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Cardiorespiratory effects of intertrigeminal area stimulation in vagotomized rats

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Abstract

It has been recently shown that the pontine intertrigeminal region (ITR) plays an important role in respiratory regulation, including vagally mediated apneic reflexes. Neurons of the ITR have connections with the nucleus tractus solitarius and projections to the ventrolateral medulla. However, the functional targets of these projections are not fully defined. Stimulation of ITR neurons produced respiratory effects, but cardiovascular responses have not been explored. We investigated impact of bilateral vagotomy on respiratory and cardiovascular responses to glutamate microinjections within the ITR in ketamine/xylazine anesthetized rats. Cardiorespiratory indices, including breath duration (TT), tidal volume (VT), mean cardiac intervals (RR), systolic blood pressure (SBP), pulse pressure (PP) and their coefficients of variation (CVTT, CVVT, CVSBP, CVPP, respectively) were analyzed in 30 s segments before and after injection of glutamate (10 mM, 30 nL) into the ITR. This assessment was carried out both before and after bilateral vagotomy. Glutamate injection evoked apnea and increased CVTT, but these responses were not altered by bilateral vagotomy. In contrast, removing vagal pathways significantly increased volume variability (CVVT), making tidal volume more vulnerable to perturbation from the ITR. Vagotomy prolonged the increase of mean systolic blood pressure observed after glutamate injection and unmasked a delayed but sustained elevation of PP and CVPP after ITR stimulation. The present findings indicate a broad involvement of the ITR in autonomic regulation, including at least cardiovascular and respiratory effects.

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1. Introduction

The pontine intertrigeminal region (ITR) was recently discovered to play an important role in respiratory regulation (Chamberlin and Saper, 1998; 2003; Radulovacki et al., 2003a,b). Neuroanatomical and neurophysiological studies (Chamberlin and Saper, 1998; 2003; Radulovacki et al., 2003a,b) showed that microinjections of glutamate into the group of cells in the lateral pons among the fibers between the motor and principal sensory trigeminal nuclei evoked immediate apnea in anesthetized rats. Rostrally, the apneic sites reached the ventral border of Kölliker-Fuse nucleus, while in the caudal direction they stretched ventrally along the motor trigeminal roots (Chamberlin and Saper, 1998). Experiments with anterograde and retrograde tracers determined connections of ITR neurons with the nucleus tractus solitarius (NTS) and the ventrolateral medulla (VLM; Chamberlin and Saper, 1998; 2003). Therefore, it was suggested that the ITR may represent a key integrating site for a wide range of apneic reflexes (Chamberlin and Saper, 1998).

Important sensory inputs mediating airway protective apneic reflexes are conveyed by the branches of the vagal, trigeminal and glossopharyngeal nerves from the oropharynx, larynx, trachea and lungs (Sant' Ambrogio et al., 1995). Reflex apnea mediated by vagus nerve activation can be modeled pharmacologically by intravascular infusion of serotonin (5-HT; Yoshioka et al., 1992a,b). Thus, it has been shown that intravenous or intracarotid infusion of 5-HT produces a complex dose-dependent and long-lasting cardiorespiratory reaction, the Bezold-Jarisch reflex. This reflex is characterized by the triad of apnea, bradycardia and hypotension (Aviado and Guevara Aviado, 2001; Sutton, 1981) and is completely abolished after bilateral vagotomy above the nodose ganglia (Yoshioka et al., 1992a,b; Jacobs and Comroe, 1971; Segers and Shannon, 1985; Kopczynska and Szereda-Przestaszewska, 2003).

