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Distinct mechanisms underlie activation of hypothalamic neurosecretory neurons and their medullary catecholaminergic afferents in categorically different stress paradigms

(catecholamine neurons/c-*fos*/corticotropin-releasing factor/hypothalamo–pituitary–adrenal axis/paraventricular nucleus)

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ABSTRACT **Intermittent electrical footshock induces c-*fos* expression in parvocellular neurosecretory neurons expressing corticotropin-releasing factor and in other visceromotor cell types of the paraventricular hypothalamic nucleus (PVH). Since catecholaminergic neurons of the nucleus of the solitary tract and ventrolateral medulla make up the dominant loci of footshock-responsive cells that project to the PVH, these were evaluated as candidate afferent mediators of hypothalamic neuroendocrine responses. Rats bearing discrete unilateral transections of this projection system were exposed to a single 30-min footshock session and sacrificed 2 hr later. Despite depletion of the aminergic innervation on the ipsilateral side, shock-induced up-regulation of Fos protein and corticotropin-releasing factor mRNA were comparable in strength and distribution in the PVH on both sides of the brain. This lesion did, however, result in a substantial reduction of Fos expression in medullary aminergic neurons on the ipsilateral side. These results contrast diametrically with those obtained in a systemic cytokine (interleukin 1) challenge paradigm, where similar cuts ablated the Fos response in the ipsilateral PVH but left intact the induction seen in the ipsilateral medulla. We conclude that (*i*) footshock-induced activation of medullary aminergic neurons is a secondary consequence of stress, mediated via a *descending* projection transected by our ablation, (*ii*) stress-induced activation of medullary aminergic neurons is not necessarily predictive of an involvement of these cell groups in driving hypothalamic visceromotor responses to a given stressor, and (*iii*) despite striking similarities in the complement of hypothalamic effector neurons and their afferents that may be activated by stresses of different types, distinct mechanisms may underlie adaptive hypothalamic responses in each.**

Catecholamine-synthesizing neurons of the medulla oblongata provide a massive and functionally important innervation of multiple visceromotor cell types resident within the paraventricular nucleus of the hypothalamus (PVH) (1). This projection arises from adrenergic and noradrenergic neurons in the nucleus of the solitary tract (NTS) and the ventrolateral medullary reticular formation (1–3) and has been implicated in conveying sensory information from the thoracic and abdominal viscera to effect reflex adjustments in the output of parvocellular neurosecretory neurons that express corticotropin-releasing factor (CRF) for the initiation of pituitary-adrenal responses to stress, magnocellular neurosecretory neurons, and cells that project intracerebrally to modulate sensory and motor traffic in central autonomic pathways (4, 5). Although catecholaminergic projections make up the major known ascending input to these effector neuron populations of the PVH, the range of ascending aminergic involvement in effecting integrated visceromotor responses to stresses of different types remains uncertain.

Recognition of the ability of certain immediate-early genes, notably the c-*fos* protooncogene, to serve as sensitive and widely applicable markers of neurons activated by salient environmental events (6) has facilitated identification of individual cell groups that are responsive to stress and has fostered testable predictions as to whether and how cellular activation at one locus may be situationally dependent upon activation of another. Perhaps predictably, Fos-based approaches have revealed prominent activation of medullary aminergic neurons and their hypothalamic targets after perturbation in cardiovascular, gastrointestinal, and immune system parameters (e.g., refs. 7 and 8). Yet the fact that aminergic neurons have been reported to be equally strongly activated in stress paradigms such as restraint, immobilization, and electrical footshock (refs. 9–13; H.-Y.L. and P.E.S., unpublished results), which are apt to rely on somatosensory and/or nociceptive pathways for initial transduction, raises the possibility of a more general involvement of aminergic projections in effecting adaptive hypothalamic responses.

We have used a unilateral fiber transection approach to demonstrate a dependence of increased cellular activation and CRF mRNA expression in the PVH on the integrity of catecholaminergic inputs in a systemic interleukin 1 (IL-1) challenge model (14). Here we report on the results of parallel experiments designed to ascertain whether such dependence generalizes to footshock stress. Electrical footshock yields a pattern of cellular activation in the PVH that is strikingly similar to that induced by an acute IL-1 challenge, and, like IL-1 injection, results in marked activation of medullary catecholaminergic neurons identified as projecting to the PVH (ref. 11 and H.-Y.L. and P.E.S., unpublished results). The results have been presented in abstract form (15).

MATERIALS AND METHODS

Animals and Surgery. Male Sprague–Dawley albino rats (250–300 g) were housed under controlled temperature and lighting (12-hr light/12-hr dark cycle; lights on at 0600 hr), with food and water available freely. Protocols were approved by the Institutional Animal Care and Use Committee.

