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Short Communication

CNS neurons with links to both mood-related cortex and sympathetic nervous system

Karl E. Krout[a], Thomas C. Mettenleiter[b], Vladimir Karpitskiy[a], Xay Van Nguyen[a], Arthur D. Loewy[a,*]

[a]*Department of Anatomy and Neurobiology, Box 8108, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA*
[b]*Friedrich-Loeffler-Institute, Institute of Molecular Biology, Boddenblick 5A, D-17498 Insel Riems, Germany*

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Abstract

Cardiovascular changes occur during mental stress and in certain types of mood disorders. The neural basis for this phenomenon is unknown but it may be dependent on CNS neurons that provide branched projections to affective processing regions of the brain, such as the medial prefrontal cortex, and to the sympathetic outflow system. Because these putative neurons may be connected to these two target sites by chains of neurons, we performed double virus transneuronal tracing experiments and show here that a select subset of neurons in the medial preoptic nucleus (MPN), lateral hypothalamic area (LHA), and nucleus tractus solitarius (NTS) are co-linked to these two sites. Neurotensin MPN, orexin-containing LHA, and catecholamine NTS neurons were the major phenotypes involved in these projections. This novel class of neurons may coordinate cardiovascular changes seen in different emotional states.

Theme: Endocrine and autonomic regulation
Topic: Cardiovascular regulation

Keywords: Arousal; Blood pressure; Emotion; Hypothalamic; Nucleus tractus solitarius; Orexin; Sleep

Blood pressure changes have been documented in various mental conditions. For example, hypotension occurs in certain types of attention and mood disorders, and hypertension has been frequently observed in states of mental stress [5,7,15], but the underlying neural circuitry responsible for these responses is not understood. More than 75 years ago, Cannon [4] hypothesized that specialized CNS neurons co-regulate sympathetic and emotional responses, but no anatomical or physiological study has attempted to determine whether such neurons exist in the brain. Since one of key cortical areas implicated in mood disorders and mental stress is the medial prefrontal cortex [6,9,17], we used the double viral transneuronal tracing method to determine whether individual CNS neurons project via multisynaptic connections to this cortical area and to the sympathetic outflow that regulates the heart.

To demonstrate that single CNS neurons provide branched projections to the medial prefrontal cortex and sympathetic nervous system, two isogenic forms of Bartha pseudorabies virus (PRV) were used as transneuronal tracers in the same animal (Fig. 1A). Each virus was engineered to contain a single unique heterologous gene inserted in the nonessential glycoprotein G (gG) locus of the unique short segment of the PRV genome: β-galactosidase (β-gal)-Bartha PRV or green fluorescent protein (GFP)-Bartha PRV [11,14] (Fig. 1B). Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), GFP-Bartha PRV was first injected into the stellate ganglion ($n = 267$, 40 nl, ≈3000 virions), which is the principal source of the sympathetic innervation to the heart. Two days later, the rats were anesthetized (as above) and stereotaxic injections of β-gal-Bartha PRV (40 nl, ≈3000 virions) were made in the deep layers of the infralimbic cortex (Fig. 1C).

* Corresponding author. Fax: +1 314 362 3446.
E-mail address: loewya@pcg.wustl.edu (A.D. Loewy).

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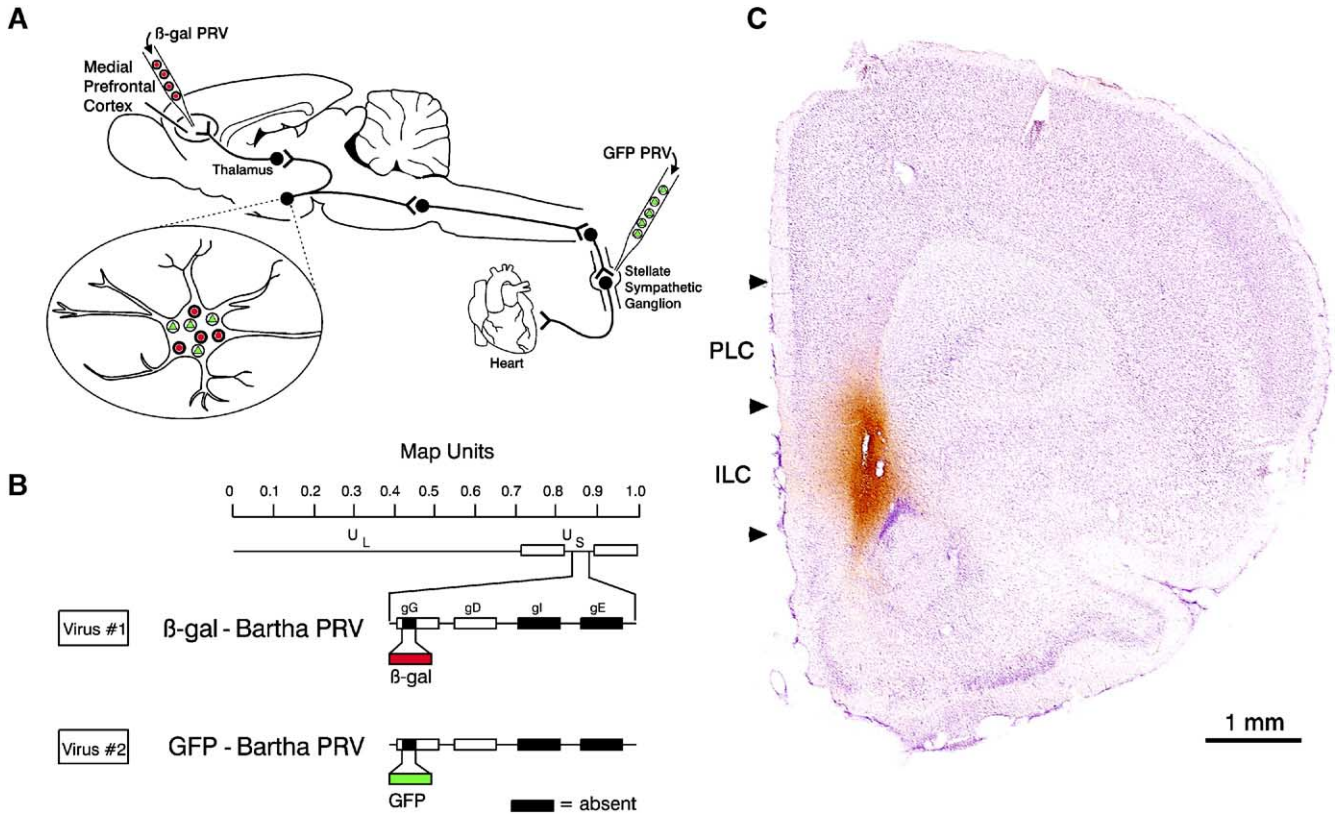

