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Short communication

Anatomical substrate for separate processing of ascending and descending visceral information in the nucleus of the solitary tract of the rat

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Abstract

We examined the possible existence of divergent visceral pathways arising from the nucleus of the solitary tract, by co-injecting axonal tracers into the parabrachial nucleus and into the ventrolateral medulla. We found that around 5% of NTS neurons projected to both sites, and that neurons projecting to VLM were larger. This parallel organization allows a differential control of the ascending versus descending visceral pathways at an early stage of processing.

Theme: Endocrine and autonomic regulation

Topic: Neuroendocrine regulation: other

Keywords: Solitarius; Parabrachial; Ambiguus; Ventrolateral medulla

We hypothesized that the NTS, as a relay for primary visceral afferents, is organized to handle separately ascending (or integrative or sensory) and descending (or homeostatic or reflex) visceral information, very much like other sensory relays, including the rostral, gustatory NTS [2]. The main NTS targets for ascending or for descending axonal projections are the parabrachial nucleus (PBN) and the ventrolateral medulla (VLM), respectively [3,12,13]. To address our hypothesis we co-injected rats with two different fluorescent retrograde axonal tracers into PBN and into VLM.

Sprague–Dawley rats (250–310 g), anesthetized with ketamine/xilazine (50/10 mg/kg i.p.), received pressure injections (50 nl) of 0.04 μm FluoSpheres (red fluorescent (580/605); yellow–green fluorescent (505/515); Molecular Probes Inc., Oregon), under stereotaxic guidance [9]. After 7 days the animals were anesthetized with 7% chloral hydrate (350 mg/kg, i.p.), and perfused with 10% neutralized formol–saline. A total of 132 50 μm coronal sections were mapped with a fluorescent microscope fitted with camera lucida.

Six additional rats, successfuly microinjected with 1% WGA–HRP into either VLM or PBN, were processed with the TMB or the DAB method [6]. The outlines of the retrogradely labeled NTS neurons were traced using a 100× oil immersion objective, and measured with the SigmaScan™ system (Jandel).

All the procedures were approved by the Committee on animal care and use of the Faculty of Biological Sciences, and done in accordance to the NIH guidelines.

Twelve experiments with two fluorescent tracers were considered successful because the injection sites included PBN and VLM in the same animal. Seven of the 12 animals received the red tracer in VLM and the green tracer in PBN; in the other five rats we reversed the tracer colors. The injections in PBN (Fig. 1) included lateral and medial PBN subnuclei at rostral, intermediate and caudal levels. The tracer injections in the VLM were always rostral to the obex, i.e. they excluded the caudal ventrolateral medulla. The tracer injections in rostral VLM involved the nucleus ambiguus, the lateral reticular nucleus, magnocellular part, the rostral (but not the caudal) ventrolateral nucleus, and adjacent medullary regions (Fig. 1).