Surgeries were carried out under ketamine/xylazine/acepromazine anesthesia. Fiber transections designed to isolate the endocrine hypothalamus from ascending catecholaminergic projections from the medulla were produced stereotaxically using a retractable wire knife fashioned from a

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Abbreviations: PVH, paraventricular nucleus of the hypothalamus; NTS, nucleus of the solitary tract; CRF, corticotropin-releasing factor; IL, interleukin; DBH, dopamine β-hydroxylase; -ir, immunoreactivity.

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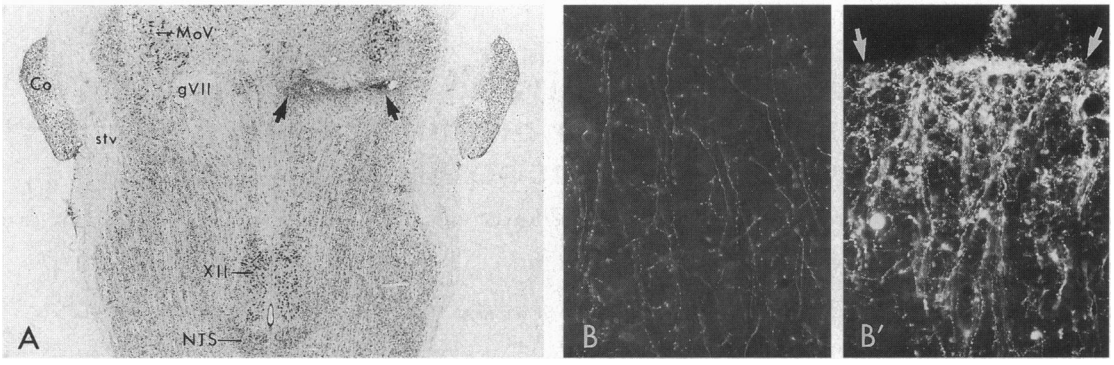

FIG. 1. Unilateral knife cut placement and effect. (*A*) Bright-field photomicrography of a thionin-stained horizontal section through the caudal brainstem showing the position and extent of a representative transection (arrows) that was effective in depleting the PVH of its catecholaminergic innervation. Cuts were positioned between the rostrocaudal levels of the facial and trigeminal (MoV) motor nuclei and extended 1.5–1.8 mm lateral from the midline and 3.0–3.3 mm dorsally from the ventral surface of the brain. (*B* and *B'*) Fluorescence photomicrographs of comparable regions on the sides contralateral (*B*) and ipsilateral (*B'*) to the transection shown in *A*. Swollen, intensely stained DBH-ir axons and varicosities (*B'*) are indicative of a "pileup" of immunoreactive materal immediately proximal to the lesion site. Rostral is to the top in all photomicrographs. Co, cochlear nucleus; stv, spinal trigeminal tract; gVII, genu of the facial nerve; XII, hypoglossal nucleus. (*A*, ×8; *B*, ×100.)

microliter syringe (16). Cuts were placed in the coronal plane at the level of the rostral pole of the facial motor nucleus and extended from the midline 2.0 mm laterally and 3.5 mm dorsally from the ventral surface (14, 17). Entry into the analysis was based on detection of accumulation (pile-up) of dopamine β-hydroxylase (DBH) immunoreactivity (ir) in fibers on the proximal side of the transection and depletion of the DBH-ir innervation of the PVH on the ipsilateral side that exceeded 60%, as assessed by rating scale comparisons of the strength of immunostaining in the PVH on both sides of the brains of unilaterally lesioned and intact animals by two independent observers blinded to the treatment status of the animals (14, 17).

Procedures. Seven days after surgery, rats were assigned to groups and adapted to the footshock chamber in seven daily 30-min sessions (between 0800 and 1000 hr) during which no shock was delivered. On the next day, experimental rats were subjected to a 30-min shock session, in which 60 shocks (1 mA) were delivered through the grid chamber floors randomly over 30 min; controls received another sham-shock session. Two hours later, the time at which we have reported maximal shock-induced Fos-ir in hypothalamus and medulla (ref. 11 and H.-Y.L. and P.E.S., unpublished results), all rats were anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5). Multiple series of 20- to 30-μm-thick frozen sections through the brain were saved. One series from each rat was stained with thionin for reference and to aid in reconstruction of knife cut placements. The others were stored in cryoprotectant at −20°C, and histochemical processing of tissue to be compared was carried out concurrently, using common batches of antisera or radiolabeled cRNA probes.

