The Relationship between FTL and NA, DMV or CVLM in Central Cardiovascular Control

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Abstract

The aim of the present study was to examine the relationship between the lateral tegmental field (FTL), a cardioinhibitory area, with other cardioinhibitory areas, i.e., the ambiguus nucleus (NA) and the dorsal motor nucleus of vagus (DMV) and the caudal ventrolateral medulla (CVLM), a vasopressor inhibitory area. In 55 cats anesthetized with chloralose (40 mg/kg) and urethane (400 mg/kg), the cardiovascular responses of heart rate (HR), systemic arterial blood pressure (SAP) and vertebral nerve activity (VNA) were recorded. The FTL, NA, DMV and CVLM were identified first by stimulation (rectangular pulses in 80 Hz, 0.5 ms, 50-100 μA) and then confirmed by microinjection of sodium glutamate (Glu, 0.25M, 70 nl). In studying the influence of NA, DMV, or CVLM lesion on the Glu-induced responses in FTL, kainic acid (KA, 24 mM, 100 nl) was microinjected into the NA, DMV or CVLM. FTL stimulation produced an average decrease of HR by 55 %. After KA lesioning of the ipsilateral NA or the DMV, the decreased HR induced by FTL was significantly diminished. After subsequent lesion of the contralateral DMV or NA, the bradycardia of FTL was abolished. The reduction of resting HR was more intense after lesioning the NA than DMV and with the left side more than that of the right side. These studies suggest that the cardioinhibitory responses of FTL are mediated through both NA and DMV with predominance of the former, while the hypotensive effect of FTL is mediated through CVLM. The precise pathway responsible for the FTL-induced bradycardia and hypotension is to be determined.

Key Words: lateral tegmental field, ambiguus nucleus, dorsal motor nucleus of vagus

Introduction

The medulla oblongata is a vital organ for regulating circulation, cardiac rhythm and baroreceptor reflex (1-2). The vagal nuclei in the medulla, i.e., ambiguus nucleus (NA) and the dorsal motor nucleus of the vagus (DMV) contribute greatly to the regulation of cardiac functions including rhythm. Cardiac vagal preganglionic neurons of the cat have been located in both NA and DMV (3-6). Projections from neurons of these two nuclei have been extensively studied using various approaches. Ciriello and Calaresu (3) reported that electrical stimulation of the right cardiac branches of the vagus nerve to antidromically activate the DMV and NA neurons in cats required a latency corresponding to the conduction velocity of B-fibers. Besides, when horseradish peroxidase (HRP) was applied to the right cardiac branches of the vagus nerve, labelled neurons were found primarily in the regions of the DMV and NA (3). Using electrophysiological recording techniques, McAllen and Spyer (5) reported that in cats vagal preganglionic motoneurons were

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found in the NA.

Neurons in NA and DMV are very different in morphology (4, 7). The size of NA neuron is larger than that of DMV. Axons from neurons in NA are A-fibers while those from DMV are C-fibers. Thus, the conduction of fibers from NA is more rapid than that from DMV.

Stuesse and Fish (8) reported that when HRP was microinjected into the NA, labelled cells were found in the ipsilateral nucleus tractus solitarius (NTS) and the contralateral paraventricular nucleus (PVN). When HRP was microinjected into bilateral NA and retroambiguus (rNA), labelled cells were found in the ventrolateral NTS (9). Activation of NA by microinjection of sodium glutamate (Glu) produced bradycardiac responses (10). Thus, when the NA was lesioned by microinjection of kainic acid (KA), the initial excitatory effect of KA produced decreases in heart rate (HR), cardiac contractility and hypotension (11-12).

In the meantime, when HRP was microinjected into the DMV, labelled cells were found in the medullary reticular formation, interpositis cerebellar nucleus, hypothalamic nuclei, central amygdala nucleus and insular cortex (13). However, there was no significant change in cardiac rhythm when KA was microinjected into the DMV (14-15).

The concept of the FTL grew out from the classic medullary "pressor area", a columnar array of cells lying 1.5-3.25 mm lateral to the midline of the reticular formation, extending from the level of the obex to the pontomedullary border (16). Gebber and Barman (17) reported that the sympathetic nerve-related activity of FTL neurons can be sympathoexcitatory or sympathoinhibitory. The sympathoexcitatory neurons project to the rostral ventrolateral medulla (RVLM), while the sympathoinhibitory neurons project to the caudal raphe. However, no projection has been found from FTL neurons to the thoracolumbar intermediolateral column (IML) which contains sympathetic preganglionic neurons (17).