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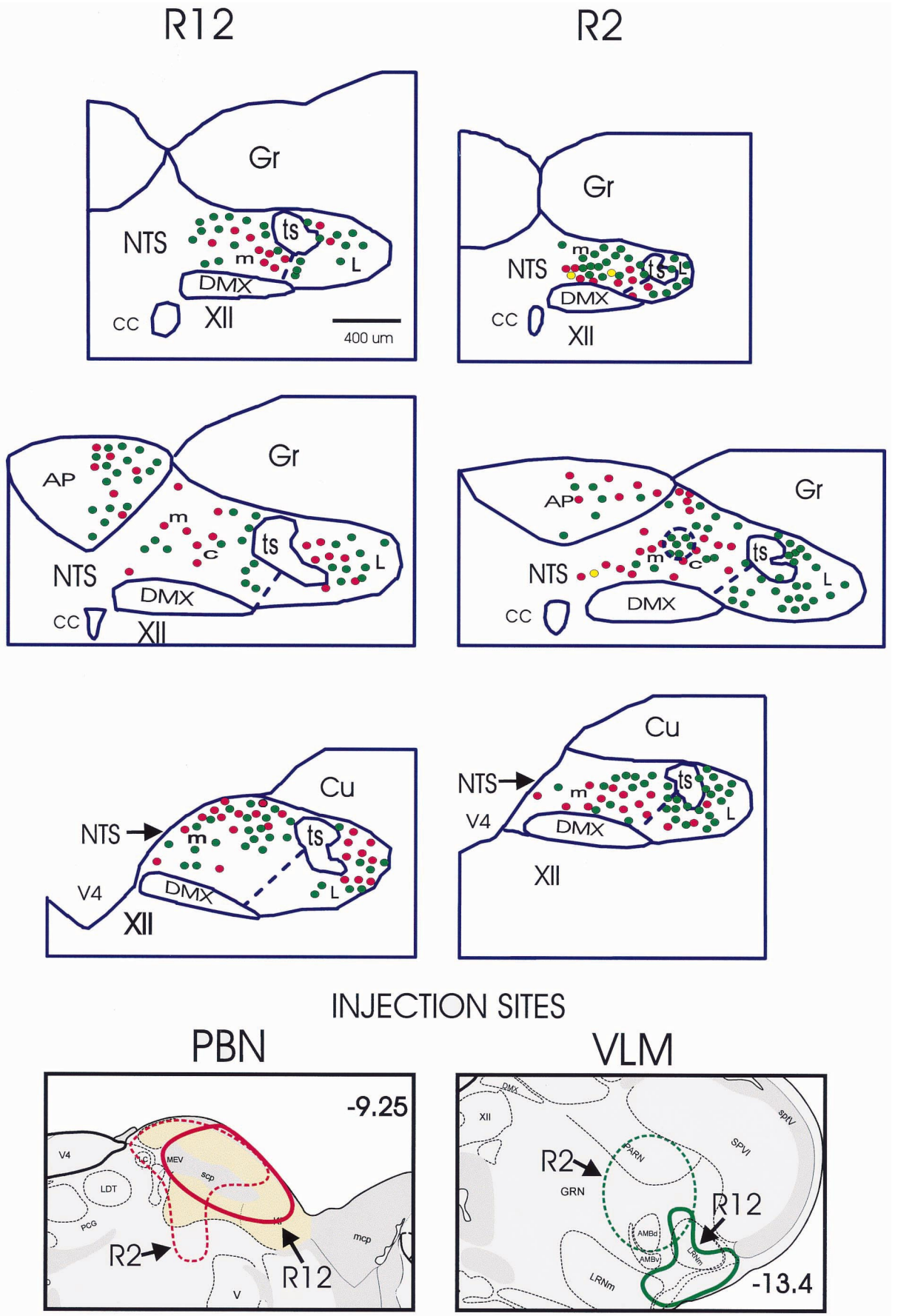
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That is, the injections in VLM included parasympathetic, sympathetic pressor and inspiratory-related premotor neurons [8,11,12].

Using a low power objective, we recorded the distribution of neurons labeled red or green, at three levels of the visceral NTS (Fig. 1). We then used a higher power objective to count the doubly labeled neurons. In this way, we analyzed 10 249 retrogradely labeled neurons in the visceral NTS and AP (see Table 1). A casual glance at the Table suggested that a higher proportion of NTS neurons projected to PBN (50.5 vs. 45.0% to VLM); however the statistical analysis revealed no differences between the two groups (Student's *t*-test); that is, the present experiments labeled similar number of neurons sending axons to both targets.

We found that, in average, 4.2±0.8% (S.E.) of the labeled neurons in the visceral NTS ($n=9058$) projected to both PBN and VLM regions. Similarly, only 4.4±1.4% of AP neurons ($n=1.191$) were doubly labeled. Taking the labeled NTS and AP neurons of each rat together, 4.4±0.7% of them (Table 1) sent collaterals to both PBN and VLM.

Given the rough viscerotopic organization of the NTS, of PBN, and perhaps of the VLM region [3], and the inevitable variability in the injection sites, it was important to test if the low fraction of NTS neurons sending axons to both PBN and VLM could be explained by injections in non-corresponding sites in those NTS targets. To this end we used several approaches. One was to have large injections, like those made in rats R2, R8 or R12, to increase the probability of hitting corresponding regions in PBN and VLM. The fraction of doubly labeled cells from experiments with larger injections was similar to that obtained in the other rats (Table 1), suggesting that our smaller injections labeled representative samples of the NTS population. We further tested whether the distribution of percentages of double labeled NTS neurons (Table 1, rightmost column) matched the pattern expected if the samples were taken from a single population. The Kolmogorov–Smirnov test indicated that our data came from a single Gaussian distribution (K–S distance=0.175; $P=0.366$), with a mean of 4.4±2.5% (S.D.) of double labeled cells, and the 75% confidence at 6.03%. This statistical analysis confirmed that the larger and the smaller injections of retrograde fluorescent tracers produced similar results in terms of doubly labeled cells.

