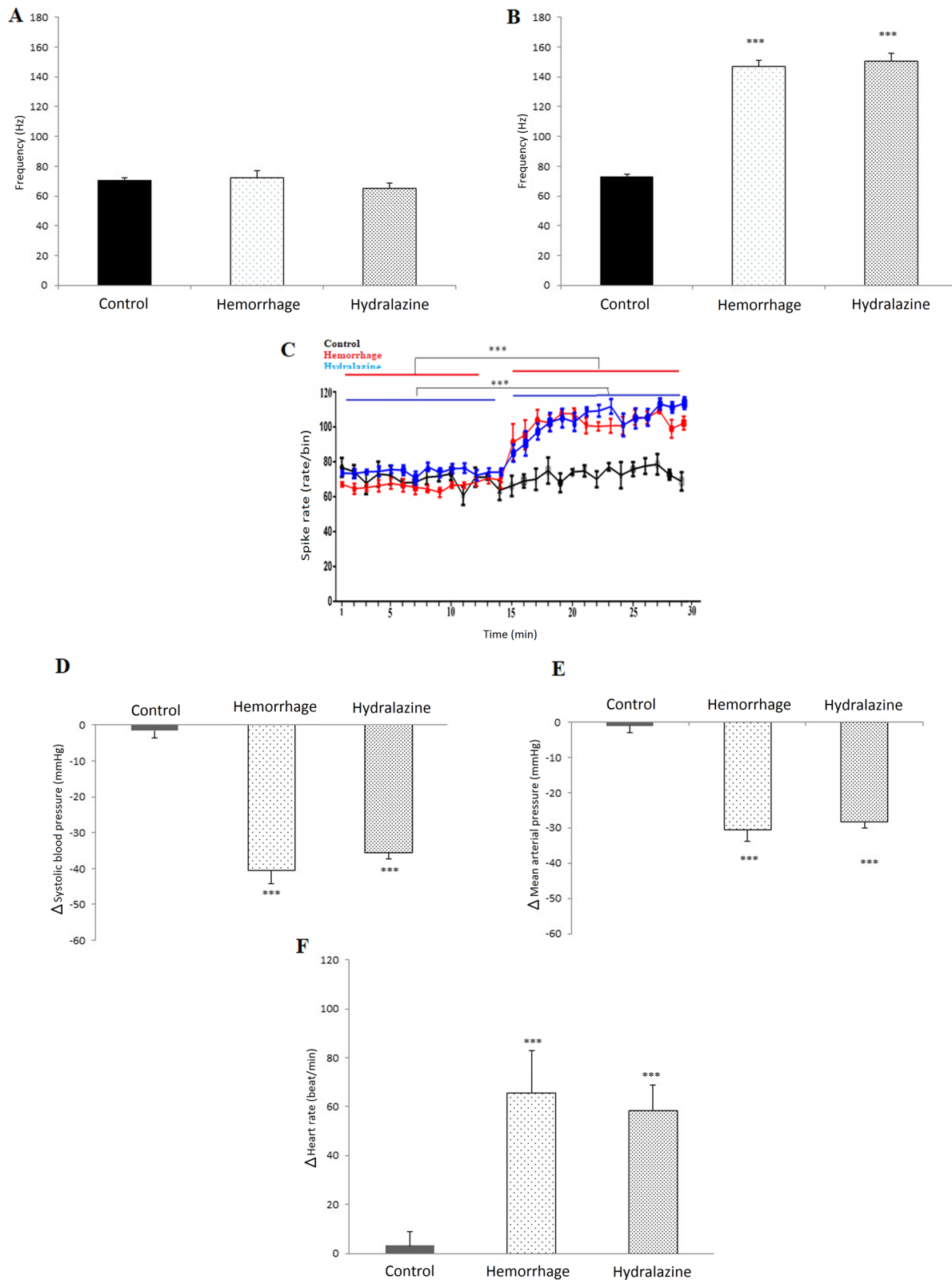


Figure 2. Representative recording of cardiovascular parameters (1, 2, 3) and neural discharge (4, 5) after injection of saline (A), injection of hydralazine (B), and hemorrhage (C).