FTL, which is a part of the lateral reticular formation of the caudal medulla, potentially participates in relaying vestibular signals to subretrofacial rostral ventrolateral medulla (sRVLM). Some FTL neurons also have projections to the cardiovascular-regulatory region in the sRVLM (18). Many FTL cells discharges synchronously with the activity of sympathetic nerve (19). Dempesy et al. (20) reported that microinjection of neurochemicals (AMPA and kynurenic acid) into the FTL of cats induced sympathetically-mediated cardiovascular responses. FTL neurons have been shown to play an important role in generating some components of spontaneous sympathetic nerve discharges (18-19), to participate baroreceptor reflexes (19, 21) and to integrate cardiovascular functions (22-24). Numerous FTL cells display increased *c-fos* expression during emesis (24). Neurons of FTL, located in the vicinity of the NA, also involved respiratory control (23, 25).

Since stimulation of the FTL produced bradycardia and hypotensive responses, the aim of the present study was to determine whether the cardioinhibitory responses of FTL are mediated through the vagal nuclei, i.e., NA and DMV, and the functional relationship between the FTL and CVLM in producing hypotensive action.

Materials and Methods

A total of 55 cats, of either sex, weighing 2.5-3.5 kg, were anesthesitized intraperitoneally with a mixture of urethane (400 mg/kg) and a-chloralose (40 mg/kg). After the trachea was intubated, the animal was artificially ventilated with the end tidal CO_2 maintained at approximately 4 %, and was further paralyzed with gallamine triethiodide (2 mg/kg/30 min). The mean systemic arterial blood pressure (MSAP), and HR were taken and displayed through the cannulated femoral artery (26). The femoral vein was cannulated for drug administration. All recordings were made on a Gould 1000S polygraph. The rectal temperature was kept at 37 ± 1 °C by a homeostatic blanket (Harvard).

The head of the animal was fixed in a David-Kopf stereotaxic instrument (Tujunga, CA, USA) with a modified mouth piece. After removal of the occipital and the parietal bones, stimulations of the brain were made through a three-barrel micropipette (World Precision Instruments) prepared through a modified Naishige puller (27). The micropipetters were inserted into the brain at an angle of 34 °. The tip of each capillary was 30-50 μm. One pipette, containing physiological saline inserted inside with a piece of silver wire, was used for electrical stimulation under rectangular pulses of constant current in 50-100 μA, 15 sec, 0.5 ms duration and 80 Hz. The current was generated from a Grass S-88 stimulator coupled with a constant current unit. This same pipette was also used for making lesion by shiffing to direct current (DC, 1 mA for 50 sec) from the same stimulator. Another pipette was filled with Glu (0.25 M

(0.25 M in artificial CSF with 0.4% pontamine sky blue, pH 7.4) for chemical stimulation (70 nl), while the remaining third pipette was filled with KA (24 mM in artificial CSF with 0.4 % pontamine sky blue, pH 7.4) for chemical lesioning (200 nl). The latter two pipettes were connected through two separate PE-50 tubings, each connecting to a pneumatic pump (Medical System, BH-2) for injection. The location and size of the area receiving stimulation were determined by postmortem histological examination. A spread of pontamine sky blue in a diameter of 0.15 mm corresponded to a microinjection of 70 nl while 0.22 mm for 200 nl.

Neural Recording

The left vertebral sympathetic nerve was exposed and its activities were recorded as has been described previously (28). In brief, under an operation microscope, the nerve was dissected free from the surrounding connective tissues, desheathed, cut distally, and placed on a bipolar platinum electrode under mineral oil. The efferent whole nerve activities were amplified (bandpass: 10-3 K Hz), rectified, and integrated by an integrator (Gould 13-4615-70) with a reset time of 1 or 5 s monitored with an oscilloscope (Tektronix 5113) and stored on a tape recorder (Neuro Data DR-886) for later analysis. To acquire the actual activity, the noise level of the recorded nerve contained in the integrating null nerve activity 10 min after the animal was killed, was subtracted from the whole nerve activity (29).

Histology

At the end of each experiment, the animal was sacrificed by i.v. saturated KCl. The brain was removed and immersed in 10 % formalin solution for 2 -3 days. The brain was frozen sectioned in 50 μm thickness by a cryostat microtome (Reichert-Jung, 2800 Frigocut). The sections were stained with cresyl violet. Sites of chemical stimulation and lesion were reconstructed from sections containing the electrode tracks and marks of the pontamine sky blue.

Data Analysis

Percent changes of SAP and perturbation of nerve activity following stimulation of the sympathoexcitatory or sympathoinhibitory site were calculated by dividing the value of maximum change with the control value (Response value-Control value) / (Control value) ′ 100 %. The control value of nerve activity was derived by averaging the integrated nerve activities from six consecutive recordings for 5 s before stimulation. The largest deviation from the control level at the period 30 s after stimulation was considered as the value of maximum change, which was positive during excitation and negative during inhibition of the nerve activity. Data were presented as mean ± standard error from the mean. P value less than 0.05 calculated from *Student's t*-test was considered statistically significant.

Results

Electrical stimulation of the FTL produced cardioinhibitory and depressor responses i.e., decrease in HR and SAP, but only slight change in VNA. Microinjection of Glu (70 nl) into the same loci in the FTL produced similar responses.

