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# DISTRIBUTION OF CATECHOLAMINERGIC PRESYMPATHETIC-PREMOTOR NEURONS IN THE RAT LOWER BRAINSTEM

**H. NAM** [a,b1] **AND I. A. KERMAN** [a*]

[a] *Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL, United States*

[b] *Cell Molecular and Developmental Biology Theme, Graduate Biomedical Sciences Program, University of Alabama at Birmingham, Birmingham, AL, United States*

**Abstract—We previously characterized the organization of presympathetic-premotor neurons (PSPMNs), which send descending poly-synaptic projections with collaterals to skeletal muscle and the adrenal gland. Such neurons may play a role in shaping integrated adaptive responses, and many of them were found within well-characterized regions of noradrenergic cell populations suggesting that some of the PSPMNs are catecholaminergic. To address this issue, we used retrograde trans-synaptic tract-tracing with attenuated pseudorabies virus (PRV) recombinants combined with multi-label immunofluorescence to identify PSPMNs expressing tyrosine hydroxylase (TH). Our findings indicate that TH-immunoreactive (ir) PSPMNs are present throughout the brainstem within multiple cell populations, including the A1, C1, C2, C3, A5 and A7 cell groups along with the locus coeruleus (LC) and the nucleus subcoeruleus (SubC). The largest numbers of TH-ir PSPMNs were located within the LC and SubC. Within SubC and the A7 cell group, about 70% of TH-ir neurons were PSPMNs, which was a significantly greater fraction of neurons than in the other brain regions we examined. These findings indicate that TH-ir neurons near the pontomesencephalic junction that are distributed across the LC, SubC, and the A7 may play a prominent role in somatomotor–sympathetic integration, and that the major functional role of the A7 and SubC noradrenergic cell groups maybe in the coordination of concomitant activation of somatomotor and sympathetic outflows. These neurons may participate in mediating homeostatic adaptations that require simultaneous activation of sympathetic and somatomotor nerves in the periphery.**


**Key words: norepinephrine, tyrosine hydroxylase, pseudorabies, brainstem, autonomic, motor.**

## INTRODUCTION

Numerous stress-elicited behaviors require simultaneous activation of somatomotor and autonomic circuits (Hilton, 1982; Jordan, 1990; Waldrop et al., 1996). Such coordination might be mediated by multiple descending projections from specific brain regions that integrate physiological functions. Our previous work utilized a retrograde trans-synaptic tract-tracing approach using attenuated pseudorabies virus (PRV) to identify neurons in the brain that send poly-synaptic collaterals to skeletal muscle and the adrenal gland. These cells, termed presympathetic-premotor neurons (PSPMNs), are located within multiple sites throughout the brainstem and hypothalamus (Kerman et al., 2003, 2006a,b, 2007; Kerman, 2008; Shah et al., 2013). Distinct populations of PSPMNs are distributed within brain regions that regulate specific aspects of homeostasis, and synthesize transmitters that integrate autonomic, motor, and behavioral aspects of adaptive behaviors (Kerman, 2008). For example, we previously defined a dense population of serotonergic PSPMNs in the ventromedial medulla within the gigantocellular nucleus pars α (GiA) and nucleus raphe magnus (Kerman et al., 2006b). Given the well-documented role of this region in coordinating motor, sensory, and autonomic responses to painful stimuli (Morgan and Whitney, 2000; Mason, 2001), as well as integrated motor and sympathetic responses as part of the cold defense (Nason and Mason, 2004; Morrison, 2011), it is possible that such neurons play multiple functional roles in homeostatic adaptations. Similarly, PSPMNs that express melanin-concentrating hormone or orexins in the lateral hypothalamus (LH) (Kerman et al., 2007) could participate in the passive vs. active coping strategies as part of the fight-or-flight response to stress (Marsh et al., 2002; Kayaba et al., 2003; Johnson et al., 2010).

Together these observations suggest that PSPMNs may mediate somatomotor–autonomic adaptive responses to a variety of stressors. Given the important role of central catecholamine circuits in broad stress integration (Sabban, 2010), we aimed to determine whether subpopulations of brainstem PSPMNs may be catecholaminergic. In our previous studies we detected

*Corresponding author. Address: Sparks Center 743, 1720 7th Avenue South, Birmingham, AL 35294, United States. Tel: +1-205-975-0310.
E-mail address: kerman@uab.edu (I. A. Kerman).
[1] Current affiliation: Department of Anatomy and Neurobiology, University of Maryland, Baltimore, MD, United States.

*Abbreviations:* BSA, bovine serum albumin; EGFP, enhanced green fluorescent protein; GiA, gigantocellular nucleus pars α; IML, intermediolateral cell column; LC, locus coeruleus; LH, lateral hypothalamus; NGS, normal goat serum; NTS, nucleus tractus solitarius; PAG, periaqueductal gray; PB, phosphate buffer; PRV, pseudorabies virus; PSPMNs, presympathetic-premotor neurons; PVN, paraventricular nucleus of the hypothalamus; SubC, nucleus subcoeruleus; TH, tyrosine hydroxylase; TX-100, Triton X-100.

