Topographic carotid vasoconstriction in the rostral ventrolateral medulla of rats

Yumi Takemoto takemotoyumi@hotmail.co.jp

Department of Physiology II, Graduate School of Biomedical and Health Sciences (Medicine), Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8551, Japan

Abstract

The vascular beds of various cranial tissues receive common carotid flow, which contributes to blood flow redistribution associated with animal behaviors such as grooming, but the medullary autonomic regulation of carotid flow resistance (CAR) is poorly understood. This study is the first to examine the response sites of CAR in the rat rostral ventrolateral medulla (RVLM) presympathetic area to chemical stimulation by the ionotropic excitatory amino acid receptors activator L-cysteine. Arterial blood pressure and CAR were monitored in anesthetized rats which had a cranial window constructed above the ventral medulla. Mapping of L-cysteine microinjection in eight rats showed carotid vasoconstriction in the caudal part alone within the RVLM pressor area, which included contributions from other vascular beds, indicating localized topographic carotid vasoconstriction. Additional testing was performed on four types of denervated rats. A similar response map was obtained in six rats that received minimal lesions during surgery as well as in 10 rats with severed internal or external carotid nerves. However, the remaining three minimally lesioned rats showed extensive vasoconstriction of the RVLM pressor area including the rostral part, indicating lack of a topographic response. The topographic response of most rats might be state-dependent. Seven rats with complete cervical denervation showed no carotid vasoconstrictor response in the RVLM pressor area, indicating cervical sympathetic mediation of the responses. The topographic carotid vasoconstriction in response to L-cysteine may suggest differential roles of presympathetic neurons in the rostral and caudal parts of the RVLM in sympathetic carotid flow regulation.

Keywords: Common carotid flow; RVLM; CVLM; L-Cysteine; Ionotropic excitatory amino acid receptors; Rats

~~1.~~1 Introduction

Grooming behavior redistributes blood flow from the muscle vascular bed to the common carotid artery (Mizuma et al., 1987), but spontaneous walking shifts blood flow inversely (Takemoto and Iriuchijima, 1989), likely through fast autonomic neural mechanisms. Central autonomic neuronal networks (Loewy and Spyer,

1990; Jänig, 2006; Llewellyn-Smith and Verberne, 2011) are considered to include mechanisms for this redistribution of blood flow. The common carotid artery supplies blood flow to the vascular beds of the neck and head including organs such as the brain and muscles, which receive sympathetic regulation (Greene, 1963; Cipolla, 2016; Reiner et al., 2018; Izumi and Ito, 1998; Izumi, 1999; Ishii et al., 2007).

To date, important roles for several neuronal groups in sympathetic regulation of the cardiovascular system have been well documented at the medullary level (Guyenet, 2006; Schreihofer and Sved, 2011; Takemoto, 1990). The rostral ventrolateral medulla (RVLM) pressor area, identified by excitatory amino acid (EAA) stimulation, includes one of the most important autonomic areas, in which presympathetic neurons regulate resting arterial blood pressure (AP), and it mediates many autonomic reflexes involved in homeostasis of the body. The caudal ventrolateral (CVLM) depressor area includes inhibitory interneurons connecting neurons in other brain areas to RVLM presympathetic neurons, and neurons in the nucleus tractus solitarius (NTS) situated in the dorsal medulla receive visceral neuronal terminals and send their information to the CVLM and RVLM. These neurons may be related to the above-mentioned cardiac redistribution that occurs during animal behaviors.

At synapses of these neuronal networks, chemicals such as neurotransmitters and neuromodulators play an important role in mediating and integrating information or signals (Schreihofer and Sved, 2011; Takemoto, 1990). These neuroactive chemicals include amino acids, peptides and gases (Llewellyn-Smith and Verberne, 2011), and others continue to be discovered. Our previous functional AP studies (Takemoto, 1995; Takemoto, 2012; Takemoto, 2013; Janáky et al., 2000) demonstrated further potential for the thiol amino acid neuromodulator L-cysteine (Takemoto, 2014a), which can activate neurons in the RVLM (Takemoto, 2014b), CVLM (Belluli and Weaver, 1991) and NTS (Mueller et al., 2011), to produce changes in AP. These AP responses to L-cysteine were blocked by antagonists of ionotropic excitatory amino acid receptors (iEAArs), indicating that L-cysteine activates only iEAArs, in contrast to the typical EAAr agonist L-glutamate, which activates both iEAArs and metabotropic EAA receptors. Antagonism of iEAArs abolishes many autonomic reflexes at the medulla level, indicating a major role of iEAArs in central autonomic regulation (Schreihofer and Sved, 2011; Takemoto, 1990) and a possible endogenous neuromodulatory role of L-cysteine (Takemoto, 2014a) in the iEAArs of medullary autonomic areas (Takemoto, 2014b; Belluli and Weaver, 1991; Mueller et al., 2011).

The RVLM contains presympathetic vascular neurons for many types of tissues including muscle and cranial organs, and when required for homeostasis, these neurons are differentially and selectively regulated by unknown mechanisms (Takemoto, 1990). To date, intermixed distributions of RVLM presympathetic neurons to several organs have been reported in rats (Farmer et al., 2019; Dampney and McAllen, 1988; McAllen and Dampney, 1990), while evidence of topographic functional presympathetic neurons exists in the cat RVLM (McAllen, 1994; Takemoto, 2020; Takemoto, 2004). Consistent with the intermingled distribution of presympathetic neurons in the rat RVLM, our previous study, which mapped muscle or hindquarter blood flow responses to L-cysteine, demonstrated widely intermingled but distinct sites of changes in muscle flow resistance, through potential activation of lumbar or adrenal sympathetic neurons (Savastano et al., 2010).