Histochemistry. Localization of Fos protein was carried out using a conventional avidin-biotin immunoperoxidase protocol (18) to localize a primary antiserum raised against an N-terminal (residues 4–17) synthetic fragment of Fos (Oncogene Sciences). Specific staining in stimulated animals was abolished by preadsorption with the synthetic peptide immunogen at 50 μM. Catecholamine neurons were detected using a mouse-derived monoclonal antibody (Chemicon) raised against rat adrenal DBH, which serves as a marker for adrenergic and noradrenergic neurons. Concurrent dual staining for Fos-ir and DBH-ir was carried out using nickel-intensified and nonintensified variants of the basic immunoperoxidase procedure.

Hybridization histochemistry localization was carried out using ^{35}S-labeled antisense cRNA probes synthesized as described (19). The CRF probe was generated from a 1.2-kb *Eco*RI fragment of a full-length rat CRF cDNA (K. Mayo, Northwestern University), subcloned into the Bluescript SK+ transcription vector (Stratagene). Labeled sense-strand runoffs failed to yield any positive signal in hypothalami of stressed or control animals.

Comparison with Systemic Cytokine Challenge Material. Because the complement of hypothalamic effector and medullary catecholamine neurons activated in response to acute footshock was found to be very similar to that seen after systemic IL-1 injection (refs. 11 and 14; H.-Y.L. and P.E.S., unpublished results), it became of interest to compare the effects of medullary knife cuts in the two paradigms. For this, we analyzed Fos material that was described, but not analyzed quantitatively, in a previous study of the dependence of IL-1-induced hypothalamic responses on aminergic inputs (14). In brief, the IL-1 experiments involved acute intravenous administration of recombinant human IL-1 (Immunex) at 1.87 μg/kg or vehicle to rats bearing knife cuts placed 2 weeks prior to testing; animals were sacrificed at 3 hr postinjection, the

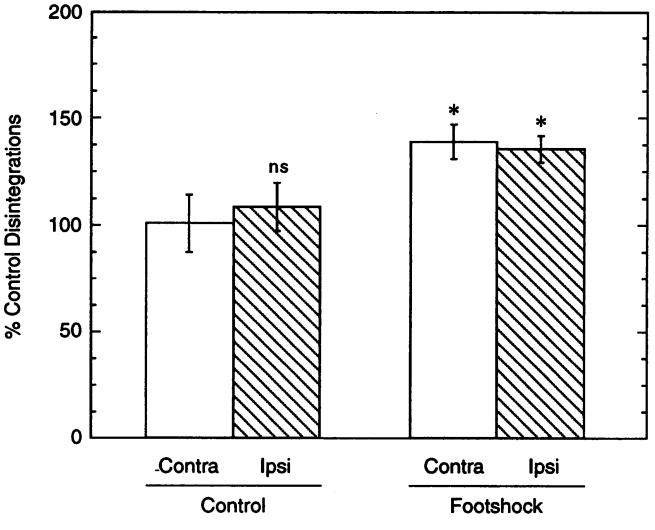

FIG. 2. Knife cut effects on CRF mRNA in control and acutely footshocked rats. Mean ± SEM relative levels of CRF mRNA in the parvocellular division of the PVH on the sides ipsilateral (Ipsi) and contralateral (Contra) to medullary knife cuts in rats sacrificed 2 hr after acute exposure to sham shock (control) of footshock procedures are given. Data are expressed as a percentage of values obtained on the contralateral side of control rats. Knife cuts affect neither resting levels of CRF mRNA nor shock-induced increases in this parameter. *, $P < 0.05$ versus contralateral side of control rats.

optimal interval for IL-1-induced Fos expression (14). The IL-1 and footshock experiments employed rats of the same strain and age and made use of the same coordinates, knife construction, and procedures for stereotaxic surgery.

Analysis. Densitometric data were gathered using IMAGE software (Version 1.55; W. Rasband, National Institutes of Health, Bethesda, MD) from all sections through the medial parvocellular subdivision of the PVH, defined by redirected sampling based on Nissl staining. Values were corrected for background and read from a curve relating optical density to dpm in brain paste standards (20). Fos-ir neurons were counted (21) in complete one-in-five series of sections through region(s) of interest. Lesion effects were analyzed using a two-way analysis of variance, with one within- (lesioned versus intact sides) and one between-group (stress versus control) variable and Scheffé's procedure for pairwise comparisons.