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a considerable number of PSPMNs within the locus coeruleus (LC), nucleus subcoeruleus (SubC), and the A5 cell group (Kerman et al., 2003, 2006a), which have been classically described as part of the descending norepinephrine system (Kvetnansky et al., 2009). Other brain regions with significant noradrenergic cell populations containing PSPMNs included the Köllicker-Fuse nucleus, which overlaps with the A7 noradrenergic cell group (Lyons and Grzanna, 1988), nucleus tractus solitarius (NTS), which contains the A2 noradrenergic and the C2 adrenergic cell groups (Minson et al., 1990; Rinaman, 2011), and the ventrolateral medulla, which contains C1 adrenergic and A1 noradrenergic cell groups (Kerman et al., 2003; Card et al., 2006).

Previous studies have documented projections from these catecholaminergic brainstem areas to both the intermediolateral cell column (IML) and the ventral horn of the spinal cord, suggesting existence of reticulospinal neurons that collateralize to innervate sympathetic preganglionic neurons and motoneurons. Studies utilizing monosynaptic anterograde and retrograde tracers have demonstrated that noradrenergic neurons within the A5 cell group, LC, SubC, and the A7 cell group send descending projections that terminate at different rostro-caudal levels of the spinal cord (Westlund et al., 1982, 1983; Clark and Proudfit, 1991a, b). Similarly, adrenergic neurons from within C1, C2, and C3 adrenergic cell groups project to the spinal cord and terminate within the IML (Minson et al., 1990). Tract-tracing with viral vectors containing PRS2, a noradrenaline-specific regulatory element that is activated by Phox2 transcription factor (Hwang et al., 2001), which preferentially infect noradrenergic and adrenergic neurons and are transported anterogradely have extended these observations. Bruinstroop et al. used this methodology to demonstrate that noradrenergic neurons within the LC, SubC pars $\alpha$, and the A7 cell send projections that terminate in the IML and the ventral horn (Bruinstroop et al., 2012). Similarly, adrenergic neurons within the C1 and C3 cell groups send dense projections to the spinal cord, which terminate within laminae IX and X (Card et al., 2006; Sevigny et al., 2012). Previous studies that utilized PRV as a retrograde trans-synaptic tract-tracer have demonstrated the presence of tyrosine hydroxylase (TH)-immunoreactive (ir) presympathetic neurons in the ventrolateral medulla, A5, LC, and SubC following injections of multiple sympathetically innervated organs, including skeletal muscle, adrenal gland, the pancreas, and brown fat (Strack et al., 1989; Jansen et al., 1997; Xiang et al., 2014).

Taken together, these observations suggest the existence of catecholaminergic neurons in the brainstem with poly-synaptic collaterals to skeletal muscle and sympathetically innervated peripheral organs. However, it is not clear whether any of these TH-ir cell groups contain PSPMNs. In this study we demonstrate the existence of TH-ir PSPMNs within multiple catecholaminergic populations of the lower brainstem. The greatest numbers of such TH-ir PSPMNs were found within the LC and SubC. Within the SubC and the A7 a large majority of TH-ir neurons are PSPMNs, suggesting that these cell groups are dedicated to somatomotor–sympathetic integration.

## EXPERIMENTAL PROCEDURES

### Animals

All of the procedures regarding animal use in this study were consistent with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (1996, National Academy of Sciences) and were approved by the local Institutional Animal Care and Use Committee. Trans-synaptic tract-tracing was performed in male Sprague–Dawley rats ($n$ = 12; Charles River Laboratories, Wilmington, MA, USA).

### Viral tracing

We previously observed a negative correlation between animal weight and the rate of motoneuron infection, with an optimal weight of approximately 200 g (Kerman et al., 2003). In light of this finding, we used rats that weighed between 163 g and 300 g, with an average weight of 205 ± 12 g (mean ± SEM; weighed at the time of sympathectomy; see below).

Animals were anesthetized with 5% isoflurane vaporized in 1.0–1.5 L/min of $O_2$ and were maintained at 1.5 – 2.5%. Surgical plane of anesthesia was achieved such that there was no spontaneous movement and no withdrawal responses to tail and/or foot pinch. Prior to PRV injections, surgical sympathectomy was performed as previously described (Kerman et al., 2003, 2006a) to remove sympathetic innervation of the hindlimb musculature. Briefly, a ventral laparotomy was performed and a segment of the lumbar sympathetic nerve from the level of the renal artery to the aortic bifurcation was extirpated. Neural plexuses along the abdominal aorta were stripped off under microscopic observation using fine forceps, and the aorta was swabbed with a 10% phenol solution.

Following a 2–10-day recovery period, animals were injected with PRV. We used recombinant strains of PRV that express unique reporter proteins, with PRV-152 expressing enhanced green fluorescent protein (EGFP) and PRV-BaBlu transcribing β-galactosidase (Billig et al., 2000). Both of these viral strains were derived from the attenuated strain PRV-Bartha, which is not infectious to humans but has been demonstrated to have the capability of simultaneous neuronal coinfection in rats (Standish et al., 1995; Billig et al., 2000). Viral stocks were harvested from pig kidney cell cultures at a titer of $10^8$–$10^9$ pfu/mL, aliquoted into 50 μL volumes, and stored at −80 °C until the time of inoculations when they were rapidly thawed in a 37 °C water bath. PRV injections were performed as previously described (Kerman et al., 2003, 2006b). Briefly, PRV-152 was injected throughout the lateral head of the gastrocnemius muscle in 1 μl volumes (totaling 30 μl) using a 10-μl glass syringe (Hamilton Company, Reno, NV, USA). PRV-BaBlu was similarly injected using a Hamilton syringe with a glass pipette attached to the tip with wax. A total of 2–4 μl of PRV-BaBlu was injected into the ipsilateral adrenal gland.