Mediation of the FTL-Cardioinhibitory Effect through the NA

Effects of lesioning the NA with KA on the Glu-induced cardioinhibitory responses of the FTL were studied in a total of 21 animals. In 9 of these animals, bilateral lesions were made, first in the NA ipsilateral to the FTL then the remaining (contralateral) NA. In the other 12 animals the procedure of NA lesioning were reversed, i.e., first contralateral then ipsilateral. The effects of lesioning are illustrated in Table 1 and Fig. 1. Lesioning the ipsilateral (left) NA substantially decreased the resting HR; from 214 to 104 beat × min^{-1} (-110 beat × min^{-1}, n = 9) (Fig. 1). Lesioning the contralateral (right) NA decreased the resting HR from 208 to 110 beat × min^{-1} (-98 beat × min^{-1}, n = 12). Thus, unilateral lesioning, particularly on the left side, caused a sufficient attenuation of the resting HR. However, in either case, the resting HR was not decreased further when the remaining NA was lesioned (Table 1).

Comparing the cardioinhibitory responses of the FTL induced by Glu, it was found that regardless of which side (ipsilateral or contralateral) of the NA was lesioned first, the FTL induced-bradycardia in HR was significantly attenuated (from –36 % to 0 %). After both NA were lesioned, the Glu-induced bradycardia of the FTL was almost eliminated. Before NA lesioning,

Table 1. Effects of NA Lesioning on Cardioinhibition and Decrease in SAP Induced by Glu Stimulation in FTL

	MSAP			HR		
	Control	Stimulation (mm Hg)	Change (%)	Control	Stimulation (bpm)	Change (%)
Ipsilateral lesioning (N=9)						
A	116.1 ± 9.6	100 ± 15.5	-13.1 ± 10.7	214.4 ± 18.1	161.7 ± 37.4	-24.3 ± 16.8
B	92.4 ± 17.8	115.9 ± 36.1	23.4 ± 20.9*	103.9 ± 32.2	104.1 ± 32.8	0.12 ± 0.37*
Contralateral lesioning (N=12)						
A	122.3 ± 14.2	105.8 ± 16.6	-13.3 ± 12.3	207.9 ± 23.6	136.8 ± 55.0	-35.6 ± 24.5
B	94.3 ± 18.5	104.5 ± 22.2	9.1 ± 15.2*	110.0 ± 37.7	111.7 ± 38.4	-0.2 ± 0.5*
Bilateral lesioning (N=21)						
A	121.3 ± 9.5	104.1 ± 15.5	-14.4 ± 9.2	223.8 ± 19.4	169.1 ± 44.1	-24.4 ± 18.2
B	88.1 ± 26.4	102.8 ± 31.6	17.0 ± 18.1*	86.9 ± 12.5	86.9 ± 12.5	0.0 ± 0.0*

Numbers in the table are mean ± SE; *: difference between A and B pair was statistically significant by Student's *t*-test, $p<0.01$; N, number of animals tested; MSAP, mean systemic arterial pressure; HR, heart rate; KA, kainic acid; NA, ambiguus nucleus; FTL, lateral tegmental field. A: before KA lesioning. B: after KA lesioning.

Table 2. Effects of DMV Lesioning on Cardioinhibition and Decrease in SAP Induced by Glu Stimulation in FTL

	MSAP			HR		
	Control	Stimulation (mm Hg)	Change (%)	Control	Stimulation (bpm)	Change (%)
Ipsilateral lesioning (N=7)						
A	113.0 ± 11.7	104.8 ± 24.6	-9.8 ± 16.7	206.3 ± 26.2	136.3 ± 44.8	-35.5 ± 24.7
B	96.4 ± 15.7	122.1 ± 36.7	26.2 ± 32.1	166.4 ± 44.4	162.9 ± 42.8	-1.9 ± 3.8*
Contralateral lesioning (N=9)						
A	125.8 ± 14.8	115.8 ± 27.3	-7.0 ± 18.5	219.4 ± 34.7	122.6 ± 55.7	-42.7 ± 25.2
B	100.4 ± 16.4	103.9 ± 22.9	3.49 ± 16.6	195.0 ± 60.8	192.8 ± 59.3	-1.0 ± 1.9*
Bilateral lesioning (N=16)						
A	127.7 ± 14.8	122.0 ± 26.6	-4.5 ± 17.6	223.3 ± 38.8	118.7 ± 51.0	-46.6 ± 20.9
B	79.9 ± 10.9	83.4 ± 10.4	5.69 ± 13.6	179.0 ± 25.4	172.2 ± 26.6	-1.4 ± 2.4*

Numbers in the table are mean ± SE; *: difference between A and B pair was statistically significant by statistically significant by Student's *t*-test, $p<0.01$; N, number of animals tested; MSAP, mean systemic arterial pressure; HR, heart rate; KA, kainic acid; DMV: dorsal motor nucleus of vagus; FTL, lateral tegmental field. A: before KA lesioning. B: after KA lesioning.