RESULTS

Unilateral knife cuts were patterned after, and found to be indistinguishable from, placements shown previously to be effective in depleting the PVH of its adrenergic and noradrenergic innervation and in mitigating the CRF mRNA response to a systemic IL-1 challenge (14) without affecting basal levels of expression (17, 22). Transections were localized between the caudal and rostral poles of the trigeminal and facial motor nuclei, respectively, and their measured dimensions were 1.5–1.8 mm lateral to the midline and 3.0–3.3 mm dorsal from the ventral surface of the brain (Fig. 1). All lesions were situated caudal and ventral to the locus coeruleus. The shallow U shape of the knife's cutting edge precluded damage to the pyramidal tract, and lesioned animals displayed no obvious motor, or other behavioral, impairment throughout the 2-week postlesion period. Well-placed cuts produced a frank pile-up of swollen DBH-ir axons and varicosities immediately proximal to the transection site (Fig. 1), as well as depletion of the DBH-ir innervation of the PVH on the ipsilateral side (17, 22, 23). By these criteria, cuts were judged to have been effective in seven rats subjected to a single acute footshock session and four lesioned controls.

Knife-cut animals sacrificed at 2 hr after footshock, the time of maximal Fos induction seen using the parameters in force here (ref. 11; H.-Y.L. and P.E.S., unpublished results), displayed comparably ($P > 0.10$) elevated levels of CRF mRNA in the contralateral and ipsilateral representations of the PVH to values that averaged 139% and 135%, respectively, of those measured on the contralateral side of lesioned sham-shocked rats ($P < 0.05$; Figs. 2 and 3). Control animals similarly displayed no side-to-side differences in the abundance of CRF transcripts in the PVH ($P > 0.10$), indicating that the knife cut procedure affected neither basal nor footshock stress-induced alterations in this parameter. In line with previous findings