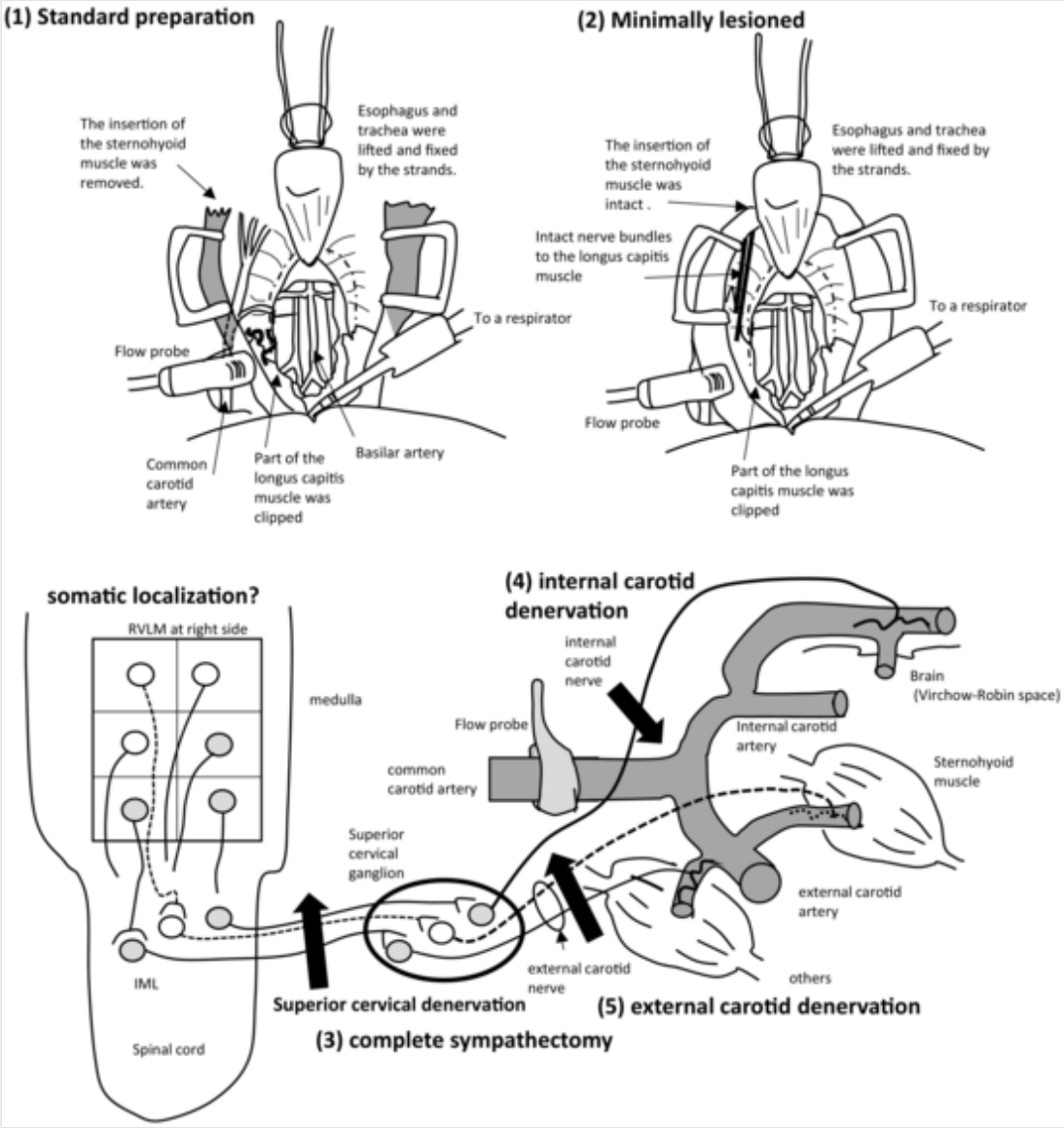
Unlike the simple sympathetic regulation of the muscle vascular bed, the common carotid artery supplies blood flow to various functional types of vascular beds such as those of the brain, muscle, glands and skin, which receive dual autonomic regulation by sympathetic and parasympathetic nerves (Greene, 1963; Cipolla, 2016; Reiner et al., 2018; Izumi and Ito, 1998; Izumi, 1999; Ishii et al., 2007). However, the RVLM presympathetic sites responsible for common carotid flow regulation have not yet been examined. Hence, this study mapped carotid flow resistance (CAR) response sites to L-cysteine microinjection in the VLM. AP changes were used to indicate changes in total peripheral resistance that reflect resistance changes in other vascular beds, as changes in

muscle vascular resistance occur in parallel to AP changes produced by chemical stimulation in the rat RVLM and CVLM (Savastano et al., 2010).

In the early stage of the experiment, rats were prepared as in previous studies (Takemoto, 2014b; Belluli and Weaver, 1991; Mueller et al., 2011; Savastano et al., 2010), as shown in Fig. 1 (case 1). In those rats, response mapping with L-cysteine indicated localized sites of carotid vasoconstriction within the RVLM pressor area. This topographic result of carotid vasoconstriction was different from the previous map regarding muscle blood flow, in which resistance responses were widely spread in all mapped sites of the RVLM and CVLM (Savastano et al., 2010), and from previous studies of other vascular beds (Farmer et al., 2019; Dampney and McAllen, 1988; McAllen and Dampney, 1990). To confirm this new topographic result in rats, the possible influence of cervical injury during the operation and the role of cervical sympathetic nerves were further examined in rats with (case 2) minimal lesions, (case 3) complete denervation of the cervical sympathetic trunk, and (case 4) internal or (case 5) external carotid denervation.

alt-text: Fig. 1

~~Fig. 1.~~Fig. 1



Five different preparations of cases (1 to 5). (1) A wider operating space from the ventral access was obtained by moving the sternohyoid muscles aside and cutting bundles to the longus capitis muscle. (2) The sternohyoid muscles and two nerve bundles were left intact, producing a narrow operating space. (3) to (5) The possible relationships of presympathetic neurons in the rostral ventrolateral medulla to the vascular beds and the surgically denervated locations are shown. See the text for

details. A dashed line indicates a possible lesioned pathway during the operation. IML: intermediolateral cell column, RVLM: rostral ventrolateral medulla pressor area.

Sympathetic nerves in the vascular beds of the cervical and facial muscles, but not of the cerebrum, choroid and salivary glands (Cipolla, 2016; Reiner et al., 2018; Izumi and Ito, 1998; Izumi, 1999; Ishii et al., 2007), could play a tonic role, and therefore an effect of lesioned nerves on muscle vascular beds was hypothesized in rats (case 2), as shown in Fig. 1. Internal (case 4) or external (case 5) carotid denervation was performed on the basis of a simplified strategy that assumed that the internal carotid nerve mainly innervates cerebral blood vessels on the surface of the brain (Cipolla, 2016), and the external carotid nerve mainly innervates blood vessels in the cervical muscles, skin, and glands (Izumi and Ito, 1998; Izumi, 1999; Ishii et al., 2007).

~~2.~~2 Methods and materials

All protocols and surgical procedures employed in the current study were approved by the President of Hiroshima University, and were performed in accordance with the guidelines of 1) the Committee of Animal Experimentation, and 2) the Committee of Research Facilities for Laboratory Animal Science in the Natural Science Center for Basic Research and Development in Hiroshima University. On the basis of these guidelines, experiments were performed with the smallest sample sizes possible to reduce the number of animals used.

All procedures were performed as previously described (Takemoto, 2014b; Belluli and Weaver, 1991; Mueller et al., 2011; Savastano et al., 2010; Weijnen et al., 2000), with several modifications. Briefly, 33 male Wistar rats (333–378 g) were anesthetized with urethane (1.0–1.2 g/kg intraperitoneally; I.P.) and α-chloralose (50 mg/kg I.P.). The animals were placed in an electrically isolated stereotaxic frame and were intubated with plastic tubing coated on the outside with atropine sulfate ointment (Santen, Osaka, Japan) to prevent mucosal secretion.

A window was opened above the ventral medulla after the esophagus, trachea, thyroid and parathyroid were retracted as shown in Fig. 1. When creating the window, five different preparations were performed (Fig. 1): (case 1) a wide window was used for the standard preparation, and (case 2) a small window was used for minimal lesions, (case 3) complete cervical sympathetic denervation, and (case 4) internal or (case 5) external sympathetic denervation.

In detail, the longus capitis muscle was partly removed, and two nerve bundles were cut (case 1) or left intact (case 2). The inserted part of the sternohyoid muscle was removed (case 1) or left intact (case 2), and part of the atlas anterior arch and the dens of the axis were removed with a rongeur. Part of the ventral occipital bone was removed with a dental drill, creating a window above the VLM on the right side. Some rats underwent complete severing of the superior cervical sympathetic trunk (case 3 in Fig. 1) (Gordon and McCann, 1988), and the internal (case 4) or external carotid nerves (case 5) were cut in some rats (Nakai et al., 1993), but two nerve bundles and the sternohyoid muscle were left intact.

The animal was cannulated by inserting tubing into the left iliac artery and vein to allow for AP measurement and drug injection, respectively. Then, an electromagnetic flow probe (1 mm in diameter, Nihon Kohden, Tokyo, Japan) was placed around the right common carotid artery, which was covered with isotonic saline-wetted cotton and further covered with paraffin film after plugging the slit of the probe with a plastic insert. A respirator system (Model SN-480-7, Shinano, Tokyo, Japan) with a capnograph was connected to the tracheal tubing and controlled to maintain normocapnia ($PaCO_2$: 35–45 mmHg) as detailed in a previous study (Weijnen et al., 2000). The AP and common carotid flow were measured with an AP-601G pressure transducer and an MFV1100 electromagnetic blood flowmeter, respectively (Nihon Kohden, Tokyo, Japan). Zero values of carotid flow were

obtained by stopping the blood flow by euthanizing animals after the experiments. AP and carotid flow recordings were sampled at 100 Hz with a LabScribe 3 digital system (iWorx, NH, USA). Carotid flow resistance (CAR) (mmHg/(ml/min/100 g) was calculated by the quotient of AP/flow after normalized by rat body weight and then expressed as the percent change. Rectal temperature was maintained between 36.5 and 37.5 °C. Arterial blood oxygen saturation was intermittently monitored with a pulse oximeter (MouseOx, Starr Life Sciences, PA, USA).