Fig. 1. Hypothalamic and brainstem neurons send multisynaptic efferent projections to both the medial prefrontal cortex and stellate sympathetic system. (A) Double-labeled neurons were identified after injections of β-gal-Bartha PRV in the medial prefrontal cortex and GFP-Bartha PRV in the stellate sympathetic ganglion. (B) Location of the β-Gal and GFP cassette insertions into the nonessential glycoprotein G (gG) locus of the unique short segment (U_S) of the PRV genome. U_L, unique long segment. (C) β-Gal-PRV injections were targeted in the deep layers of the infralimbic cortex (ILC). Prelimbic cortex, PLC.

After leveling the skull, the stereotaxic coordinates used for the infralimbic cortex injections were bregma +2.2 mm, lateral 0.8 mm, and deep 4.4 mm. Cholera toxin β-subunit (CTb; List Biological, Campbell, CA), a retrograde neuronal tracer, was mixed with the PRV and in the post hoc analysis, its cortical spread was used to define the injection site [13]. After 3 days, 100 μg of colchicine/10 μl of sterile saline was injected the lateral cerebral ventricle to increase neuropeptide levels for better quality immunohistochemistry. After 24 h, each rat was perfused with a 4% paraformaldehyde-buffered solution.

The double virus injection protocol described above was developed empirically, using >100 rats to obtain the optimal conditions for experiments in which two isogenic forms of Bartha PRV were injected in the stellate ganglion and cerebral cortex [13]. In the present study, a total of 267 rats was used. Eight cases were selected for the data analysis presented here that met two criteria: (1) the cortical injections were centered in the deep layers of the infralimbic cortex and (2) the stellate PRV experiments produced a pattern of transneuronal labeling in the brainstem and hypothalamus that were similar to previously reported data [10,13].

Histological processing was identical to methods used in earlier studies from this laboratory [13]. The brains were cut on a freezing microtome at 50 μm, and collected as a 1-in-5 series in 0.1 M sodium phosphate buffer containing 0.1% sodium azide. Brain sections were immunostained by a triple-color method (see Ref. [13] for full details) for GFP, β-gal, and either a neurotransmitter enzyme (viz., choline acetyltransferase, phenylethanolamine-*N*-methyltransferase, tyrosine hydroxylase; Chemicon, Temecula, CA) or a neuropeptide (orexin, Phoenix, Belmont, CA; neurotensin, Immunostar, Hudson, WI) and examined by indirect immunofluorescence microscopy. Control tissues were used to maximize the staining procedure and to ensure no bleed through occurred. None of the antibodies used here produced false-positive staining.

Each section from a 1-in-5 series of the entire brain was analyzed for either double- or triple-labeled neurons. The cytoarchitectonic distribution of these neurons was recorded. Since the purpose of the present study was to determine whether individual central neurons exist that are potentially co-linked to the medial prefrontal cortex and sympathetic nervous system, most of the data presented below provide an approximation of this labeling pattern as a percentage of the total double-infected neuronal population. However, the raw data for the key findings are given below as the mean ± standard error of the mean (SEM).

Double-infected neurons (viz., GFP + β-gal positive) were localized primarily in three brain regions: medial preoptic nucleus (MPN), lateral hypothalamic area (LHA), and nucleus tractus solitarius (NTS) (Fig. 2) (ANOVA, $P <$