HR by 24-36 %. After bilateral NA lesioning, the slowing of HR induced in FTL became not significant (from – 24 % to 0 %) (Table 1). VNA did not change much as compared to a 3 % increase before NA lesioning (Fig. 1).

Mediation of the FTL-Cardioinhibitory Effect through the DMV

The effects of DMV lesioning on the Glu-induced cardioinhibitory responses of FTL in 16 cats are

Table 3. Effects of CVLM Lesioning on Hypotensive and Decrease in HR Induced by Glu Stimulation in FTL

	MSAP			HR		
	Control	Stimulation (mm Hg)	Change (%)	Control	Stimulation (bpm)	Change (%)
Ipsilateral lesioning (N=11)						
A	109.5 ± 11.6	84.5 ± 12.7	-22.1 ± 12.6	172.2 ± 31.9	89.0 ± 29.0	-46.6 ± 19.5
B	89.5 ± 10.1	110.5 ± 17.4	23.2 ± 12.8*	128.2 ± 45.5	131.1 ± 46.1	2.5 ± 3.4*
Contralateral lesioning (N=7)						
A	111.7 ± 13.5	85.4 ± 13.5	-23.8 ± 5.2	171.4 ± 33.0	95.7 ± 39.5	-43.8 ± 22.8
B	93.6 ± 19.0	111.4 ± 22.5	19.6 ± 11.0*	103.6 ± 28.1	103.6 ± 28.1	0.0 ± 0.0*
Bilateral lesioning (N=18)						
A	125.0 ± 2.9	90.8 ± 15.4	-27.3 ± 12.1	185.8 ± 31.4	95.8 ± 32.8	-48.5 ± 15.1
B	92.5 ± 16.5	103.3 ± 16.2	12.2 ± 0.52*	132.8 ± 41.2	132.8 ± 41.2	0.0 ± 0.0*

Numbers in the table are mean ± SE¡F*: difference between A and B pair was statistically significant by statistically significant by Student's t-test, $p<0.05$; N, number of animals tested; MSAP, mean systemic arterial pressure; HR, heart rate; KA, kainic acid; CVLM: caudal ventrolateral medulla; FTL, lateral tegmental field. A: before KA lesioning. B: after KA lesioning.

The effects of DMV lesioning on the Glu-induced cardioinhibitory responses of FTL in 16 cats are summarized in Table 2 and illustrated in Fig. 2. Among these 16 cats, 7 were subjected to DMV lesion, first on the same (left) side, then followed by contralateral (right) side of FTL. In other 9 of these cats, the process of DMV lesioning was reversed. Similar to that of the NA, microinjection of KA into the DMV produced an initial but larger decrease in HR than application of Glu into the same area. Lesioning the ipsilateral (left) DMV substantially decreased the resting HR; from 206 to 166 beat × min^{-1} (-40 beat × min^{-1}, n = 7). Lesioning the contralateral (right) DMV decreased the resting HR from 219 to 195 beat × min^{-1} (-24 beat × min^{-1}, n = 9). The decrease was more pronounced after lesioning the left than the right DMV (Table 2). The reduction of resting HR was less than that of the NA (in a range from 98 to 110 beat × min^{-1}) after lesioning the DMV (in a range from 24 to 40 beat × min^{-1}).

DMV lesioning, regardless of which side first, produced a significant decrease in the Glu-induced cardioinhibitory responses of the FTL. The extent of decrease was large despite unilateral lesioning. The HR decreased from DMV activation was reduced from 70-96 to 2-3 beat × min^{-1}, against an increase of the VNA. After the remaining DMV (bilateral) was further lesioned, the Glu-induced cardioinhibitory responses were almost eliminated (Table 2), but the VNA had no significant change.

FTL and CVLM Hypotensive Effects

Effects of lesioning the CVLM with KA on the Glu-induced hypotensive responses of the FTL were studied in a total of 18 animals. In 11 of these animals, bilateral lesions were made, first in the CVLM ipsilateral to the FTL then the remaining (contralateral) CVLM. In the other 7 animals, the procedure of CVLM lesioning was reversed, i.e., first contralateral, then ipsilateral. The effects of lesioning are illustrated in Table 3 and Fig. 3. Lesioning the ipsilateral (left) CVLM, substantially decreased the resting SAP from 111 to 90 mmHg (–22 mmHg, n = 11) (Fig. 3). Lesioning the contralateral (right) CVLM decreased the resting SAP from 112 to 94 mmHg (–18 mmHg, n = 7). Bilateral CVLM lesions caused a more pronounced decrease of SAP from 125 to 93 mmHg (–32 mmHg, n = 18) (Table 3).