A functional role of the ITR in regulation of respiratory reflexes and its involvement in the mechanisms of vagally mediated reflex apnea was suggested by the observation that blockade of ITR neurons by kynurenic acid – a broad-spectrum glutamate receptor antagonist – prolonged the duration of reflex apnea produced by an intravenous injection of serotonin (Radulovacki et al., 2003a). Additionally, acute ponto-medullary transection, isolating the ITR from the ventrolateral medulla evoked a dramatic potentiation of 5-HT induced apnea (Radulovacki et al., 2003a). In addition to its role in the mechanisms of reflex apnea it was also shown that local unilateral lesion of ITR neurons increased the appearance of sleep apneas in rats, indicating a potential convergence in the mechanisms underlying reflex apneas and central sleep apneas (Radulovacki et al., 2003b).

While these findings support an important role of the ITR in respiratory control, the possible involvement of the ITR in cardiovascular regulation has not been explored. This is of interest since the ITR also sends projections to areas of ventrolateral medulla, which is associated with cardiovascular regulation (Chamberlin and Saper, 1992). Our aim here was to test cardiovascular effects of ITR stimulation and to determine the role of vagal pathways in ITR regulatory influences. Therefore, we investigated cardiorespiratory responses to glutamate microinjections into the ITR before and after bilateral vagotomy.

2. Results

The general features of the cardiorespiratory response to ITR glutamate injection are illustrated in Fig. 1. Microinjections of glutamate into the intertrigeminal area elicited immediate apnea 3–10 s duration (Fig. 1A). The apneic reaction was used for a functional verification of the injection site location within the ITR. Visual scoring of the record revealed also a transient hypertensive response with a secondary brief compensatory decrease of blood pressure. The blood pressure reaction appeared simultaneously with the apnea within the first 30 s after the glutamate injection (Fig. 1A). Subsequently, a small but constant increase of blood pressure appeared 60–90 s post-injection (Fig. 1A) with a concomitant increase of respiratory intervals. Similar early and late responses of respiration and blood pressure to the glutamate stimulation of the ITR were registered after the vagotomy (Fig. 1B).

2.1. Respiratory responses to glutamate microinjections into the ITR: effects of vagotomy

The apneic response to the glutamate injection into the ITR was followed by destabilization of the respiratory pattern (Fig. 1A) and changes of respiratory frequency. However, the mean value of respiratory intervals (TT) did not show significant alteration (Fig. 2A).

A similar effect was observed after the vagotomy (Fig. 2B), which also revealed the significant secondary increase in mean breath duration (TT) ($p = 0.05$) (Fig. 2A).

Respiratory timing variability, measured as coefficient of variation of total breath duration (CVTT) increased significantly during the first 30 s immediately following the injection both before ($p = 0.00004$) and after ($p = 0.02$) vagotomy (Fig. 2B). Vagotomy did not affect the propensity of CVTT response to ITR glutamate stimulation (pre- and post-vagotomy values of CVTT did not differ by paired t-test at any time interval; Fig. 2B).

Respiratory volume variability (CVVT) showed a significant increase in the 0–30 s time interval ($p = 0.05$) with a subsequent decrease at 90–120 s post-injection ($p = 0.004$) only in vagotomized animals (Fig. 2C). The mean respiratory volume (VT) did not change significantly after the injection of glutamate either before or after vagotomy (data not shown).

2.2. Cardiovascular effects of ITR stimulation and the impact of vagotomy

2.2.1. Cardiac intervals (RR)—Neither the mean RR nor CVRR was altered at any time interval by ITR glutamate injection. This was the case both before and after vagotomy (data not shown).

The periodic appearance of extra-long (at least 20 percent above the mean for the 30 s interval) and extra-short (less than 60 percent of the mean for the 30 s interval) RR intervals, which possibly represented respiratory-related arrhythmia, was observed in the electrocardiogram (ECG). Counts of such arrhythmic beats were analyzed in relation to glutamate injection and vagotomy.

ITR stimulation by glutamate had no effect on the occurrence of extra-long RR intervals either before or after vagotomy (Table 1). In contrast, the number of extra-short RR intervals was significantly reduced by glutamate injection into the ITR; an effect that was not altered by vagotomy (Table 1).