Microinjections were performed at a depth of 0.7 mm in the RVLM area and 0.8 mm in the CVLM area using a glass micropipette containing L-cysteine solution (30 mM, 34 nl) connected to a microsyringe via polyethylene tubing filled with distilled water; the syringe was controlled with a micromanipulator (IM-3, Narishige, Japan). The L-cysteine concentration was determined according to previous studies (Takemoto, 2014b; Belluli and Weaver, 1991; Mueller et al., 2011; Savastano et al., 2010). Care was taken to avoid blood vessels. L-cysteine was freshly dissolved in commercial artificial cerebrospinal fluid (Artcereb, Otsuka Pharmaceutical Co., Ltd., Otsuka, Japan) and filtered with a disposable syringe membrane filter unit (0.45 μm, Dismic-3cp, Advantec, Tokyo, Japan). The injection interval was longer than 4 min to allow the variables to return to the original levels.

Responses were mapped to the ventral medulla, and the rostral-caudal axis at the location where the basilar artery is initiated was set as zero. When the position of zero was apparently different from the normal position, the rostral end of the second rootlet of cranial nerve XII was used as the point at 1.11 mm from the zero point (Weijnen et al., 2000).

Supplemental doses of urethane and α-chloralose (intravenous; I.V.) were administered as needed. Adequate depth of anesthesia was assessed based on AP stability and/or the absence of a withdrawal response to a firm toe pinch.

At the end of the experiments, Coomassie Brilliant Blue solution was microinjected to mark the injection site. The rat was euthanized with an overdose of pentobarbital sodium (I.V., 65 mg/kg), and the zero value of carotid flow was obtained. The upper body was transcardially perfused with isotonic saline containing heparin (20 ml) followed by an 8% formaldehyde saline solution (50 ml). The brainstem was removed and kept in the same solution at 4 °C. The sliced coronal sections (50 μm) were imaged with a digital camera system (DP70, Olympus, Tokyo, Japan).

The values of variables are expressed as the mean ± SD.

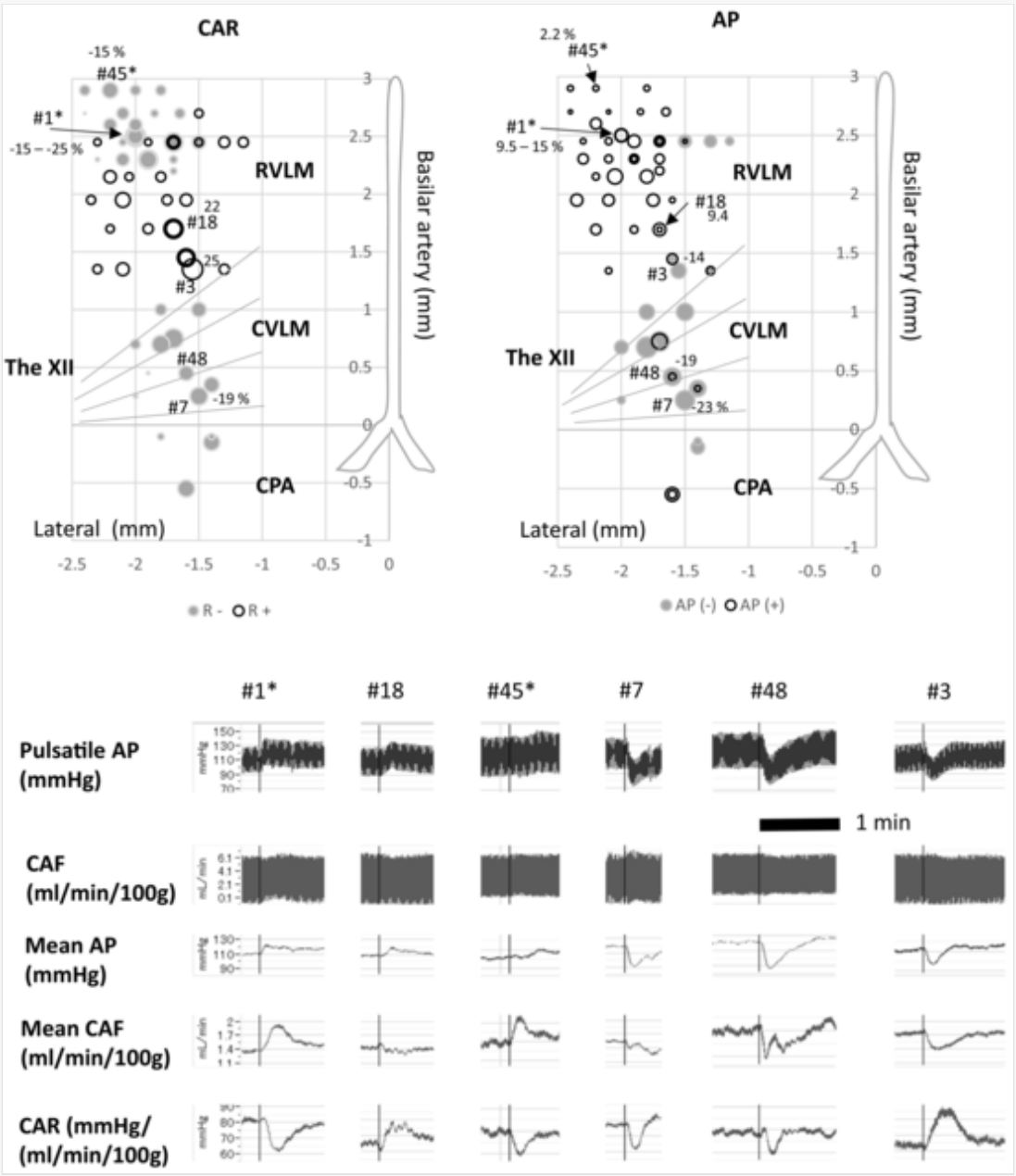
~~3.~~3 Results

Example of CAR and AP response maps with their typical recordings in a rat with the standard preparation.

Fig. 2 shows examples of response maps in a rat with the standard preparation (case 1 in Fig. 1), showing sites of changes in CAR and AP on the right side of the ventral medulla surface, and recordings at marked sites (#) of the two maps when L-cysteine (30 mM, 34 nl) was microinjected. The circle sizes correspond to both CAR and AP responses and a level was considered significant when the change was greater than 10%. The small numbers near #X indicate the percent change in both maps. The basal values before L-cysteine microinjections (n = 68) were 68.9 ± 6.6 mmHg/ (ml/min/100 g) for CAR and 105 ± 8.2 mmHg for AP.

alt-text: Fig. 2

~~Fig. 2.~~Fig. 2



Example of carotid flow resistance and arterial blood pressure response maps of the right side of the ventral medulla surface and typical recordings in a rat with a standard preparation (case 1 in Fig. 1). The circle sizes represent the magnitudes of responses. CAF: common carotid flow, CAR: carotid flow resistance, AP: arterial blood pressure, RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area, CPA: caudal pressor area, XII: 12th cranial nerve. See the details in text.

Pressor response sites of the AP map were found at the rostral ventrolateral part of the medulla surface, referred to as the RVLM, and at the caudal pressor area (CPA) (Boczek-Funcke et al., 1992), and depressor sites were located at the caudal lateral part or CVLM. In the RVLM of the CAR map, the caudal part exhibited vasoconstriction as shown at site #18, but its rostral part illustrated vasodilation as shown at site #1*. The more rostral site #45* demonstrated equi-pressure vasodilation. At many CVLM sites, CAR responses were parallel to those of the AP, as shown at site #7 and #48, but the response was opposite at #3.