The hypotensive responses of the FTL in SAP induced by Glu before CVLM lesioning was-22 – 27%. After CVLM lesioning, the same Glu activation in FTL induced hypertensive than hypotensive action. However, regardless of which side (ipsilateral or contralateral) of the CVLM was lesioned first, subsequent activation of FTL produced a similar hypertensive responses increase in SAP; 20-23 %. After both CVLM were lesioned, the

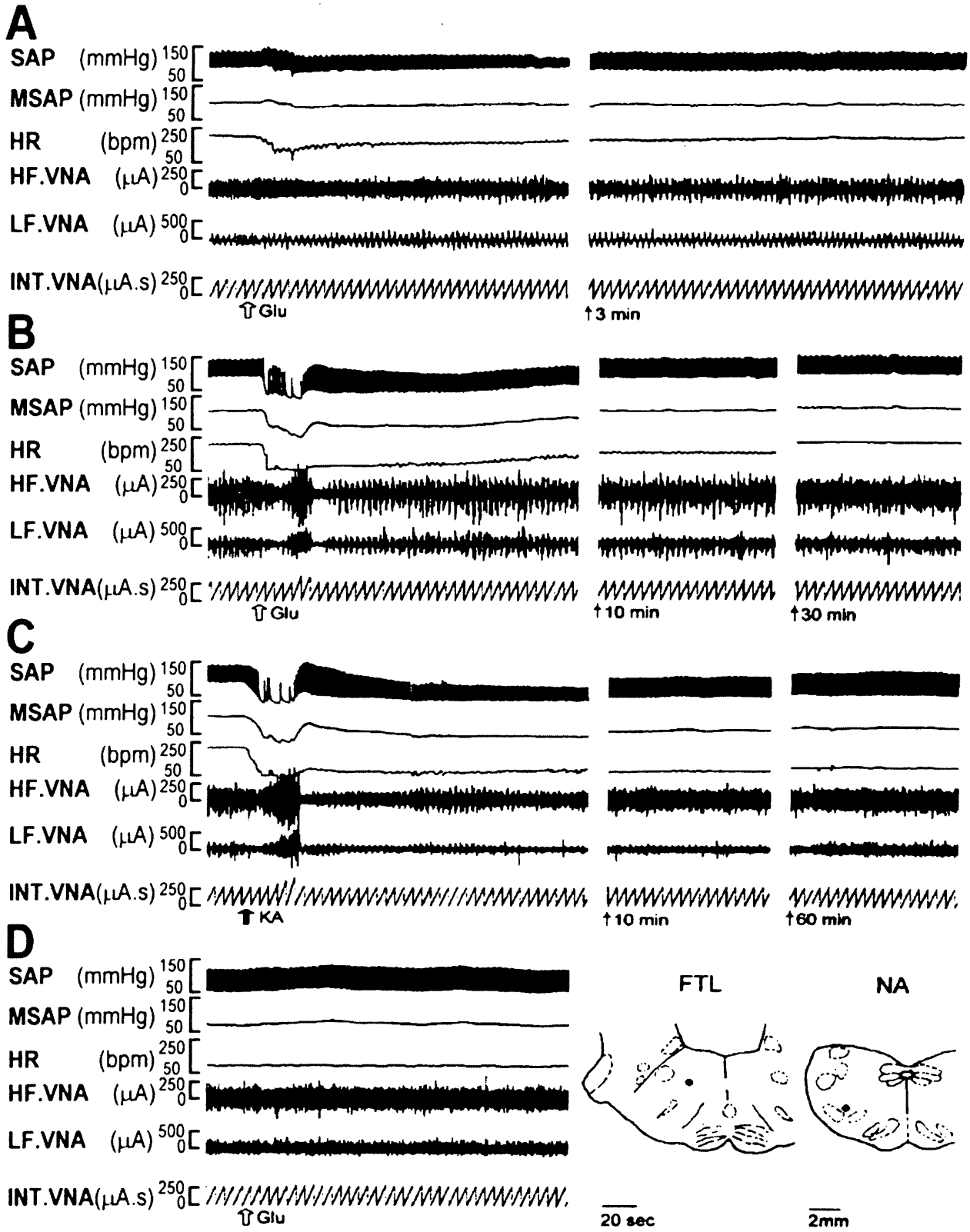

Fig. 1. Cardioinhibitory responses of the FTL induced by Glu were markedly reduced after lesioning the ipsilateral NA. A. Microinjection of Glu (0.25 M, 70 nl) into the left FTL produced decreases in HR and SAP with little change in the VNA. B. Glu (0.25 M, 70 nl) stimulation of the left NA produced pronounced cardioinhibition and decrease in SAP but associated with a brief and slight increase of the VNA. C. The initial excitatory effect of KA (24 mM, 50 nl) produced similar responses when it was microinjected during lesioning the NA. D. Two hrs after NA lesioning, the same dose of Glu into the same point in the FTL produced no cardioinhibitory responses. Instead, the SAP was slightly increased. Abbreviations: SAP, systemic arterial blood pressure; MSAP, mean SAP; HR, heart rate; INT.VNA, integrate vertebral sympathetic nerve activity; HF.VNA, high frequency VNA; LF.VNA, low frequency VNA.