2.2.2. Characteristics of blood pressure—Mean systolic blood pressure (SBP) revealed a significant elevation 30–60 s after ITR glutamate injection (Fig. 3A; $p = 0.05$ versus pre-injection for each post-injection 30 s time interval). Post-vagotomy SBP also increased during 60–90 s and 90–120 s after injection in comparison to pre-injection and pre-vagotomized values ($p = 0.04$ and $p = 0.05$, respectively; Fig. 3A).

In distinction to the delayed increase of mean SBP, its variability (CVSBP) was immediately (0–30 s) but transiently increased after the glutamate injection both in control and vagotomized conditions ($p = 0.004$ and $p = 0.01$, respectively; Fig. 3B).

Prior to vagotomy ITR injection exerted no effects on either mean pulse pressure (PP) or its coefficient of variation (CVPP; Fig. 3C and D). Vagotomy per se increased mean PP in comparison to controls (Fig. 3C; $p = 0.01$). After vagotomy glutamate stimulation of the ITR produced a delayed but significant increase of mean PP (30–60 s, 60–90 s, 90–120 s and 120–150 s intervals) in comparison to the pre-injection control interval ($p = 0.01$, 0.01, 0.008 and 0.05, respectively; Fig. 3C). Vagotomy also unmasked an immediate but transient (0–30 s time interval) increase of pulse pressure variability (CVPP) following glutamate stimulation of the ITR ($p = 0.04$; Fig. 3D).

2.3. Histological localization of injection sites

Histological analysis of the injection sites verified that all functionally identified apneic points were within the anatomical boundaries of the ITR (9.3–9.84 mm posterior to bregma), between the principal sensory and motor trigeminal nuclei. The injections were located in the ventral part of the ITR.

Fig. 4A provides a photographic example of one ITR injection site, showing the micropipette trace. A schematic summary mapping of the ITR injection sites is presented in Fig. 4B.

In summary, stimulation of the ITR by glutamate induced alterations of both respiratory and cardiovascular indices. The immediate short-term reactions and late long-lasting responses were observed both before and after the vagotomy. The variability of both respiratory timing (CVTT) and systolic blood pressure (CVSBP) increased within the first 30 s after injection. Vagotomy did not alter this response, but promoted similar immediate increases of respiratory volume (CVVT) and pulse pressure (CVPP) variability. In contrast, mean values of systolic blood pressure exhibited a late prolonged elevation, which was most prominent after vagotomy and which also unmasked a concomitant late augmentation of mean pulse pressure and mean breathe time.

3. Discussion

The present study demonstrates for the first time a significant modulation of blood pressure by the pontine ITR. Additionally, our observations reveal that supranodose vagotomy exaggerates and prolongs the perturbation of both blood pressure and respiratory timing produced by ITR stimulation. Although respiratory timing modulation by the ITR had been previously shown (Chamberlin and Saper, 1994; 1998; Radulovacki et al., 2003a,b; 2007) the present study further demonstrates an ITR stimulation-induced respiratory volume modulation after bilateral vagotomy. As shown in Figs. 2 and 3, in vagotomized animals amplification or unmasking of the ITR-induced autonomic variability (variability of respiratory timing and pulse pressure) was immediate, whereas increases in mean parameters of respiratory timing and pulse pressure tended to be delayed.