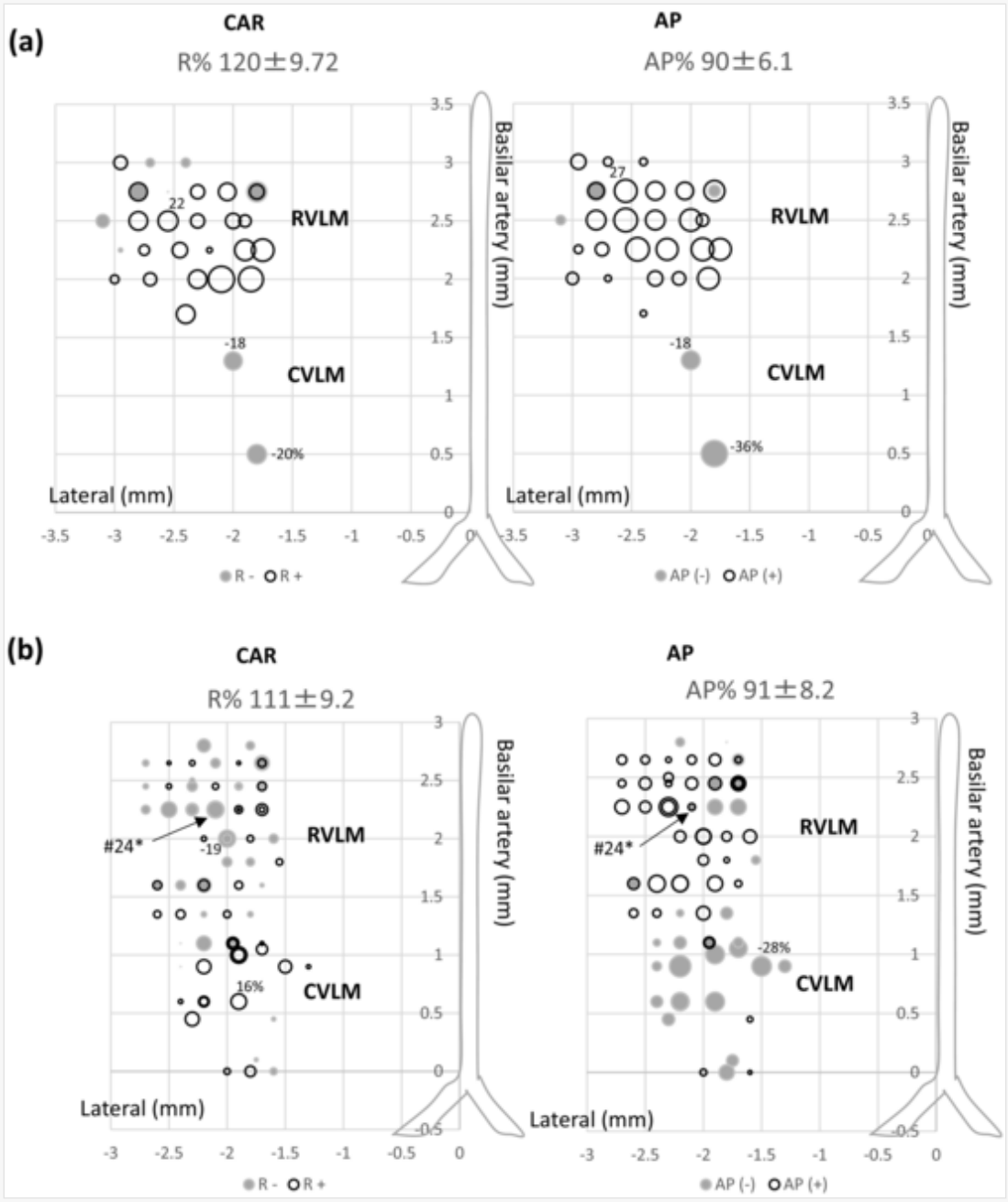
In summary, Fig. 2 indicates that L-cysteine microinjections produced carotid vasoconstriction in the caudal part of the RVLM pressor area and vasodilation in the CVLM depressor area in the rats that received the standard preparation. This type of topographic RVLM vasoconstriction map was obtained in most rats as mentioned below.

~~3.1.~~3.1 Examples of other typical response maps

Fig. 3 illustrates other typical maps in rats with minimal lesions of case 2 in Fig. 1 and in a completely denervated rat in case 3. As seen in Fig. 3a, one rat with minimal lesions showed a relatively large number of vasoconstriction sites corresponding to the pressor response of the RVLM, including its rostral part. Two depressor sites in the CVLM caused vasodilation. The map type in Fig. 3a was obtained from three of nine rats with minimal lesions. The remaining six rats exhibited a topographic vasoconstriction map as shown in Fig. 2.

alt-text: Fig. 3

~~Fig. 3.~~Fig. 3



Examples of response maps to L-cysteine in case 2 (a) or case 3 (b) rats. Equi-pressure vasodilation was obtained at site #24*. Circle sizes represent magnitudes of responses. Means and SDs before microinjections are shown after "R%" or "AP%" in individual maps; the units indicate mmHg/(ml/min/100 g) for carotid flow resistance (CAR) and mmHg for arterial blood pressure (AP). RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area.

In one complete cervical-sympathetic-denervated rat (Fig. 3b), all CAR sites in both the RVLM and CVLM showed an opposite or independent response to changes in AP. At site #24*, L-cysteine produced equi-pressure vasodilation. Carotid flow levels were altered depending on AP changes induced by L-cysteine microinjection without autoregulation.

~~3.2.~~3.2 Summarized maps from the five individual preparations

Figs. 4–7 illustrate maps summarizing CAR and AP responses of the ventral medulla surface from the five individual preparations (~~case~~ cases 1–5) in Fig. 1. Changes of less than 10% in both CAR and AP were classified as no response for simplicity, and the magnitude of the response is expressed by the circle size. Representative values are shown near some circles. Note that the RVLM frame in this study is depicted by a pressor response to L-cysteine, unlike the extended frame that relies on the response to ~~L~~L-glutamate in a previous study (Savastano et al., 2010).