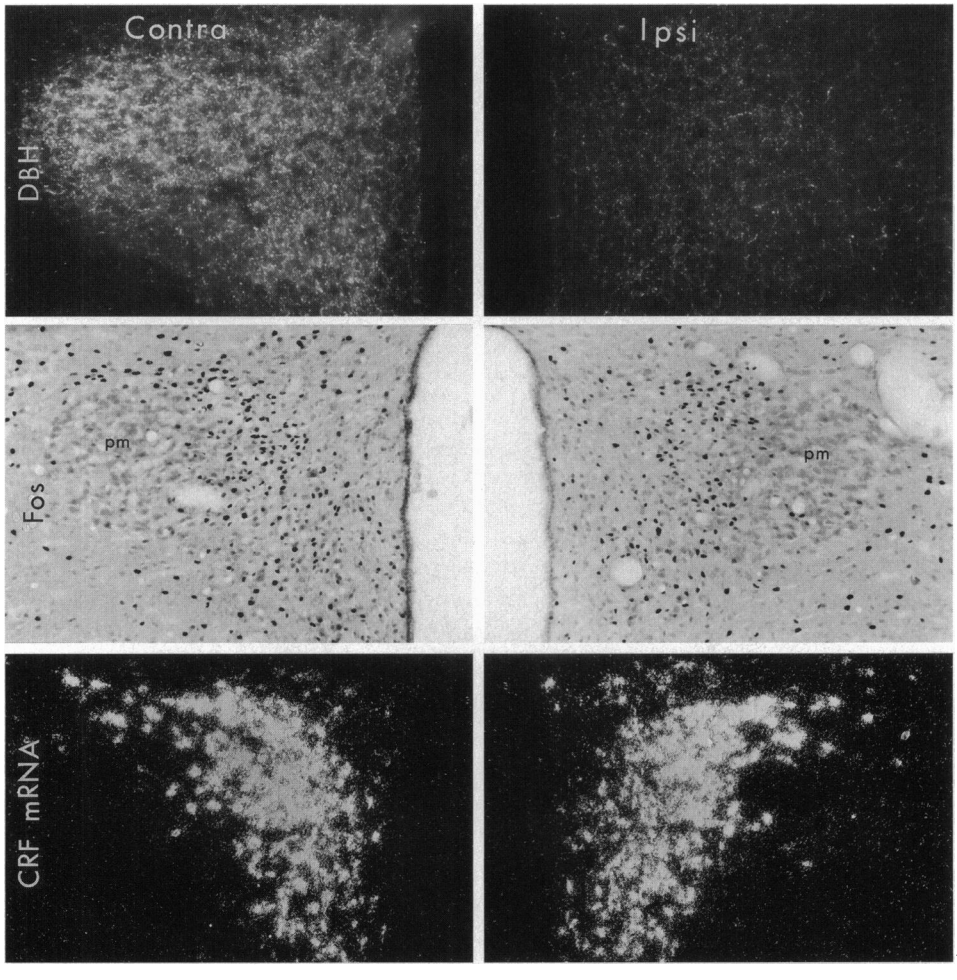

FIG. 3. Catecholamine-depleting knife cuts fail to modify footshock-induced alterations in Fos protein or CRF mRNA expression. Fluorescence (*Top*), bright-field (*Middle*), and dark-field (*Bottom*) photomicrographs illustrate the impact of unilateral medullary knife cuts on the DBH-ir innervation (DBH) and footshock-induced alterations in Fos-ir and CRF mRNA, respectively, on the intact (contra; *Left*) and lesioned (Ipsi; *Right*) sides of the PVH. Despite a pronounced reduction in the aminergic innervation on the ipsilateral side, stress-induced up-regulation of Fos-ir and CRF mRNA appears comparable to that seen contralaterally. These results contrast with those reported previously in an IL-1 challenge paradigm, where similar cuts markedly attenuated Fos and CRF mRNA responses on the lesioned side. pm, posterior magnocellular part of the PVH. (×90.)

(ref. 11; H.-Y.L. and P.E.S., unpublished results), Fos expression in the PVH of chamber-adapted control animals was low to undetectable, with no lateralized differences. Stressed rats displayed a robust Fos-ir induction that was centered in the hypophysiotropic (CRF-rich) medial parvocellular part of the PVH, with secondary accumulations in subdivisions of the nucleus that project to autonomic-related cell groups in the brainstem and spinal cord, as well as in aspects of the magnocellular division of the nucleus in which oxytocin-expressing cells are massed (4, 24). Despite a massive unilateral depletion of its catecholaminergic innervation, stress-induced Fos-ir expression in the PVH was comparable in strength and distribution on the intact and lesioned sides. Thus, independent indices fail to support a dependence of footshock stress-induced activation of hypothalamic visceromotor neurons on the integrity of their medullary aminergic afferents.

This outcome stood in stark contrast with the results of a study in which an identical approach was used to probe the circuitry underlying responses to systemic cytokine (IL-1) injection, which activates a similar complement of PVH effector and medullary catecholaminergic neurons (14). It was therefore of interest to ascertain what, if any, impact the transection may have had on footshock-induced cellular activation beyond the hypothalamus and how this compared in the shock and IL-1 challenge paradigms. Examination of the brainstems of animals exposed to acute footshock revealed a striking diminution in the number of Fos-ir neurons in the NTS and ventrolateral medulla on the ipsilateral side to levels that in the NTS averaged only 34% of those seen contralaterally ($P < 0.01$); this contrasts with the lack of any reliable lesion effect on shock-induced Fos-ir in the PVH of these same animals ($P > 0.10$; Figs. 4 and 5). This decrement in shock-induced medullary Fos expression on the side of a transection could be a simple result of cell loss consequent to axotomy of neurons whose major or sole projections had been severed. Two sets of observations argue against this possibility. First, costaining for Fos and DBH revealed that the complement of catecholaminergic neurons in the NTS and ventrolateral medulla on the side ipsilateral to the knife cut was intact ($P > 0.10$ versus the contralateral side) and that the lateralized diminution in shock-induced Fos expression was localized overwhelmingly to catecholaminergic (i.e., DBH-ir) neurons (Fig. 4). Second, parallel cell counts undertaken of material from five IL-1-injected rats bearing similar unilateral transections revealed a significant ($P < 0.01$) decrement in shock-induced Fos-ir in the PVH (to 20% of control values) but not in the medulla ($P > 0.10$). Therefore, in distinct stress paradigms, this common lesion exerts opposite effects on cellular activation in hypothalamic effector neurons and their medullary aminergic afferents.

The impact of the transection in the footshock situation was not limited to aminergic neurons of the medulla. Activation of noradrenergic cells of the locus coeruleus encountered in this paradigm (refs. 9 and 11; H.-Y.L. and P.E.S., unpublished results) was also attenuated on the transected side (data not shown). Fos expression in other cell groups found reliably to harbor neurons identified as both displaying shock-induced cellular activation and projecting to the region of the PVH (ref. 11; H.-Y.L. and P.E.S., unpublished results) was ostensibly unaffected by the lesion. These included ones associated with somatosensory and/or nociceptive information processing (pedunculopontine and laterodorsal tegmental nuclei, periaqueductal gray), the limbic region of the telencephalon (bed nucleus of the stria terminalis, lateral septal and medial amygdaloid nuclei), and associated regions of the hypothalamus (dorsomedial and perifornical nuclei).