The precise mechanisms and pathways by which the ITR modulates cardiorespiratory behaviors cannot be directly inferred from the present functional data. Several possibilities are supported by previous investigations. The possible circuitry, mediated by ITR in regulation of respiratory and cardiovascular reflexes comprises at least nucleus tractus solitarius, ITR and ventrolateral medulla (Fig. 5). Nucleus tractus solitarius receives afferent inputs from the lungs and upper airways as well as chemoreceptor and baroreceptor inputs, via the vagus (X) and glossopharyngeal (IX) nerves. NTS, in turn, sends projections to ITR (Fig. 5). Respiratory inputs from upper airways are also transferred by the ethmoidal branch of trigeminal nerve

(V), which is relayed to the pontine respiratory structures, including ITR via spinal trigeminal nucleus of the medulla (Fig. 5; Chamberlin and Saper, 1994;1998;Panneton, 1991;Duchmann and Herbert, 1998; Herbert et al., 1990). Both NTS and ITR neurons project to the ventrolateral medullary region along its entire ventrocaudal extent (Fig. 5), which is involved in generation of respiratory rhythm and pattern and cardiovascular integration (Chamberlin and Saper, 1998, 2001) and which send sympathetic outputs to medi thoracic spinal cord area and exert parasympathetic influence via descending branches of vagus.

The present data, together with previous findings (Chamberlin and Saper, 1998, 2001; Radulovacki et al., 2003a,b; 2007) support a role of ITR in modulation (i.e. attenuation) of respiratory and cardiovascular reflexes. It is known that these reflexes are exaggerated following ponto-medullary transection (Radulovacki et al., 2003a; McCrimmon, 2006), which suggest a net dampening effect of pontine structures on the medullary circuits that subserve cardiorespiratory reflex regulation. The medullary projections of ITR can play an important role in modulation of respiratory timing, switching of inspiratory/expiratory phases and stabilizing the respiratory pattern, possibly via influences of GABAergic neurons widely represented in ITR (Li and Wu, 2005). Thus, it is reasonable to propose that these pathways may be central to the cardiovascular and respiratory effects of ITR stimulation observed in the present study.

In addition to the medullary projections of ITR, its influences on respiratory timing may be transmitted via connections with other pontine parabrachial/Kölliker-Fuse neurons known to exhibit respiratory rhythm (Alheid et al., 2004; St John, 1987; Dick and Bellingham, 1994; Bertrand and Hugelin, 1971; Song et al., 2006) and which also send both phasic and tonic outputs to medullary respiratory structures (Fig. 5; Dick and Bellingham, 1994; Song et al., 2006). In accordance, the dendritic invasion of the ITR by respiratory neurons of the Kölliker-Fuse nucleus has been recently demonstrated (Song et al., 2006). It was reported that in vagotomized decerebrated animals, pontine respiratory neurons fire with a slower rhythm compared with medullary respiratory units and exhibit varying degrees of respiratory modulation (Bertrand et al., 1973; Bianchi and St John, 1982; Shaw et al., 1989; Dick et al., 1994). This may partly explain the increase of ITR-induced variability in respiratory indices after vagotomy.

The mechanisms underlying the cardiorespiratory modulation by the ITR are possibly based on glutamatergic transmission within the ITR. We previously showed that glutamatergic activity within the ITR influenced both apnea duration (TT) and the respiratory pattern disturbance (CVTT) following induction of the Bezold-Jarisch reflex by intravenous infusion of serotonin (Radulovacki et al., 2003a). Injection of kynurenic acid, a broad spectrum glutamate receptor antagonist in the ITR exacerbated 5-HT induced apneas (Radulovacki et al., 2003a; 2007). Administration of selective blockers of ionotropic NMDA and AMPA receptors does not produce any changes in serotonin-induced apneas, but suppresses (NMDA antagonists) or attenuates (AMPA antagonists) apneas following glutamate stimulation of ITR (Radulovacki et al., 2007; Isenovic, 2007). In contrast, injection of a type 1 metabotropic glutamate receptor antagonist increased 5-HT induced apnea, augmenting both respiratory timing and volume variability (CVTT and CVVT; Stojkovic et al., in press). These findings provide additional evidence regarding the role of ITR in modulating vagal reflexes apnea and suggest the importance of glutamatergic neurotransmission within ITR for this function.