Table 1
Number of neurons in the visceral NTS (including area postrema) labeled from the rostral ventrolateral medulla (VLM), or from the parabrachial nucleus (PBN) or from both regions

	VLM (n)	PBN (n)	Both (n)	VLM (%)	PBN (%)	Both (%)
Rat #						
R1	449	569	21	43.2	54.8	2.0
R2	640	510	114	50.6	40.3	9.0
R3	428	278	41	57.3	37.2	5.5
R4	531	628	53	43.8	51.8	4.4
R5	374	282	18	55.5	41.8	2.7
R6	382	324	64	49.6	42.1	8.3
R7	59	11	12	32.4	61.0	6.6
R8	424	735	55	34.9	60.5	4.5
R9	262	733	40	25.3	70.8	3.9
R10	193	310	13	37.4	60.1	2.5
R11	274	372	17	41.3	56.1	2.6
R12	598	324	11	64.1	34.7	1.2
Total	4614	5176	459			

In addition, we assessed the spatial segregation of NTS neurons differentially projecting to PBN or to VLM, by measuring the distance to the nearest neighbor (a neuron labeled red or green) for each retrogradely labeled neuron. We then applied a χ^2-test to determine whether the proportion of near neighbors departed from chance [10]. The idea was that neuronal populations sharing the same NTS regions should receive inputs from the same visceral afferents [1]. We found no segregation of red and green labeled cells in most sections of the 12 rats studied. In just two rats (R6 and R7, which had the smallest PBN injections), and only within intermediate rostro–caudal NTS levels, we found statistically significant ($P<0.05$) segregation of red- and green-labeled neurons. None of the 12 rats showed a statistically significant segregation in the caudal (for example see upper Fig. 1), or in paraventricular NTS (lower Fig. 1). The absence of segregation indicated that the scarcity of double labeled neurons could hardly be explained by injections in non-corresponding sites in PBN and VLM.

The above results showed that the neurons with efferents

Fig. 1. Schematic drawings of the injection sites (lower row) and of the NTS labeling (upper rows) from rats R12 and R2. *Upper 3 rows:* transverse sections through the dorsal medulla, showing the spatial distribution of NTS and AP neurons that projected *ipsilaterally* to PBN (red), to VLM (green), or to both (yellow). Upper to lower rows=caudal to rostral visceral NTS levels. The statistical analysis (see text) of these animals indicated no spatial segregation of red and green neurons, except for lateral NTS of R2. Note that rat R2 was not injected into the Kölliker Fuse nucleus, which is the PBN region connected to lateral NTS [3]. Note in the middle row the cluster of green labeled neurons in the central subnucleus of the NTS; this subnucleus was not labeled in rat R12, because VLM injection spared the nucleus ambiguus. *Lower row:* Injection sites (based on Swanson [14]) into PBN (labeled yellow in left side) or into VLM (right side). The injections in PBN were outlined with red, and those in VLM were outlined with green The negative numbers in each picture indicate the rostro–caudal level respect to bregma. Abbreviations: AP=area postrema; CC=central canal; c=central NTS subnucleus; Cu, cuneate nucleus; DMX=dorsal motor nucleus of the vagus; Gr=nucleus gracilis; L=lateral NTS; m=medial NTS subnucleus; NTS=nucleus of the solitary tract; ts=solitary tract; V4=fourth ventricle; XII=hypoglossal nucleus.

to either PBN or VLM share the same NTS subnuclei. An expected exception was the central subnucleus (''c'' in middle Fig. 1), connected to the VLM but not to the PBN region [3,12]. We excluded the central subnucleus from the data shown in Table 1.