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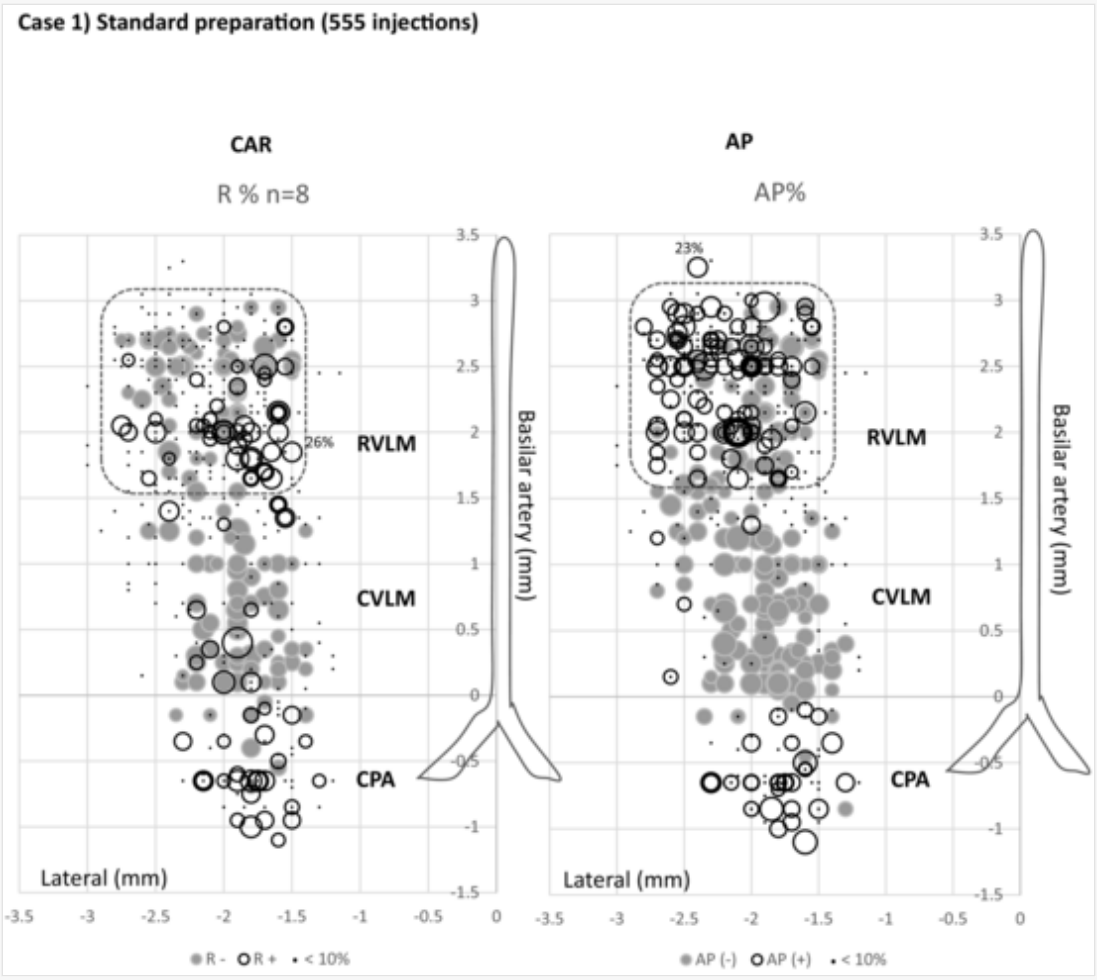
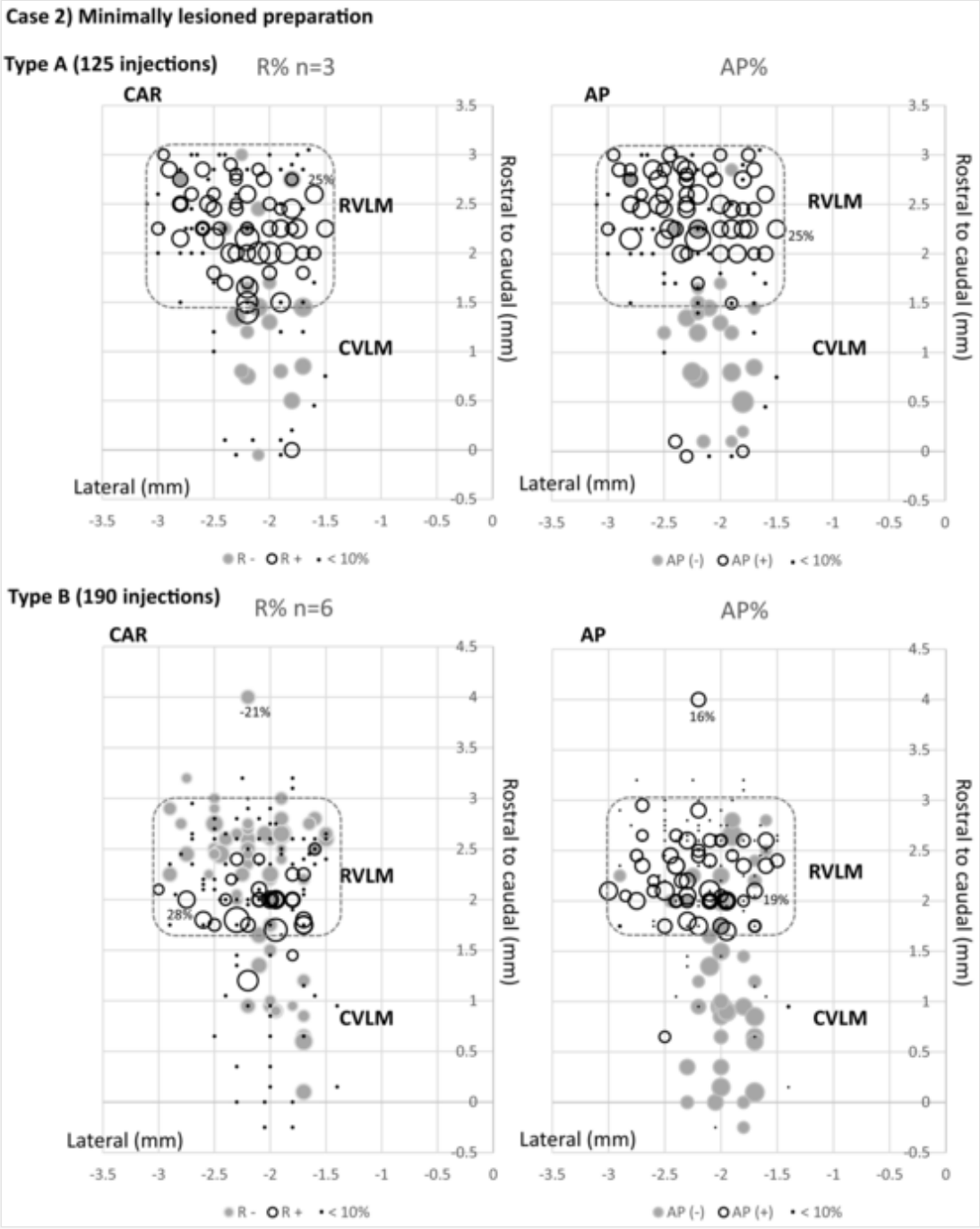
~~Fig. 4.~~Fig. 4



Summarized response maps of the ventral surface for a standard preparation (case 1). Circle sizes, except for those <~~-~~10%, represent the magnitudes of responses. "n = X" indicates the number of rats used. RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area, CPA: caudal pressor area. The basal values before microinjections are 84 ± 26 mmHg/(ml/min/100 g) for carotid flow resistance (CAR) and 88 ± 11 mmHg for arterial blood pressure (AP).