Vagal afferents play a particularly important role in the regulation of respiratory volume (Kubin et al., 2006; McCrimmon, 2006). Vagotomy eliminates this proprioceptive feedback to the NTS (Kalia and Mesulam, 1980a,b; Kalia and Sullivan, 1982; Kalia and Richter, 1988) and consequently to pontine respiratory neurons (Jhamandas and Harris, 1992; Herbert et al., 1990), including the ITR. Consistent with this view, our study shows that vagotomy increased

tidal volume instability following ITR perturbation (Fig. 2), a result likely due to the deficiency of vagal volume feedback.

The present data provide the first evidence of a cardiovascular regulatory role of the ITR. As with respiratory timing variability, ITR activation by glutamate evoked an immediate increase in variation of systolic blood pressure (CVSBP, Fig. 3B), which was not affected by vagotomy. Although pulse pressure was unaffected by glutamate injections into the ITR before vagotomy, ITR stimulation produced an immediate increase in pulse pressure variability (CVPP) after vagotomy (Fig. 3D).

These observations are consistent with the view that glutamate activation of ITR neurons, projecting directly to respiratory and cardiovascular neurons of the ventrolateral medulla (Chamberlin and Saper, 1992; 1998) produces disturbances in ongoing cardiorespiratory rhythms which, in the absence of vagal proprioceptive feedback become more severe. This possibility, however, remains to be directly demonstrated by future studies.

ITR stimulation also evoked a delayed increase of mean systolic blood pressure; vagotomy prolonged this phenomenon (Fig. 3A) and unmasked a similar delayed increase of mean pulse pressure (Fig. 3C). These delayed responses may suggest that stimulation of ITR neurons produces activation of unmyelinated vasomotor sympathetic outputs, which act more slowly in comparison to myelinated vagal pathways. Although a direct pathway from ITR to sympathetic premotor neurons remains to be demonstrated, such neurons are known to exist within the medullary projection fields of the ITR (Chamberlin and Saper 1992; Ross et al. 1984; Morrison et al., 1988; Reis et al., 2007) (Fig. 5). Additionally, humoral circulating factors of vascular regulation (arginine/vasopressin and angiotensin systemic factors) may become perturbed following the ITR evoked primary response, and may also influence the delayed blood pressure increase.

It is reasonable to propose that vagal afferents play a role in providing a feedback from vascular baroreceptors (baroreceptors of the aortic arch), which can contribute to attenuation of the hypertensive effects following glutamate stimulation of the ITR. Loss of these afferents after vagotomy may contribute to the larger amplitude fluctuations of mean blood pressure observed in this study. We may further suggest that the delayed increase of mean breath duration following ITR stimulation in vagotomized animals reflects baroreceptor (glossopharyngeal nerve) mediated respiratory inhibition due to the increased systolic blood pressure which occurs at the same time (Figs. 2 and 3). An existence of mutual relationships between baroreflex and chemoreflex were reported by many authors (e.g. Heistad et al., 1974; 1975; Mancina et al., 1976; Bishop, 1974). The activation of baroreflex by mechanical factors and humoral pressor agents can influence chemoreflex which leads to alteration of respiratory timing and volume (Walker and Jennings, 1996; 1998; Alexander and Lumbers, 1981). It is noteworthy, that these effects are not observed in baroreceptor denervating animals, e.g. in vagotomy conditions (Alexander and Lumbers, 1981; Walker and Bizziee, 1990). Still these possible mechanisms remain speculative confirmation which will require further investigations.

In summary, the present study demonstrates participation of ITR in both respiratory and cardiovascular control. Bilateral vagotomy amplified or unmasked immediate ITR-induced cardiorespiratory variability, whereas delayed increases of mean blood pressure and breath duration were exaggerated by vagotomy. Taken together, our results allow us to suggest an even broader role of ITR in modulation and integration of respiratory and cardiovascular reflexes, than has been previously suspected.