**Figure 3.** The spike rate and cardiovascular parameters analysis was performed from the Cuneiform nucleus in different experimental groups. Data represent BIN size spike rate (count/time) in the control, hemorrhage, and hydralazine groups (A, B, C). A paired t-test was used for statistical analysis. \*\*\* $p < 0.01$  indicates differences between before (A) and after (B) induction in each group. A comparison of the mean spike rate per BIN between different groups was measured before and after hypotension induction (C) ( $n = 5$ /each group). The data is expressed as mean  $\pm$  SEM. One-way ANOVA was used for statistical analysis. \*\*\* $p < 0.001$  indicates significant differences with the control group. Effects of hydralazine and hemorrhage on maximal change ( $\Delta$ ) of SBP (D), MAP (E), and HR (F) in anesthetized rats ( $n = 5$ /each group) are shown. The data were compared with the control group and presented as mean  $\pm$  SEM. One-way ANOVA was used for statistical analysis. \*\*\* $p < 0.001$  indicates significant differences with the control group.

## Single unit recording

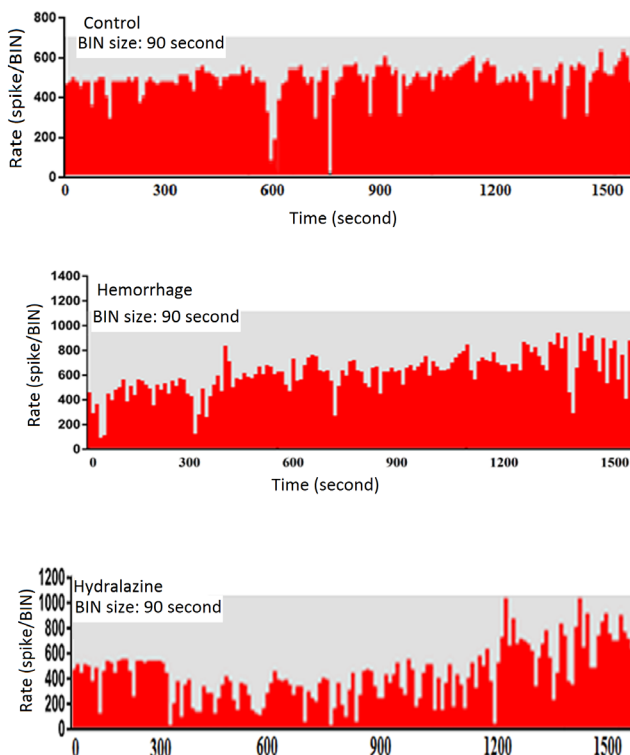
No significant differences in the CnF firing rate were observed between different groups before the induction of hypotension. After the induction of hypotension by hemorrhage or hydralazine, a significant increase in the firing rate was observed ( $p < 0.001$ ). Furthermore, a significant enhancement of PSTH was recorded after the induction of hypotension ( $p < 0.01$ ). The analysis of the amplitude changes and the repetition rate of the population firing showed a significant enhancement of more than 20% in the hemorrhage and hydralazine groups after the induction of hypotension (Figures 2, 3, and 4). However, there was no significant correlation between the changes in the cardiovascular parameters and neural firing pattern.

The linear regression analysis showed that the firing rate was significantly correlated with increased time in the hemorrhagic and hydralazine groups but was stable in the control. ((Control: Slope=0.1130, F: 1.273,  $p=0.2612$  deviation from zero); (Hemorrhage: Slope=4.429, F: 258.9,  $p < 0.0001$ ); (Hydralazine: Slope=4.171, F: 292.1,  $p < 0.0001$ )) (Figure 5).