alt-text: Fig. 5

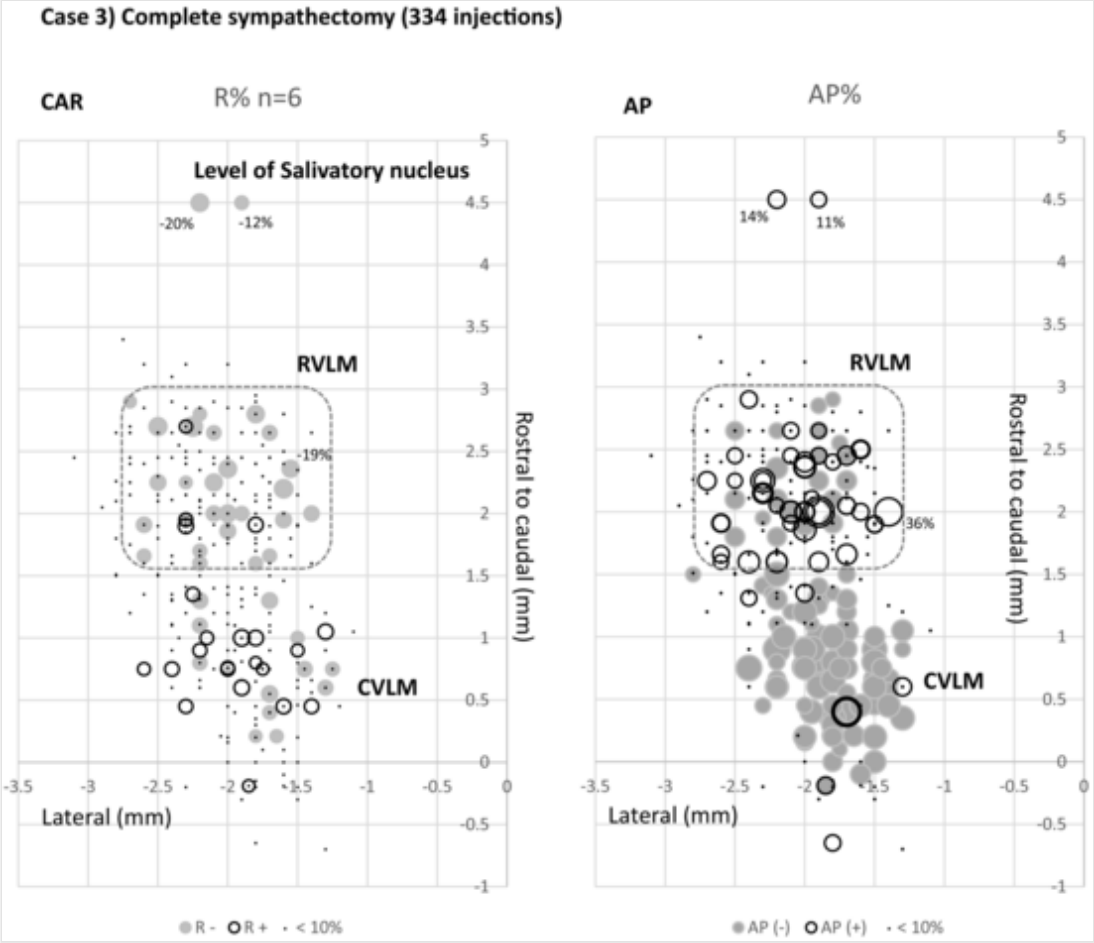
~~Fig. 5.~~Fig. 5



Summarized response maps of the ventral medulla for the minimally lesioned preparation (case 2). Circle sizes, except for those <-10%, represent the magnitudes of responses. “n = X” is the number of rats used. RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area. Basal values before microinjections: Type A, 127 ± 14 mmHg/(ml/min/100 g) for carotid flow resistance (CAR) and 91 ± 9.4 mmHg for arterial blood pressure (AP) and Type B, 113 ± 31 mmHg/(ml/min/100 g) for CAR and 96 ± 11 mmHg for AP.

alt-text: Fig. 6

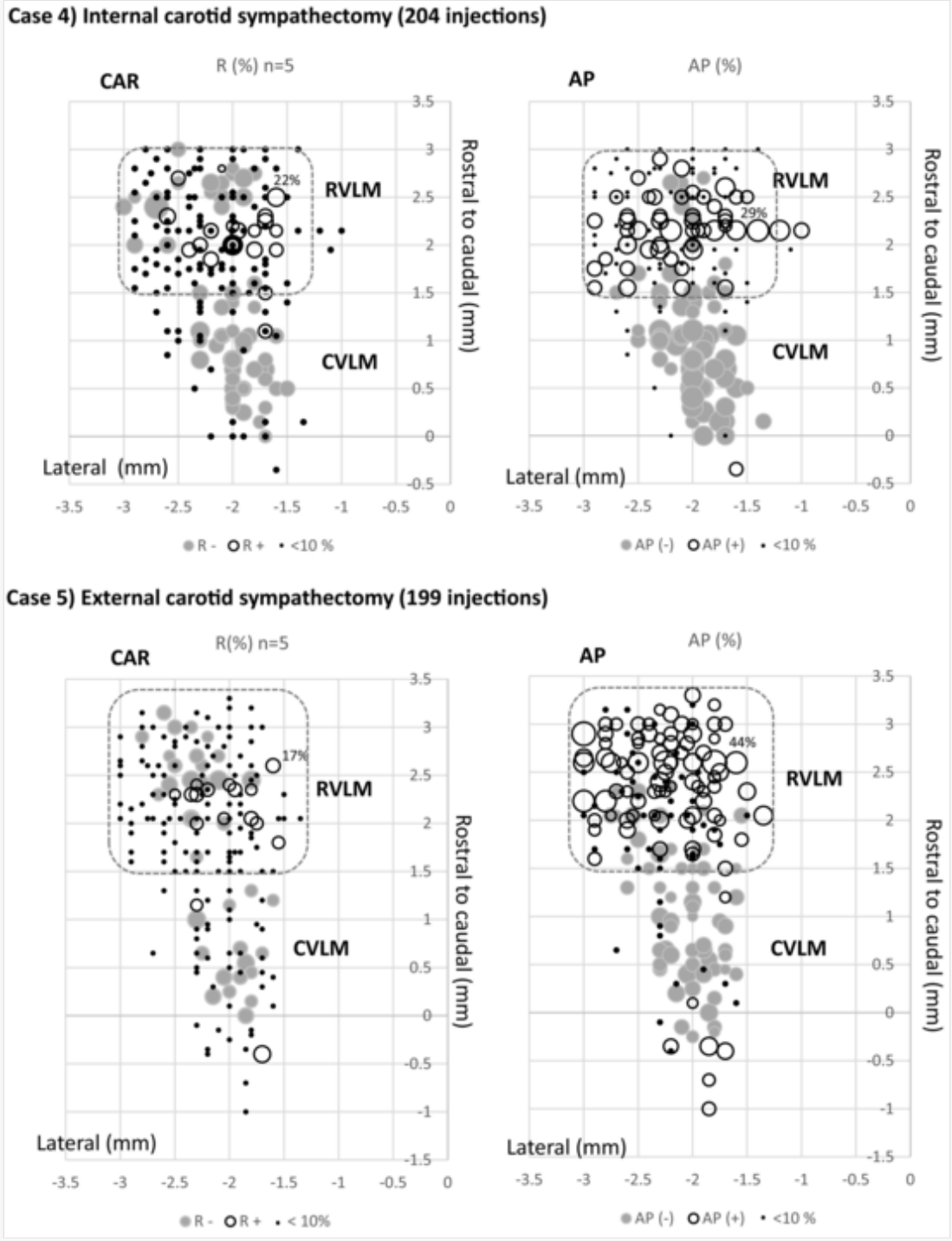
~~Fig. 6.~~Fig. 6



Summarized response maps for complete sympathectomy (case 3). Circle sizes, except for those <–10%, represent the magnitudes of responses. “n = X” indicates the number of rats used. RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area. Basal values before microinjections are 87 ± 22 mmHg/(ml/min/100 g) for carotid flow resistance (CAR) and 93 ± 11 mmHg for arterial blood pressure (AP).

alt-text: Fig. 7

~~Fig. 7.~~Fig. 7



Summarized response maps of the ventral surface for partial sympathectomy (cases 4 and 5). Circle sizes, except for those < 10%, represent the magnitudes of responses. “n = X” indicates the number of rats used. RVLM: rostral ventrolateral medulla pressor area, CVLM: caudal ventrolateral medulla depressor area. Basal values before microinjections in case 4 are

68 ± 16 mmHg/(ml/min/100 g) for carotid flow resistance (CAR) and 94 ± 9.9 mmHg for arterial blood pressure (AP) and in case 5 are 97 ± 26 mmHg/(ml/min/100 g) for CAR and 82 ± 7.8 mmHg for AP.

Fig. 4 summarizes maps from eight rats with the standard preparation (case 1 in Fig. 1). The RVLM frame for AP is drawn to include pressor sites but also contains depressor sites for L-cysteine microinjection, while the CVLM area mainly contains depressor sites, which is consistent with our previous study (Savastano et al., 2010). The standard preparation allowed injection into the CPA.

Regarding CAR responses, stimulation of the caudal part of the RVLM pressor area produced carotid vasoconstriction, but stimulation of the rostral part produced no response or occasional vasodilation. Stimulation of most parts of the CPA produced carotid vasoconstriction. Part of the CVLM depressor area contained vasodilation sites.