4. Experimental Procedure

Experiments were performed on 12 spontaneously breathing, anesthetized, adult, male Sprague–Dawley rats (270–300 g, Harlan, Indianapolis, IN). All procedures complied with the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996.

Rats were anesthetized with a combination of 80 mg/kg ketamine (Abbott Laboratories, North Chicago, IL) and 5 mg/kg xylazine (Phoenix Scientific Inc., St. Joseph, MO) given by intraperitoneal injection. After achieving a stable plane of anesthesia, which was controlled by the absence of a toe-pinch reflex rats were tracheostomised. The vagus nerves were exposed bilaterally above the nodose ganglia and loose silk ligatures were placed over them. These ligatures were tightened to affect vagotomy at the appropriate point in the protocol. Effective vagotomy was functionally verified by abolition of the apneic response to intravenously infused 5-HT (Yoshioka et al., 1992a,b; Jacobs and Comroe, 1971; Segers and Shannon, 1985; Kopczynska and Szereda-Przestaszewska, 2003). For this purpose, a catheter was inserted into the left femoral vein for bolus infusions of 5-HT (5-hydroxytryptamine hydrochloride MP Biomedical LLC, Aurora, OH) using a Hamilton syringe and infusion pump (model KDS210, KD Scientific Inc., Hollister, MA; 0.05 M; 0.25 μ L). Another catheter was inserted into the left femoral artery for blood pressure monitoring and secured by a suture. A single lead ECG was acquired from needle electrodes placed in the left axillary and right flank regions.

After the surgical preparation, rats were placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL). Incisor bar was set at the same level as the ear bars (interaural zero). A unilateral osteotomy was made to allow access to rostral lateral pons, and the dura was carefully removed. Two-barrel micropipettes were made using standard filament glass (1 mm $\times$ 0.25 mm, A-M Systems, Carlsberg, WA) and a vertical puller (model no. 50–239, Harvard Apparatus Ltd., Kent, England) to obtain an overall tip diameter of 10–20 μ m. The micropipette was introduced into the brain on a dorso-ventral axis to allow pressure microinjections into the ITR (AP = –9.30 from bregma; ML = 2.4; DV = –8.0; Paxinos and Watson, 1986). The surface of the brain was used as the zero point for the dorso-ventral stereotaxic coordinates.

A pulse pressure injector (model Picospritzer II, General Valve Co., Fairfield, NJ) was used to inject glutamate (l-glutamic acid monosodium salt, 10 mM, 30 nL, ICN Biomedicals, Aurora, OH), or oil red O-dye (Sigma, St. Louis, MO; solution of 7 mg in 1 mL ethanol) into the ITR. The dose of the glutamate was chosen according the previous works (Chamberlin and Saper, 1992; 1994; 1998; Radulovacki et al., 2003a,b; 2007), which showed the effectiveness of this amount to evoke a prominent apneic reaction from the ITR. All drugs were dissolved in 0.2 M PBS. Injection volumes were determined by measuring the displacement of the pipette fluid meniscus with a calibrated eyepiece reticle in a binocular stereozoom microscope (model 48920-10, Cole-Parmer, Vernon Hills, IL). In all cases, the target injection volume was 30 nL.

4.1. Recording procedure

Each experimental protocol was performed with four-channel recording: 1. arterial blood pressure registered using a Transpac IV transducer (Hospira, Lake Forest, IL); 2. electrocardiogram (ECG); 3. respiration recorded by a thoracic piezoelectric sensor (Velcro^R Tab-Infant-Ped; Sleepmate^R Technologies); 4. injection marker (logic level pulse provided by the pressure injector). The respiration recording system comprised a 1 cm² crystal attached to an elastic band that was fixed around the animal at a substernal level. The crystal provided quantitative measurements of respiratory timing and relative uncalibrated measurements of respiratory volume. An automated adaptive threshold algorithm was used to detect and quantify the inspiratory and expiratory peaks as well as uncalibrated inspiratory tidal volume of each breath, allowing us to track changes in timing variability and estimated tidal volume variability,

measured as breath by breath coefficients of variation. After conventional amplification and filtering (1–50 Hz band-pass; Grass Model 12, West Warwick), the analog data were digitized (sampling frequency 200 Hz) and recorded using BrainWave for Windows software (Datawave Systems, Longmont, CO).