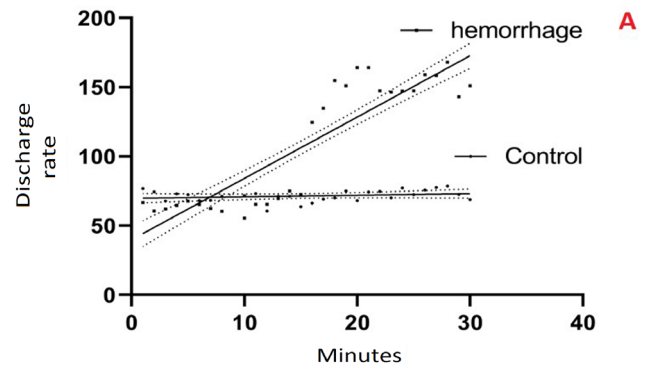
## Correlation

The Pearson test showed a non-significant correlation between cardiovascular parameters and neural frequency (Table 1).

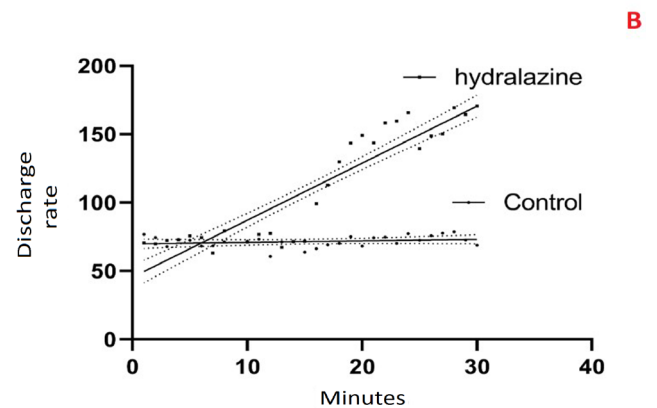
Figure 6 shows the electrode sites determined according to a rat brain atlas.



**Figure 4.** Pre-stimulus time histogram (BIN size=90 seconds recorded by “eLAB C apparatus Science Beam. Co.”) in the control, hemorrhage, and hydralazine groups showed a significant increase in firing rates after induction of hemorrhage or injection of hydralazine.