Fig. 5 shows two types of response maps in nine rats with minimal lesions (case 2 in Fig. 1). Three rats showed no topographic carotid vasoconstriction in the RVLM pressor area (Type A in Fig. 5) including the rostral part, but six rats exhibited a few vasoconstriction sites localized in the caudal part of the RVLM (Type B), similar to the topographic response pattern of the standard preparation (Fig. 4). The correlation coefficient between changes in the CAR and AP in Type A was 0.6722 ($p < 0.01$), indicating a significant relationship between these changes. Some CVLM depressor sites exhibited vasodilation for both ~~Type~~ Types A and B. Type B also showed a pressor vasodilation site on the ventral surface at a depth of 0.7 mm in the pons corresponding to the salivatory nucleus as reported by Nakai et al. (1993).

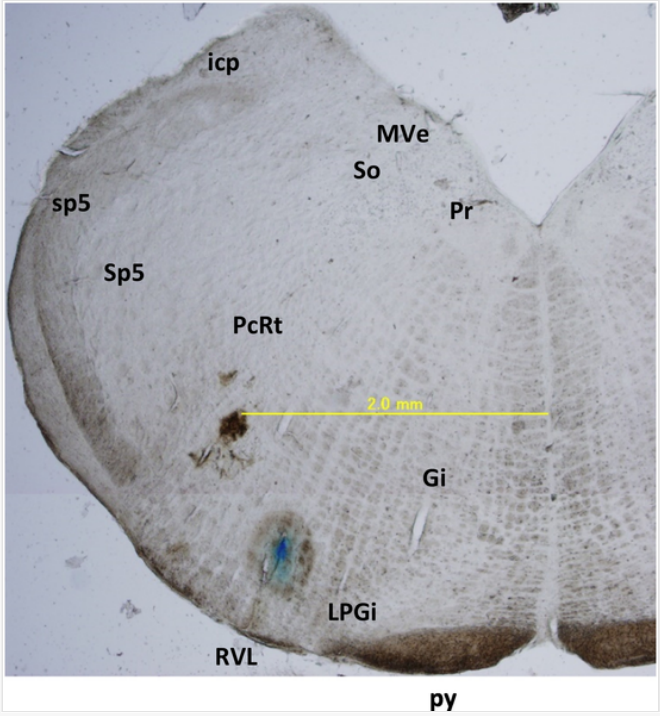
Fig. 6 shows response maps of the CAR and AP to L-cysteine microinjection in six rats with complete denervation of the cervical sympathetic nerve trunk (case 3 in Fig. 1). Both sides of the trunk in two rats were severed, but no clear difference was observed in the results. The RVLM frame in the AP map includes the pressor and depressor sites, and the CVLM primarily includes depressor sites, as in other cases. However, all CAR sites in the RVLM and CVLM were unresponsive or exhibited opposite responses. L-Cysteine microinjection into the ventral surface of the pons at the level of the salivatory nucleus (Li and Horn, 2006) produced pressor vasodilation as in Type B in Fig. 5. An equi-pressure carotid vasodilator response was observed at several sites in the RVLM pressor area.

Fig. 7 demonstrates response maps in each of the five rats with internal or external carotid sympathetic denervation (case 4 or 5 in Fig. 1). Both CAR maps show fewer but significant localized vasoconstriction sites in the caudal part of the RVLM and vasodilation sites in part of the CVLM in response to L-cysteine.

Fig. 8 shows a photograph of the coronal section, which includes a pressor carotid vasoconstrictor site in response to L-cysteine microinjection, marked by Coomassie brilliant blue staining.

alt-text: Fig. 8

~~Fig. 8.~~Fig. 8



A coronal section of the pressor vasoconstriction site showing the response to L-cysteine microinjection at 2 mm rostral, 1.95 mm lateral and 0.7 mm dorsal to the ventral medulla surface zero point on the map. Mve: medial vestibular nucleus, So: solitary nucleus, PcRt: parvicellular reticular nucleus, Sp5: spinal trigeminal nucleus, sp5: spinal trigeminal tract, RVL: rostroventrolateral reticular nucleus, Gi: gigantocellular reticular nucleus, LPGi: lateral paragigantocellular nucleus, py: pyramidal tract, icp: inferior cerebellar peduncle, Pr: prepositus nucleus, according to the Chemoarchitectonic Atlas of the Rat Brainstem (1999).

~~3.3.~~3.3 Equi-pressure carotid vasodilator sites in the RVLM pressor area

Vasodilation without a change in AP was occasionally obtained in the rostral part of the RVLM as shown at #45 in Fig. 2. Carotid flow increased by 21 ± 7.4% in a total of 15 sites of 10 rats, including all cases except external carotid denervation. Equi-pressure vasodilation sites were located in the rostral part of the RVLM pressor area or 2–3 mm rostral and 1.5–3 mm lateral to the zero point of the map.

~~4.~~4 Discussion

This study is the first to explore the functional contribution of the rat ventrolateral medulla (VLM) to sympathetic vascular regulation of common carotid flow, by mapping the carotid flow responses to L-cysteine microinjection in the RVLM and CVLM. The results uncovered topographic carotid vasoconstriction in the caudal part of the RVLM pressor area.

The carotid vascular responses in a pilot study demonstrated a map of topographic vasoconstriction limited to the caudal part of the RVLM pressor area (Figs. 2 and 4), which was different from the map of muscle vasoconstriction that showed parallel changes of the AP in all examined sites of the VLM (Savastano et al., 2010). Because changes in the AP in response to L-cysteine are produced by the total peripheral resistance of various

peripheral vascular beds, pressor response sites without carotid vasoconstriction could indicate the effects on other vascular beds. Therefore, only presympathetic neurons in the caudal part of the RVLM pressor area appeared to be involved in the regulation of vascular beds in the neck and head. In rats, intermingling of presympathetic neurons in the RVLM has been reported for the adrenal gland and the visceral, muscle and kidney vascular beds (Farmer et al., 2019; Dampney and McAllen, 1988; McAllen and Dampney, 1990; Savastano et al., 2010). To confirm this seemingly new result in rats, additional experiments were carefully performed while taking the possibility of injury and the relevance of cervical sympathetic nerves into account.

The first possibility was that an unintended sympathetic injury of the nerves projecting to the vascular bed in the cervical muscles occurred during the operation performed to access the VLM. Common carotid vascular tone in cats (Izumi and Ito, 1998) and cervical muscle vascular tone in rats (Ishii et al., 2007) have been reported, and potential presympathetic neurons for hindquarter muscle vascular beds are widely distributed in the RVLM pressor area (Savastano et al., 2010). This evidence suggests a possible regulatory role of presympathetic neurons projecting to the vascular beds in the facial and neck muscles, which receive a large part of the carotid blood flow. These vasoconstriction sites could be spread in the RVLM pressor area in rats with minimal muscle lesions. Therefore, somatic localization of cervical muscle presympathetic neurons was hypothesized, as shown in Fig. 1.

The insertion part of the sternohyoid muscle and two nerve bundles that connect to the longus capitis muscle were left intact in nine rats with minimal lesions, as shown in Fig. 1 (case 2). The resulting map shows widely spread vasoconstriction sites in the RVLM in three rats (Type A in Fig. 5); however, the remaining six rats exhibited localized vasoconstriction sites in the caudal part of the RVLM pressor area (Type B) similar to those observed in rats that underwent the standard preparation (Fig. 4). Because Type B responses were obtained, the somatic localization hypothesis regarding cervical muscle vascular beds was disregarded at this stage.

Contrary to the disproved simple explanation of presympathetic topography, the presence of a wide distribution of vasoconstriction sites in the RVLM pressor area indicates that presympathetic neurons of the head and neck can also be spread and intermingled similar to those of the other vascular beds (Farmer et al., 2019; Dampney and McAllen, 1988; McAllen and Dampney, 1990; Savastano et al., 2010). This wide existence of vasoconstriction sites in three rats demonstrates a possibility that the topographic vasoconstriction in most rats examined is state-dependent and suggests specific differential roles between the rostral and caudal parts of the RVLM in carotid flow regulation.