4.2. Experimental protocol

Each experiment started with a 10 minute baseline recording prior to any injections. In order to identify an ITR apneic site, the pipette was introduced into the stereotaxically defined intertrigeminal area and advanced ventrally in 100 μ m increments until glutamate injection produced an apnea at least 2.5 s in duration. Data only from the apneic sites were collected.

After 10 minutes of recording, which was sufficient for the visually assessed respiratory pattern to return to baseline, for the purposes of additional control 30 nL of PBS was injected into the same ITR site at which response to glutamate was observed. In the following 10 minutes interval injection of glutamate was repeated to test the reproducibility of the reaction. After an additional 10 minutes an intravenous bolus of 5-HT (0.05 M, 0.25 μ L over 5 s) was infused, which induced apnea, indicating the integrity of the nodose ganglia and vagal afferent system. Ten minutes later vagotomy was produced by tightening the ligatures placed around the vagi. After a 30 minutes interval, sufficient for visually evident stabilization of the respiratory pattern, another bolus of 5-HT was infused to confirm the vagal transection rostral to the nodose ganglia. To determine the effect of vagotomy on glutamate stimulation of ITR, two additional glutamate injections followed each time by 30 nL control injections of PBS were made at 10 minute intervals. Finally, oil red O-dye was microinjected into the functionally identified ITR apneic site to aid in histological verification.

4.3. Data analysis

Only the data from the animals with histologically and physiologically proven location of the injection within the ITR apneic sites were included in the analysis.

Offline analysis of respiratory and cardiovascular parameters was performed using Experimenter's Workbench software (Datawave Systems, Longmont, CO). An automated adaptive threshold algorithm was used to detect and quantify the timing, duration (TT), and uncalibrated inspiratory tidal volume (VT) of each breath, heart period (RR) of the ECG, systolic blood pressure (SBP), and pulse pressure (PP). The mean values of all parameters, as well as their corresponding coefficients of variation (CVTT, CVVT, CVRR, CVSBP, CVPP, respectively), were computed from 30 s epochs. Each animal served as its own control by a matched comparison of each post-injection interval to the specific immediate 30 s pre-injection interval. To define the temporal evaluation of cardiorespiratory responses to ITR glutamate administration, we examined 8 post-injection time intervals: 0–30 s, 30–60 s, 60–90 s, 90–120 s, 120–150 s, 150–180 s, 180–210 s and 210–240 s following the injection. Effects of injections and vagotomy were examined by paired t-tests. In all cases, statistical significance was inferred for $p < 0.05$.

4.4. Histology

At the end of each experiment rats were deeply anesthetized and transcardially perfused with normal saline (pH 7.4) at a rate of 40 mL/min until the liver cleared. This was followed by a 4% paraformaldehyde solution (in 0.1 M PBS; 200 mL at 40 mL/min, then 30 mL/min) and finally by 10% sucrose solution (in 0.1 M PBS; 200 mL at 30 mL/min). The fixed brain was extracted en-bloc, cleared of meninges, post-fixed in 4% paraformaldehyde overnight, and then stored in 30% sucrose (in 0.1 M PBS) until it sank to the bottom of the vial. Frozen brain tissue was cut in 40 μ m thick sections in a transverse plane using a cryostat microtome (model Leica

CM1900, Nussloch, Germany). To identify the location of microinjection sites the sections were stained using cresyl violet Nissl stain.