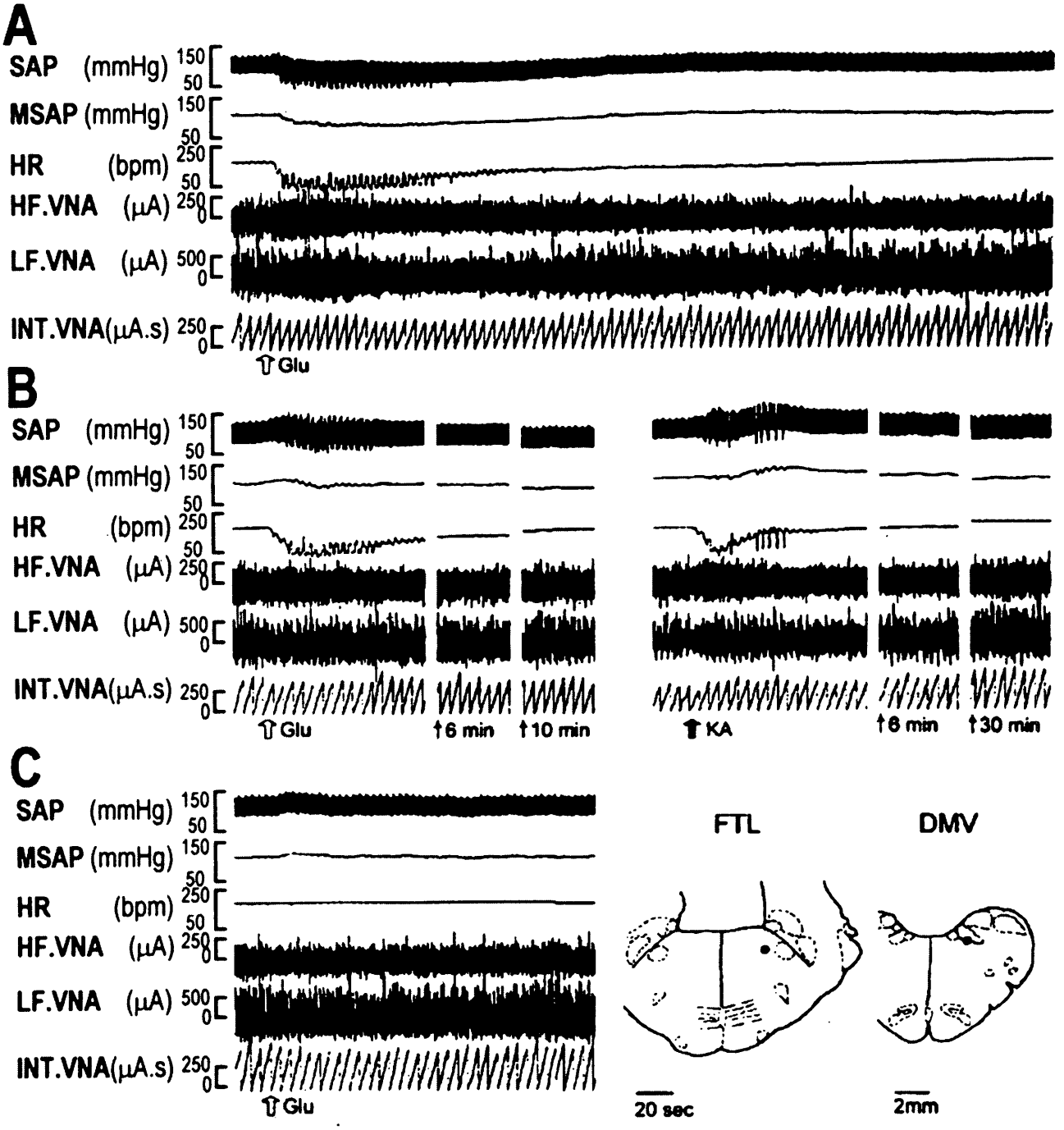

Fig. 2. The cardioinhibitory responses of the FTL induced by Glu were markedly reduced after lesioning the ipsilateral DMV. A. Microinjection of Glu (0.25 M, 70 nl) into the right FTL produced cardioinhibition and decrease in SAP with little change in VNA. B. The ipsilateral (right) DMV was explored first by microinjection of Glu (0.25 M, 70 nl) and then lesioned by KA (24 mM, 50 nl). C. Two hrs after DMV lesioning, a same dose of Glu in the same point of FTL did not produce cardioinhibitory responses.

Glu-induced hypertensive responses of the FTL were decreased (from 23 to 12 %)(Table 3). VNA did not change as compared to a 3 % increase before CVLM lesioning (Fig. 3).

Discussion

The present study shows that: 1. The cardioinhibitory responses of FTL are mediated through NA and DMV. 2. The hypotensive action induced by FTL is mediated through CVLM.