We measured the soma size of NTS neurons labeled by retrograde axonal transport of WGA–HRP, and counterstained with cresyl violet. Neurons projecting to VLM were larger (n=111; mean=390 μm^2; median=371 μm^2; S.E.±13 μm^2. $P<0.001$, Mann–Whitney test) than those projecting to PBN (n=189; mean=330 μm^2; median=326 μm^2; S.E.±6.7 μm^2). In sections developed with TMB, to better examine the shape of the WGA–HRP labeled neurons, we found no obvious differences in the soma shape or in the number or orientation of primary dendrites between neurons projecting to VLM or PBN. Our results on the size difference between VLM and PBN projecting neurons in the visceral NTS agree with the interpretation offered by Kawai and Senba [5] of their intracellular injections into NTS neurons. They proposed that a population of smaller NTS neurons possibly projecting to PBN, have local axonal arborizations. A second population of larger neurons, without local arborizations, projects to VLM, among others known targets of NTS.

The present study showed that neurons in the visceral NTS and the AP sent projections to either premotor regions in the ventrolateral medulla, or to the parabrachial nucleus in comparable numbers, and that less than 5% of the labeled neurons sent projections to both regions. This percentage may even be lower, since ascending NTS axons transverse VLM [3], and some of them may have taken up the tracer there. The outputs of the rostral, gustatory NTS region are also organized in a similar, parallel fashion [2].

The output of reflex and of integrative networks may lead to opposite changes in physiological variables during affective behaviors. For instance, in the defense reaction the reflex network tends to keep the arterial blood pressure within narrow limits, while the animal might need a raise, or even a slight fall, in arterial pressure under some circumstances [4]. In such circumstances it would be useful to have a differential control of the transmission at the NTS level, so as to have a faithful representation of the bodily state, while independently altering the function of visceral reflexes.

We conclude that different populations of NTS neurons distribute visceral information to either premotor autonomic (including respiratory) circuits or to ascending, sensory, circuits. This dichotomy makes it possible that the activity of individual NTS neurons can be differentially modulated, according to their sensory or reflex function, by central or peripheral inputs [7].

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References

[1] W.W. Blessing, in: The Lower Brainstem and Bodily Homeostasis, Oxford University Press, New York, 1997, p. 575.
[2] C.B. Halsell, S.P. Travers, J.B. Travers, Ascending and descending projections from the rostral nucleus of the solitary tract originate from separate neuronal populations, Neuroscience 72 (1996) 185–197.
[3] H. Herbert, M.M. Moga, C.B. Saper, Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat, J. Comp. Neurol. 293 (1990) 540–580.
[4] D. Jordan, Autonomic changes in affective behavior, in: A.D. Loewy, K.M. Spyer (Eds.), Central Regulation of Autonomic Functions, Oxford University Press, New York, 1990, pp. 349–366.
[5] Y. Kawai, E. Senba, Organization of excitatory and inhibitory local networks in the caudal nucleus of tractus solitarius of rats revealed in in vitro slice preparation, J. Comp. Neurol. 373 (1996) 309–321.
[6] M.-M. Mesulam, Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neuronal pathways–axonal transport, enzyme histochemistry and light microscopic analysis, in: M.-M. Mesulam (Ed.), Tracing Neuronal Connections with Horseradish Peroxidase, Wiley-Interscience, Chichester, 1982, pp. 1–151.
[7] S.W. Mifflin, R.B. Felder, Synaptic mechanisms regulating cardiovascular afferent inputs to solitary tract nucleus, Am. J. Physiol. 259 (1990) H653–H661.
[8] P.A. Nuñez-Abades, A.M. Morillo, R. Pásaro, Brainstem connections of the rat ventral respiratory subgroups: afferent projections, J. Auton. Nerv. Syst. 42 (1993) 99–118.
[9] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, Sydney, 1986.
[10] E.C. Pielou, in: An Introduction to Mathematical Ecology, Wiley-Interscience, New York, 1969, pp. 179–184.
[11] D.W. Richter, K.M. Spyer, Cardiorespiratory control, in: A.D. Loewy, K.M. Spyer (Eds.), Central Regulation of Autonomic Functions, Oxford University Press, New York, 1990, pp. 189–207.
[12] C.A. Ross, D.A. Ruggiero, D.J. Reis, Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla, J. Comp. Neurol. 242 (1985) 511–534.
[13] C.B. Saper, Central autonomic system, in: G. Paxinos (Ed.), The Rat Nervous System, Academic Press Inc., 1995, pp. 107–135.
[14] L.H. Swanson, in: Brain Maps: Structure of the Rat Brain, 2nd Edition, Elsevier, Amsterdam, 1999, p. 267.