DISCUSSION

Challenges as distinct as electrical footshock and systemic IL-1 injection activate essentially indistinguishable populations of hypothalamic visceromotor effector neurons and their medullary catecholaminergic afferents (refs. 11 and 14; H.-Y.L. and P.E.S., unpublished results), observations that could be construed as supporting a measure of nonspecificity in the manner in which the brain mobilizes adaptive visceromotor responses to novel stresses. The present findings reveal, to the contrary, a differential dependence of IL-1- and footshock-

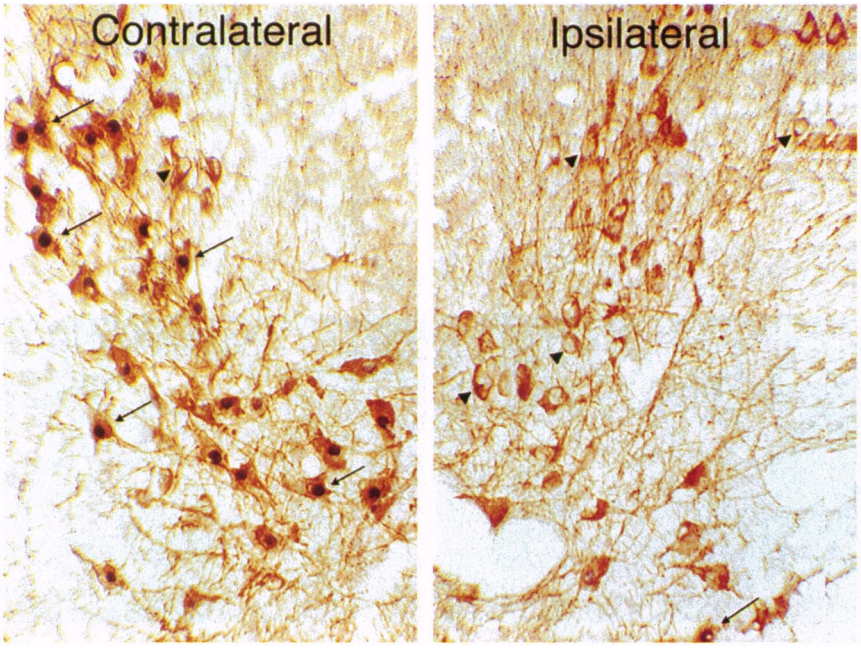

FIG. 4. Medullary knife cuts attenuate footshock-induced Fos expression in catecholaminergic neurons. Bright-field photomicrographs of a horizontal section through the NTS show dual immunoperoxidase staining for footshock-induced Fos-ir and DBH-ir on the sides of the brain contralateral and ipsilateral to a medullary knife cut. Shock-induced nuclear Fos expression (black) is severely attenuated in aminergic cells (red-brown cytoplasmic labeling) on the lesioned side of the brain. Examples of DBH-ir neurons that concurrently do (arrows) or do not (arrowheads) display nuclear Fos-ir are indicated. (×200.)