The FTL is included in the dorsomedical pressor area originally described by Wang and Ranson (30) in cats, and further characterized by Dempesy in rabbits

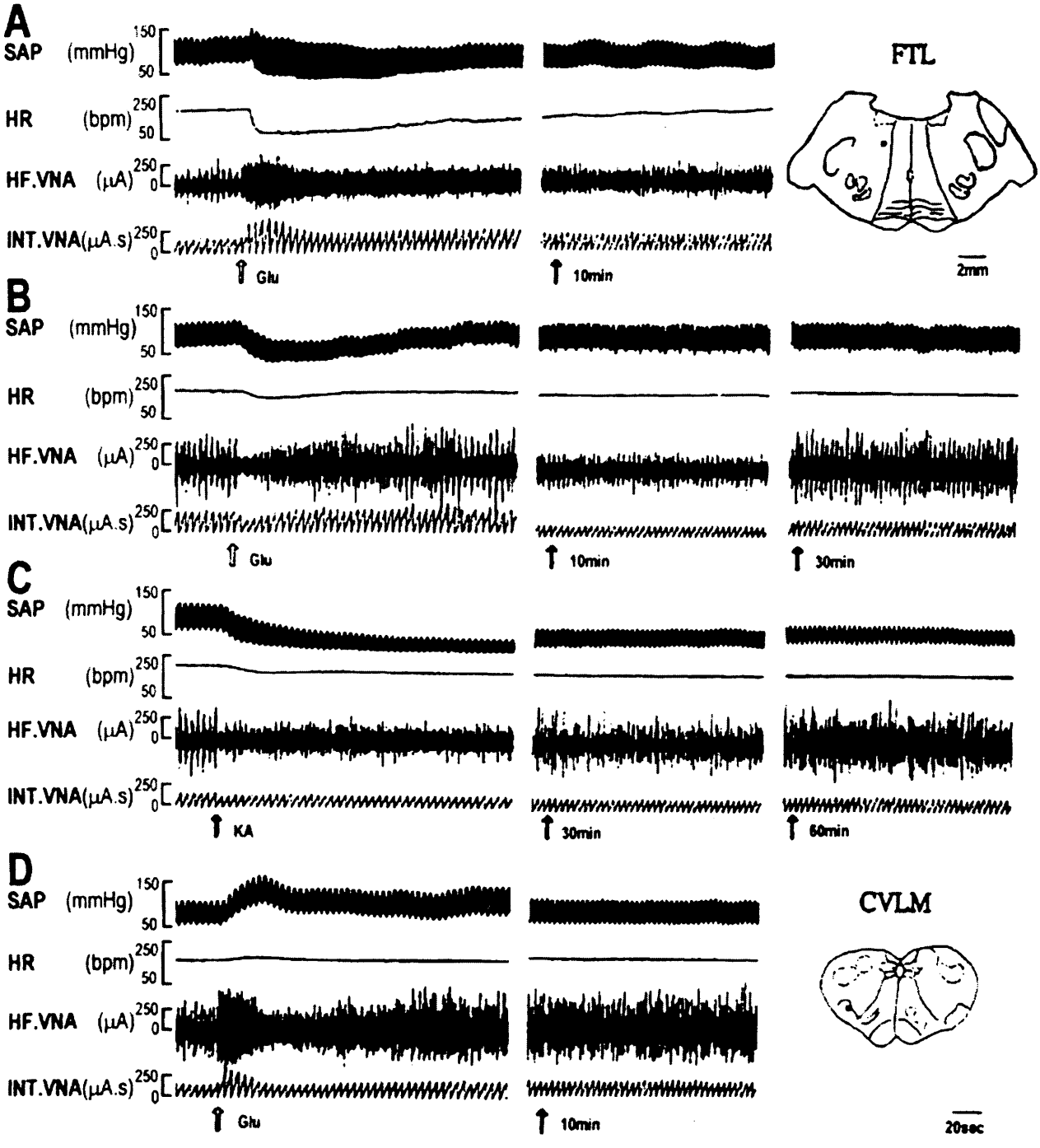

Fig. 3. Hypotensive responses of the FTL induced by Glu were markedly reduced after lesioning the ipsilateral CVLM. A. Microinjection of Glu (0.25 M, 70 nl) into the left FTL produced decreases in SAP and HR with little change in the VNA. B. Glu (0.25 M, 70 nl) stimulation of the left CVLM produced marked decrease in SAP but associated with a brief and slight increase of the VNA. C. Similar responses were produced when KA (24 mM, 50 nl) was microinjected during the process of lesioning the CVLM. D. Two hrs after CVLM lesioning, a same dose of Glu into the same point of FTL produced no cardioinhibitory response. Instead, the SAP was increased.