It is unclear what would have caused the rostral sites in the RVLM pressor area of most rats to be insensitive to exogenous L-cysteine if widely distributed vasoconstriction sites corresponded to presympathetic neurons. Considering the evidence that L-cysteine stimulates only iEAARs (Takemoto, 2014b; Belluli and Weaver, 1991; Mueller et al., 2011), it can be speculated that iEAARs on presympathetic neurons in the rostral part of the RVLM are occupied by tonic release of endogenous EAA agonists from presynaptic neurons, leaving no iEAARs sensitive to exogenous L-cysteine. Some conditions may cause excitatory neuronal excitation or inhibitory neuronal suppression in potential presympathetic neurons in the rostral part of the RVLM during carotid flow regulation. Such a condition could include accidental denervation of neuronal fibers during surgery. Another possibility is the onset of reflexes involving baroreceptor, sensory and/or parasympathetic vasodilator neurons. However, there remains a possibility that the difference between Types A and B is rat-dependent: those individual animals may simply have developed with different wiring. Future studies are needed to determine the details of the functional roles of the two parts of the RVLM pressor area in common carotid flow regulation.

In rats that underwent complete cervical sympathectomy, corresponding changes in CAR and AP were not observed. The carotid flow did not exhibit autoregulation and completely followed the changes in AP produced by L-cysteine microinjection in both the RVLM pressor area and the CVLM depressor area. The results showed that RVLM carotid vasoconstriction and CVLM vasodilation in other groups of rats were mediated by the sympathetic nerves, which made important contributions to autoregulation of carotid flow. Both the RVLM pressor area and the CVLM depressor area include presympathetic and related neurons that regulate sympathetic neurons of the head and neck vascular beds.

The superior cervical ganglion relays sympathetic signals to various tissues through the internal and external carotid nerves, which run along the internal and external arteries, respectively (Greene, 1963; Nakai et al., 1993). The vascular bed of the brain could be the major tissue innervated by internal carotid nerves, and the vascular beds of cervical and facial muscles, glands and skin could be major tissues innervated by external carotid nerves. To examine whether the distribution of vasoconstriction sites in the RVLM is different from that of vascular areas, response mapping was performed in rats with internal (case 4) or external (case 5) carotid denervation. Internal carotid denervation leaves the external carotid nerves intact. The changes in CAR produced by L-cysteine can be produced through excitation of the external carotid nerves. In rats with internal carotid denervation, as expected, the carotid vasoconstrictor response was obtained at several sites in a relatively caudal part of the RVLM (case 4 in Fig. 7), similar to the map from rats that underwent the standard preparation. As shown in electrophysiological studies of cervical sympathetic neurons in cats (Flett and Bell, 1991) and rats (Saeki et al., 1989; Maeda et al., 1991a), blood vessels in the muscles and skin of the head and neck may receive sympathetic regulation initiated from these RVLM vasoconstriction sites.

The cerebral vascular bed appears to be uniquely regulated by the internal and external neural systems, and limited areas or pial arteries and arterioles at the surface of the brain are thought to receive innervation from sympathetic neurons as part of the external neural system (Cipolla, 2016). Because of the minor sympathetic role in the resting brain vascular bed (Cipolla, 2016), no carotid vasoconstriction in the RVLM pressor area was expected in rats with external carotid denervation (with the internal carotid nerves left intact). However, several significant vasoconstriction sites were observed (case 5 in Fig. 7), indicating a possible role of carotid presympathetic neurons of the RVLM in cerebral blood flow. Internal carotid nerves innervate the pineal, eye, and forehead vascular beds (Nakai et al., 1993). When an emergency situation occurs such as acute hypertension, these presympathetic neurons that project to the internal carotid nerves might be recruited to protect the brain and eyes from the damage caused by high blood pressure, as explained by references (Cipolla, 2016; Reiner et al., 2018). The location of vasoconstriction sites in the caudal part of the RVLM is similar to that in rats with internal carotid denervation (case 4 in Fig. 7). No difference in vasoconstriction regions was identified in the RVLM for the two carotid nerve innervating areas. In other words, a robust carotid vasoconstriction topography was present in most examined rats.

With regard to cerebral blood flow, several groups have reported responses to L-glutamate microinjection into a limited area of the rat RVLM, resulting in inconsistent carotid flow changes (Saeki et al., 1989; Maeda et al., 1991a, 1991b; Underwood et al., 1992; Underwood et al., 1994; Chida et al., 1995). They defined the RVLM by a pressor response to L-glutamate, but many pressor sites did not show a CAR response to microinjection of L-cysteine in the RVLM of rats with intact internal carotid neurons, as shown in case 5 of Fig. 7. Discrepant results could have been observed because different pressor sites were examined.

All of the experimental preparations for this study caused inevitable dysfunction of the trachea, esophagus, thyroid glands and parathyroid glands in addition to some cervical tissues, together with the associated neurons.

Sites with no carotid response corresponding to pressor or depressor response sites may be caused by injury of sympathetic fibers to these organs.

The current study occasionally obtained equi-pressure vasodilator responses to L-cysteine in the RVLM as seen at #45* in Fig. 2 of rats with the standard preparation and at #24* in Fig. 3b of rats with complete cervical sympathetic denervation. Carotid flow increased by 21 ± 7.4% in a total of 15 sites of 10 rats with the four types of preparations. Therefore, their responses to L-cysteine were likely not related to changes in AP sympathetically produced by other vascular beds. Nakai et al. (1993) found that in the salivatory nucleus in the brainstem, which is a parasympathetic cerebral vasodilator center in rats, microinjection of the multiple EAA receptor agonist L-glutamate increased cerebral cortical blood flow. Considering this possible parasympathetic nervous activation in the pons, CAR responses to L-cysteine were tested at the level of the salivatory nucleus. As shown in Fig. 5 (Type B) and Fig. 6, pressor vasodilator responses to the iEAAr activator L-cysteine were obtained even at more ventral sites of the salivatory nucleus, 1–1.5 mm rostral to the RVLM. An anatomical study using a retrograde tracer reported a widely spread parasympathetic origin in the brainstem with long dendrites (~0.8 mm) (Contreras et al., 1980), but no functional mapping studies of CAR using chemical stimulation have been performed. The iso-pressure vasodilation may be produced by parasympathetic excitation with L-cysteine in the RVLM reaching dendrites from that parasympathetic origin. A detailed examination is required to determine whether this vasodilation can be caused by parasympathetic activation in the brainstem (Contreras et al., 1980).

To summarize, mapping of the responses to microinjection of the ionotropic EAAr activator L-cysteine showed persistent topographic carotid vasoconstriction in the caudal part of the RVLM pressor area in most examined rats, but no topographic response was obtained in some rats. Rats that underwent cervical sympathectomy showed no AP-dependent carotid resistance changes in response to L-cysteine or no autoregulation of carotid flow, both in the RVLM pressor area and the CVLM depressor area. Occasionally, the rostral part of the RVLM included a site of equi-pressure vasodilation. The results suggest that sympathetic carotid flow regulation involves ventrolateral medullary presympathetic neuronal networks possibly with unique mechanisms related to iEAARs. The rostral and caudal parts of the RVLM pressor area may play differential roles in carotid flow regulation.

Uncited references

Dampney, 2016

Takemoto, 2014c

Declaration of competing interest

The author declares that there are no conflicts of interest.

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Highlights

- Common carotid flow responses to L-cysteine were mapped in rat ventrolateral medulla.
- Topographic vasoconstriction was obtained in RVLM of most rats but no topography in some rats.
- ~~Hence,~~ Ttopography might be ~~is~~ state dependent.
- No resistance changes parallel to arterial pressure was obtained in cervical sympathectomized rats.
- It suggests a different role of RVLM topographic area for sympathetic carotid flow regulation.

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