	Control	hemorrhage
Slope	0.1130	4.429



	Control	hydralazine
Slope	0.1130	4.171

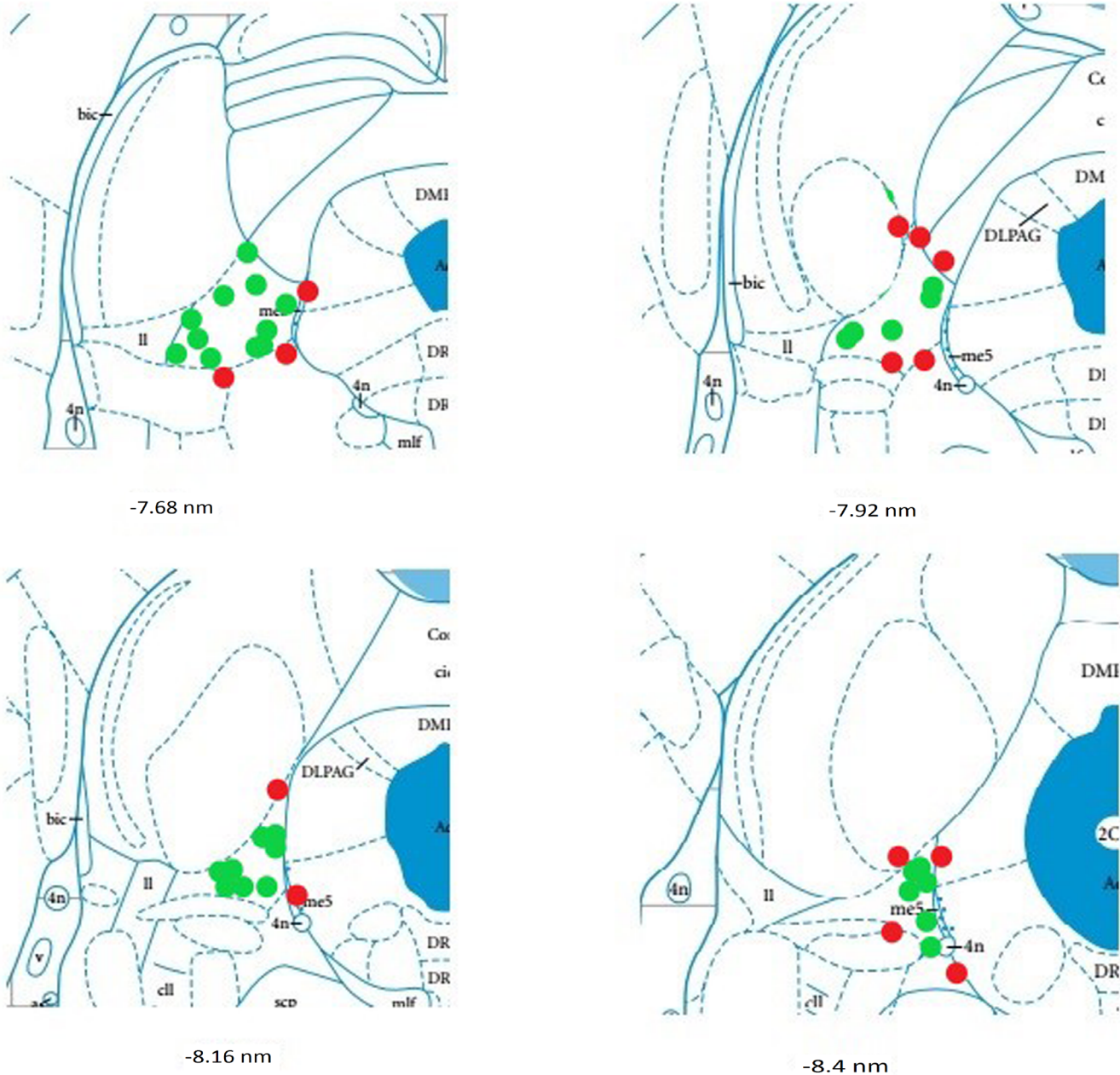
**Figure 5.** The linear regression of control vs hemorrhagic and hydralazine groups (Control vs hemorrhage 0.1130/4.429 (A) and control vs hydralazine 0.1130/4.171 (B)).

**Table 1.** Correlation between cardiovascular parameters and neural frequency.

		HR	SBP	MAP	Frequency
HR	Pearson correlation	1	-0.970	-0.978	0.763
	p-value		0.156	0.134	0.448
SBP	Pearson correlation	-0.970	1	0.999	-0.897
	p-value	0.156		0.022*	0.291
MAP	Pearson correlation	-0.978	0.999*	1	-0.881
	p-value	0.134	0.022		0.314
Frequency	Pearson correlation	0.763	-0.897	-0.881	1
	p-value	0.448	0.291	0.314	

\*Correlation is significant at the 0.05 level (2-tailed p value). HR: heart rate, SBP: systolic blood pressure, MAP: mean arterial pressure.





**Figure 6.** Schematic section of the rat brain adapted from Paxinos and Watson atlas. The electrode sites in the CnF are shown: green points are adequate and red points are out of range.