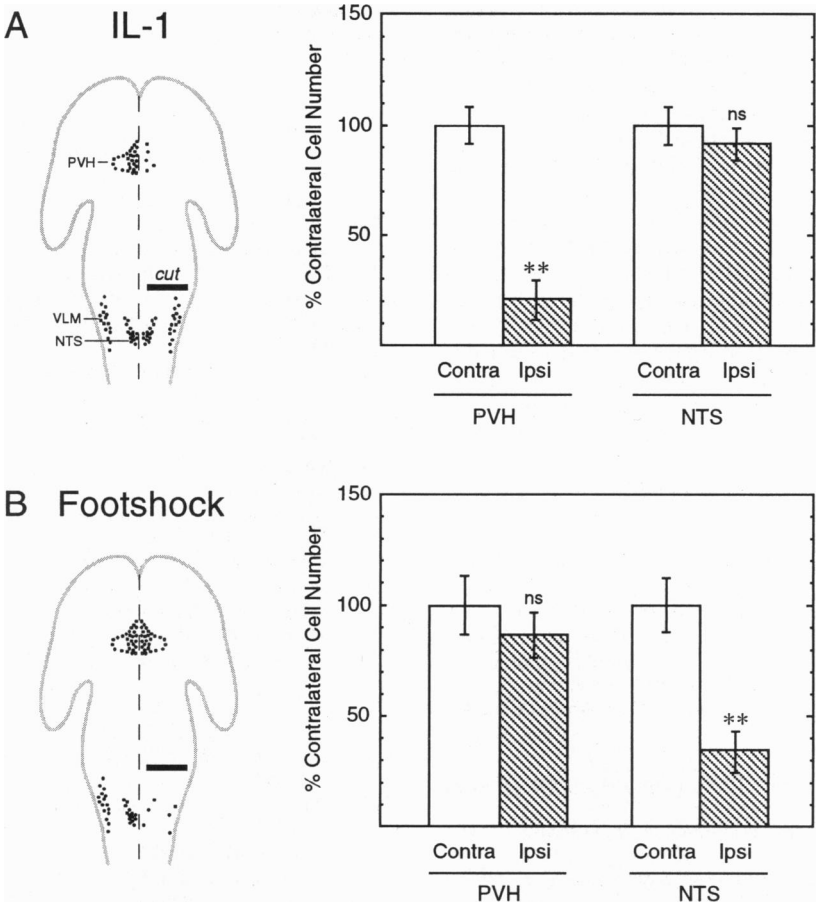

FIG. 5. Medullary knife cut effects on IL-1 and footshock-induced Fos expression in hypothalamus and medulla. Diagrammatic summary and mean ± SEM number of Fos-ir neurons in the NTS and PVH on the side of the brain ipsilateral to a medullary knife cut, expressed as a percentage of immunoreactive cells on the contralateral side after acute IL-1 (*A*) and footshock (*B*) challenges are shown. Lesion effects are summarized in a schematic drawing of a horizontal section through the brain at the left of each graph. In the IL-1 model, transections significantly reduced Fos induction in the PVH, while leaving the activation of medullarly aminergic neurons intact. Opposite effects were noted in the footshock paradigm. **, $P < 0.01$ versus contralateral side; ns, not significant.

induced increases in Fos protein and CRF mRNA expression in the PVH on the integrity of aminergic projections originating in the caudal medulla. Disruption of this pathway markedly attenuates hypothalamic responses to an acute IL-1 challenge but does not significantly affect footshock-induced alterations in these same parameters. These results complement data summarized elsewhere to support a mediating (as opposed to a merely permissive) role for aminergic projections in the activation of PVH regulatory mechanisms by IL-1 (14). The lack of any comparable dependence in the footshock model leaves open the critical circuitry subserving PVH responses in this paradigm. More generally, our findings emphasize the need to experimentally test inferences concerning situation-specific functional relatedness that may be drawn from patterns of neuronal activation and connectivity.

An unanticipated effect of knife cuts was the pronounced diminution of footshock-induced activation of medullary aminergic neurons on the ipsilateral side. Because this was not accompanied by loss of cells exhibiting the catecholamine phenotype and was not evident in animals bearing similar cuts but challenged with IL-1, we conclude that activation of catecholaminergic cells in the footshock paradigm is a secondary consequence of stress that is dependent upon a projection that originates distal (i.e., rostral) to, and is disrupted by, the ablation. Candidates for this role would include cell groups that exhibit footshock responsivity and are known to project to the NTS and/or ventrolateral medulla. These criteria are met by neurons associated with the limbic system (bed nucleus of the stria terminalis), nociceptive information processing (periaqueductal gray), and, intriguingly, by the PVH itself (11). Both anatomical and physiological evidence support a capacity of long descending projections of the PVH to interact directly with the very medullary cell groups that issue the bulk of its aminergic innervation (25, 26).

The footshock and IL-1 paradigms may be viewed as representative of two distinct classes of stress models, which are commonly termed neurogenic (or emotional or psychological) as opposed to systemic (homeostatic or physiological) paradigms (27). As generally conceived, neurogenic and systemic stresses differ principally as to the means by which effective stimuli are registered and processed (i.e., via visceral versus somatosensory/nociceptive mechanisms) and the extent to which they are consciously appreciated and/or accompanied by affective responses (5). While this distinction is not universally accepted, it finds support in the few available comprehensive surveys of the loci of stress-induced Fos expression in brain. Thus, cellular activation patterns elicited by such systemic challenges as hemorrhage (28) and IL-1 injection (14, 29) display many fundamental similarities that distinguish them from neurogenic models like footshock (refs. 9–11; H.-Y.L. and P.E.S., unpublished results), immobilization (12), or restraint (13). This raises the possibility that the activation of PVH visceromotor neurons encountered in other neurogenic paradigms may share with footshock a common underlying substrate, which does not include a critical dependence on ascending aminergic projections. The handful of cell groups

identified previously as reliably exhibiting shock-induced Fos expression and as projecting to the region of the PVH (ref. 11; H.-Y.L. and P.E.S., unpublished results), and in present experiments as exhibiting no decrement in stress-induced cellular activation as a consequence of knife cuts, remain as viable candidate afferent mediators of footshock influences on PVH mechanisms. These include ones associated either with processing of somatosensory/nociceptive information (periaqueductal gray, pedunculopontine and laterodorsal tegmental nuclei) or with the limbic region of the telencephalon and associated hypothalamic structures (bed nucleus, lateral septal, medial amygdaloid and dorsomedial hypothalamic nuclei). In view of evidence supporting a critical involvement of the limbic region in affixing an emotional valence to environmental events (30), further analysis should provide insight into the relationship between limbic system mechanisms governing the evaluative and experiential aspects of emotion, on one hand, and hypothalamic visceromotor neurons that constitute an important avenue of emotional expression, on the other.