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Abbreviations

ITR	intertrigeminal region
NTS	nucleus tractus solitarius
VLM	ventrolateral medulla
TT	total breath duration
VT	tidal volume
SBP	systolic blood pressure
PP	pulse pressure
RR	cardio interval
CVTT	coefficient of variation of total breath duration
CVVT	coefficient of variation of tidal volume
CVSBP	coefficient of variation of systolic blood pressure
CVPP	coefficient of variation of pulse pressure
5-HT	serotonin
ECG	electrocardiogram

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Fig. 1. Characteristic apnea and reaction of blood pressure evoked by glutamate injection in the ITR. A. Prior to vagotomy. B. Post-vagotomy responses. Note the early and the late cardiorespiratory reactions. Upper tracing is arterial blood pressure; lower tracing is respiration. Note the apnea evoked immediately after the glutamate injection (pulse in lowest tracing).

Fig. 2.

Group averages at 30 s prior glutamate stimulation of ITR (control) and in subsequent 30 s intervals after the glutamate injection. Data represent glutamate injection trials before vagotomy (PreVagX) and after vagotomy (PostVagX). A. Total breath time (TT, seconds). B. Respiratory timing variability measured as coefficient of variation of breath time (CVTT). C. Respiratory volume variability measured as coefficient of variation of tidal volume (CVVT). Bars reflect group means $\pm$ SE (N = 12). * $p \leq 0.05$ with respect to control.

Fig. 3.

Group mean cardiovascular responses to ITR stimulation presented in the format of Fig. 2. A. Systolic blood pressure (SBP, mmHg). B. Systolic blood pressure variability measured as its coefficient of variation (CVSBP, mmHg). C. Pulse pressure (PP, mmHg). D. Pulse pressure variability represented as its coefficient of variation (CVPP, mmHg). Results are group means $\pm$ SE (N = 12). * $p \leq 0.05$ with respect to control interval. † $p < 0.05$ with respect to pre-vagotomy condition.

Fig. 4.

A. Histologic verification of an apneic ITR site. ITR: intertrigeminal region; Mo5: motor trigeminal nucleus; Pr5: principal sensory trigeminal nucleus. The tip of the pipette track at the microphotograph is located in the ITR at the ventral edge of Pr5. B. Schematic diagram of the histologically verified injections into apneic ITR sites.

Fig. 5. Schematic diagram of possible ITR regulatory pathways. ITR: intertrigeminal region; KF: Kölliker-Fuse nucleus; PB: parabrachial region; NTS: nucleus tractus solitarius; VLM: ventrolateral medulla; Sp5c: spinal trigeminal nucleus; V: trigeminal nerve; IX: glossopharyngeal nerve; X: vagus nerve.

Table 1

The number of extra-long and extra-short RR-intervals under pre- (PreVagX) and post- (PostVagX) vagotomy conditions prior to (Pre-injection) and following glutamate injection (Post-injection) in the ITR

	Extra-long intervals		Extra-short intervals	
	PreVagX	PostVagX	PreVagX	PostVagX
Pre-injection	2.15±0.62	1.40±0.47	3.00±0.87	3.80±1.20
Post-injection				
0–30 s	1.92±0.56	0.90±0.30	2.15±0.62*	3.20±1.01
30–60 s	1.77±0.51	1.80±0.60	2.31±0.67	3.10±0.98
60–90 s	2.46±0.71	1.60±0.53	2.46±0.71	2.50±0.79
90–120 s	2.62±0.75	1.80±0.60	1.62±0.47*	2.00±0.63*
120–150 s	2.46±0.71	1.50±0.50	1.54±0.44*	0.60±0.19*
150–180 s	2.38±0.69	1.50±0.50	0.85±0.24*	0.80±0.25*
180–210 s	2.23±0.64	1.30±0.43	1.15±0.33*	0.80±0.25*
210–240 s	1.62±0.47	1.50±0.50	1.23±0.36*	0.60±0.19*

Table shows group means ± standard errors (N=12), $p \leq 0.05$ with respect to control condition.