## DISCUSSION

In this study, we used the single-unit electrophysiological recording to evaluate responses of the CnF neurons in hypotension induced by hypovolemic hemorrhage and non-hypovolemic (hydralazine) conditions. Our results showed that the firing rate of the CnF neurons increased in both conditions, which confirms the role of this nucleus in cardiovascular regulation. In previous studies, the modulatory effect of the CnF on the cardiovascular system in normotensive rats has evaluated<sup>13,14</sup>. Shafei et al. have shown that micro-injection of glutamate into the CnF increased BP and that

the cholinergic system has an inhibitory effect on the cardiovascular system<sup>11</sup>. In addition, it has been reported that CnF do not project directly to the spinal cord or areas involved in cardiovascular regulation specially RVLM and that these projections are mediated via areas such as KF and dIPAG. In line with these results, Nasimi et.al in an electrophysiological study in normotensive rats indicated that chemical stimulation of the CnF produces several patterns of neural activity and these responses correlate with various blood pressure responses<sup>4</sup>. Activation of the CnF neurons during hypotension in this study correlated with neural activity and blood pressure but not significantly. The reasons for this are not

clear but we speculate that CnF has neurons that respond to both isovolemic and hypovolemic hypotension. Neural activation of CnF during hemorrhage is in line with our previous studies that indicate the involvement of CnF in the regulation of cardiovascular parameters during hypovolemic hypotension<sup>17</sup>. However, the lack of correlation may be due to the multi-synaptic neural pathway of the CnF nucleus. For example, it has been shown that the cardiovascular effect of CnF is mostly mediated by the KF nucleus. Therefore, CnF may activate the neuron population in KF and these neurons can be responsible for the regulation of blood pressure. In addition, CnF is a heterogeneous nucleus with numerous neurons with different effects. Therefore, we speculate that during hypotension all of these neurons activate and cause several responses that disrupt those correlations. However, further studies are needed to investigate these effects.

In a hypotension condition, baroreflex, as a compensatory reflex, is activated by baroreceptors located in the carotid body and arch of the aorta. Because the CnF is involved in the sympathetic part of the baroreflex (NTS, CVLM, RVLM), we hypothesize that the autonomic effect of the CnF is mostly mediated by the sympathetic system<sup>18</sup>. In non-hypovolemic hypotension induced by hydralazine, the cardiovascular effect of the CnF is mediated by the NTS-CVLM-RVLM pathway. It has been reported that the projection of the CnF to RVLM is indirect and probably mediated by PAG, KF, and raphe nuclei. We believe that the CnF-dIPAG-RVLM pathway is involved in the regulation of the cardiovascular system in conditions such as stress and exercise<sup>19,20,21</sup>.

The neuronal pathway that regulates cardiovascular parameters in a hypovolemic condition is not well

defined. However, besides RVLM, numerous brain areas, such as the supra-optic nucleus (SON), the paraventricular nucleus (PVN), and raphe nuclei may be implicated in brain response to hypotension<sup>20</sup>. Furthermore, it has been indicated that the CnF has a relationship with PVN and raphe nuclei. Thus, the CnF may exert its effect on the cardiovascular system via its connections with the PVN and/or raphe nuclei.

The CnF is a heterogeneous nucleus and contains several neural groups such as glutamatergic<sup>22</sup>, GABAergic<sup>23</sup>, cholinergic<sup>24</sup>, and opioidergic<sup>22</sup> and serotonergic neurons. Our previous studies indicated that serotonergic and opioid pathways of the CnF are involved in the regulation of the cardiovascular system during hemorrhage<sup>13,14</sup>. It has also been reported that the CnF is an integrative area that mediates the cardiovascular response to a stressor such as hypotension. Therefore, hypotension signals from baroreceptors, chemoreceptors, and volume receptors project to the CnF, and this nucleus, via other areas, precipitate the cardiovascular responses for the maintenance of hypovolemic conditions.

An important limitation of this study is that we did not discriminate individual neuronal groups. If responses of individual neuronal groups were examined separately, their effects on hypotension could be better evaluated.

In summary, the current preliminary findings revealed that neurons of the CnF responded to both hypovolemic and non-hypovolemic hypotension and this neural activity did not differ between these conditions. However, further studies are needed to evaluate the exact mechanisms of these responses.

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