cardiovascular control and emesis (24). For the past three decades, despite neuronal responses of pain to noxious stimuli have been observed in FTL, little is known about the function of this diffused structure (32). More recently, the FTL has been proposed as a site for the generation of basal sympathetic nerve activity (18-19). Application of chemical or electrical stimulation to the FTL increases the activity of the postganglionic inferior cardiac sympathetic nerve (19, 33). The FTL of the cats contains neurons with naturally occurring activity

inferior cardiac sympathetic nerve (19, 33). The FTL of the cats contains neurons with naturally occurring activity synchronized with the cardiac-related rhythm in sympathetic nerve discharge (SND) (19, 34-35). The FTL contains a heterogeneous mixture of sympathoexcitatory and parasympathoexcitatory neurons (36). It is of interest to observe that the FTL hypotensive effect is reversed to a hypertensive one after CVLM lesion. This is a functional illustration regarding colocalization of sympathoexcitatory and sympathoinhibitory neurons in the same FTL.

The CVLM plays an important role in autonomic regulation. The CVLM contains vasodepressor neurons that serve as a critical component of the baroreceptor reflex pathway. Stimulation of the CVLM produces selective changes in blood pressure (37-38). Stocker et al. (37) reported that CVLM neurons project to FTL in an area extending between the caudal obex and the rostral medulla. Neurons in CVLM also project to RVLM (36, 39-40). This explains that inputs from CVLM to FTL are important in cardiovascular regulation. Results of the present study showed that the depressor responses elicited from FTL were suppressed following KA lesioning in the CVLM. KA lesioning of the FTL also blocked the baroreceptor-mediated inhibition of sympathetic activity (33). These data suggest that the FTL-depressor responses probably are mediated through excitation of the depressor neurons in CVLM. A direct pathway exists between the CVLM and RVLM (41), through which projects to the IML of the spinal cord where sympathetic preganglionic neurons reside.

The NA consists of two components, i.e., the ventral motor nuclei of the glossopharyngeal nerve and vagus nerve (42). The NA involves parasympathetic control of the HR (12). Neurons of NA are likely to receive cardiovascular afferent inputs through the NTS (43). The C_1 area has been identified in the reticular formation of RVLM as a cell column extending throughout the VLM and lying ventral to the NA cell group (44). The NA could exert a modulatory effect on the C_1 area and thus indirectly influence autonomic functions (12). The pathway from NTS to cardiac vagal neurons may mediate cardiovagal action, whereas to the RVLM that helps maintaining the discharge of preganglionic sympathetic neurons may mediate the vasodepressor responses through sympathoinhibition (45).

The majority of preganglionic parasympathetic fibers terminating in the heart originate from the NA (4, 46). Inhibition of cardiac function, mediating through efferent vagal pathways, is an important component of cardiovascular adjustments (11, 47). The region of the NA may be capable of inhibiting sympathetic outflow from the adjacent C_1 area in the RVLM (11-12). Gerrits and Holstege (16) reported that injections of HRP on the rNA revealed labelled neurons in FTL. The rNA in cats receives afferents from the FTL of the caudal pons and medulla (16). The interneurons of the rNA project mainly via a contralateral pathway to NA motoneurons (16). Our findings of KA lesion on NA are in line with Machado and Brody (11) who reported that destruction of cell bodies in NA can alter the inhibitory influence on sympathetic vasomotor tone. Similarly, we observed that after both NA were lesioned, the Glu-induced bradycardiac responses of the FTL were almost eliminated.

Similar events were observed when both DMVs were lesioned, but the reduction of the resting HR was less intensive than that after NA lesioning. Unilateral lesion in NA caused an attenuation of HR effect in a range from 98 to 110 beat $\times$ min^{-1} (Fig. 1, Table 1). The decrease from DMV was reduced by 24-40 beat $\times$ min^{-1} (Fig. 2, Table 2). Thus, the cardioinhibitory responses of FTL are mediated through NA and DMV but with predominance of the former. This is consistent with our previous findings that microinjections of HRP into the cardiopulmonary nerve revealed labelled cells in both NA and DMV but with predominance in the former. Besides, significantly higher portion (91 %) of HRP labelled cells was found in the NA when HRP was injected into the heart tissue (4). Thus, the present study supports the thesis that the cardiac vagal preganglionic neurons originate predominantly from the NA in cats. Another interesting finding of the present study concerns the relative importance of the NA vs. DMV in the vagal innervation of the heart. The bradycardiac response elicited from the FTL was suppressed when KA was microinjected into the DMV. This suggests that the FTL-bradycardiac responses probably are mediated through excitation of the vagal neurons in DMV.

In summary, findings of the present experiment are compatible with the hypothesis that FTL-bradycardiac responses probably are mediated through excitation of the vagal neurons in NA and/or DMV. The FTL modulates sympathetic outflow possibly through its projections to the region of inhibitory CVLM. This will help clinical diagnosis in correlating the cardiovascular symptoms of lesion in the region of FTL or CVLM. Whether the NA has other functions await further study.

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