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1. Sawchenko, P. E. & Swanson, L. W. (1982) *Brain Res. Rev.* **4,** 275–325.
2. Cunningham, E. T., Jr., & Sawchenko, P. E. (1988) *J. Comp. Neurol.* **274,** 60–76.
3. Cunningham, E. T., Jr., Bohn, M. C. & Sawchenko, P. E. (1990) *J. Comp. Neurol.* **292,** 651–667.
4. Swanson, L. W. & Sawchenko, P. E. (1983) *Annu. Rev. Neurosci.* **6,** 269–324.
5. Sawchenko, P. E., Brown, E. R., Chan, R. K. W., Ericsson, A., Li, H.-Y., Roland, B. L. & Kovács, K. J. (1995) *Prog. Brain Res.*, in press.
6. Morgan, J. I. & Curran, T. (1991) *Annu. Rev. Neurosci.* **14,** 421–451.
7. Hoffman, G. E., Smith, M. S. & Verbalis, J. G. (1993) *Front. Neuroendocrinol.* **14,** 173–213.
8. Chan, R. K. W., Brown, E. R., Ericsson, A., Kovács, K. J. & Sawchenko, P. E. (1993) *J. Neurosci.* **13,** 5125–5138.
9. Pezzone, M. A., Lee, W.-S., Hoffman, G. E., Pezzone, K. M. & Rabin, B. S. (1993) *Brain Res.* **608,** 310–318.
10. Pezzone, M. A., Lee, W.-S., Hofman, G. E. & Rabin, B. S. (1992) *Brain Res.* **597,** 41–50.
11. Li, H.-Y. & Sawchenko, P. E. (1995) *Soc. Neurosci. Abstr.* **20,** 938.
12. Senba, E., Umemoto, S., Kawai, Y. & Noguchi, K. (1994) *Mol. Brain Res.* **24,** 283–294.
13. Cullinan, W. E., Herman, J. P., Battaglia, D. F., Akil, H. & Watson, S. J. (1995) *Neuroscience* **64,** 477–505.
14. Ericsson, A., Kovács, K. J. & Sawchenko, P. E. (1994) *J. Neurosci.* **14,** 897–913.
15. Li, H.-Y., Ericsson, A. & Sawchenko, P. E. (1995) *Soc. Neurosci. Abstr.* **21,** 1386.
16. Gold, R. M., Kapatos, G. & Carey, R. J. (1973) *Physiol. Behav.* **10,** 813–815.
17. Sawchenko, P. E. (1988) *Neuroendocrinology* **48,** 459–470.
18. Sawchenko, P. E., Cunningham, E. T., Jr., Mortrud, M. T. & Gerfen, C. R. (1990) *Methods Neurosci.* **3,** 247–260.
19. Simmons, D. M., Arriza, J. & Swanson, L. W. (1989) *J. Histotechnol.* **12,** 168–181.
20. Sawchenko, P. E., Arias, C. A. & Mortrud, M. (1993) *J. Neuroendocrinol.* **5,** 341–348.
21. Abercrombie, M. (1946) *Anat. Rec.* **94,** 239–247.
22. Swanson, L. W. & Simmons, D. M. (1989) *J. Comp. Neurol.* **285,** 413–435.
23. Palkovits, M., Mezey, E., Skirboll, L. R. & Hökfelt, T. (1992) *J. Chem. Neuroanat.* **5,** 407–415.
24. Swanson, L. W. & Kuypers, H. G. J. M. (1980) *J. Comp. Neurol.* **194,** 555–570.
25. Sladek, J. R., Jr., & Sladek, C. D. (1983) *Prog. Brain Res.* **60,** 437–443.
26. Buijs, R. M., van der Beek, E. M., Renaud, L. P., Day, T. A. & Jhamandas, J. H. (1990) *Neuroscience* **39,** 717–725.
27. Allen, J. P., Allen, C. F., Greer, M. A. & Jacobs, J. J. (1973) in *Brain-Pituitary-Adrenal Interrelationships*, eds. Brodish, S. & Redgate, E. R. (Karger, Basel), pp. 99–127.
28. Li, Y.-W. & Dampney, R. A. L. (1994) *Neuroscience* **61,** 613–634.
29. Brady, L. S., Lynn, A. B., Herkenham, M. & Gottesfeld, Z. (1994) *J. Neurosci.* **14,** 4951–4965.
30. LeDoux, J. E. (1987) in *Emotion*, ed. Plum, F. (Am. Physiol. Soc., Washington, DC), pp. 419–459.