The Journal of Neuroscience, September 1994, 14(9): 5694–5707

Afferents to the Midline Thalamus Issue Collaterals to the Nucleus Tractus Solitarii: An Anatomical Basis for Thalamic and Visceral Reflex Integration

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The goal of this study was to establish a structural basis for thalamic and visceral integration. We sought to define neural networks that convey visceral or integrated environmental stimuli to the diffuse thalamocortical relay system and that link periodic changes in forebrain and visceral reflex function. Our experiments were designed to determine whether afferents to the midline-intralaminar thalamic nuclei (MIT) issue collaterals to the general viscerosensory division of the nucleus tractus solitarii (NTS). Experiments were performed on anesthetized male Sprague–Dawley rats. Two tracers, FluoroGold and rhodamine latex microbeads, were stereotaxically centered on the MIT and NTS, respectively, in each animal. Subsets of midline thalamic afferents were identified that issue collaterals to the solitary complex. In the cerebral cortex, dually labeled soma were detected in layer V of the insular and infralimbic areas. In the subcortical forebrain, the lateral septal nucleus, anterolateral area of the bed nuclei of stria terminalis, medial preoptic nucleus, medial and central amygdaloid nuclei, caudal lateral hypothalamic area, supramammillary nucleus, and parvicellular division of the paraventricular hypothalamic nucleus constitute other newly identified sources of collateral projection. In the midbrain and pons, collateral projection cells were observed in the periaqueductal gray, dorsal raphe nucleus, mesencephalic reticular formation, laterodorsal tegmental nucleus, lateral and medial parabrachial nuclei, and noradrenergic A5 area. In the lateral parabrachial nucleus, dually labeled neurons were detected in the dorsal–lateral division. In the medulla, collaterals are derived from cells in the rostral and caudal ventrolateral reticular formation and parapyramidal area. Dually labeled cells were also found in the cerebellar fastigial nucleus. Collaterals may coordinate changes in visceral reflex excitability and thalamocortical rhythms during phases of sleep–wake cycle and behavioral expression.

[Key words: midline-intralaminar thalamic nuclei, diffuse thalamocortical projection system, nucleus tractus solitarii, autonomic function, collaterals, double retrograde transport study, rat]

Homeostasis is maintained by processing multimodal afferents to form integrated patterns of autonomic response appropriate to the physiological state of the organism. Examples include modifications of cerebral electrocortical activity and cardiorespiratory reflex function that accompany severe muscular exercise, the defense–arousal response, or phases of the sleep–wake cycle (for reviews, see Jordan, 1990; Parmeggani and Morrison, 1990).

A link between the state of vigilance or wakefulness and autonomic function was observed by Jackson and Stewart (1899) in patients with epileptic automatisms associated with lesions of the uncinate region. Intimate associations between the level of arousal and activity of the autonomic nervous system are well documented, clinically and experimentally, in human and animal models. As reviewed by Pickering (1990), the contrasting effects of sleep and wakefulness on blood pressure are reflected by the progressive falls coinciding with stages 3 and 4 of deep slow wave sleep or the sympathetic vasoconstriction and elevations during periods of arousal of stages 1 and 2 and on waking.

The neural networks that link recurring periodic changes in cerebral cortical activity and visceral reflex function have not been identified. It has long been recognized that the electrical activity of the cerebral cortex during sleep, arousal, pain perception, and selective attention, and even consciousness itself is regulated by multimodal stimuli conveyed by way of afferents terminating in the diffuse or midline-intralaminar thalamus (MIT) (for reviews, see Plum and Posner, 1980; Herkenham, 1986; Jones, 1989). Unlike the specific or discriminative thalamosensory relay nuclei concerned with relaying epicritical sense, neurons in the diffuse system, *in toto,* project globally across the cortical mantle and striatum (Berendse and Groenewegen, 1991) and elicit widespread cortical recruiting responses originating in the thalamic reticular nucleus (Purpura, 1970; Yingling and Skinner, 1976; Steriade and Llinás, 1988; Steriade et al., 1990).

We speculate, based in part on axonal transport data, that neurons may exist that coordinate diffuse thalamocortical sensory processing, for example, modulation of cortical activity during phases of the sleep–wake cycle, and visceral reflex function. In support, subsets of thalamic afferents implicated in state-dependent alterations of electrocortical activity (Asanuma, 1992; Otake et al., 1993) derive from brainstem nuclei, notably, within the mesopontine tegmentum, recently identified as novel

Received Jan. 18, 1994; revised Mar. 10, 1994; accepted Mar. 24, 1994.

This work was supported by HL18974 (D.J.R., D.A.R.) and NS28200 (D.A.R.). We thank Mr. Muhammad Anwar for his expert technical assistance and Mr. Terence Ziegler for the excellent photography. K.O. is on leave from the Department of Anatomy, Faculty of Medicine, Tokyo Medical and Dental University, Tokyo, Japan, and is partly supported by the Japan Ministry of Education, Science and Culture.

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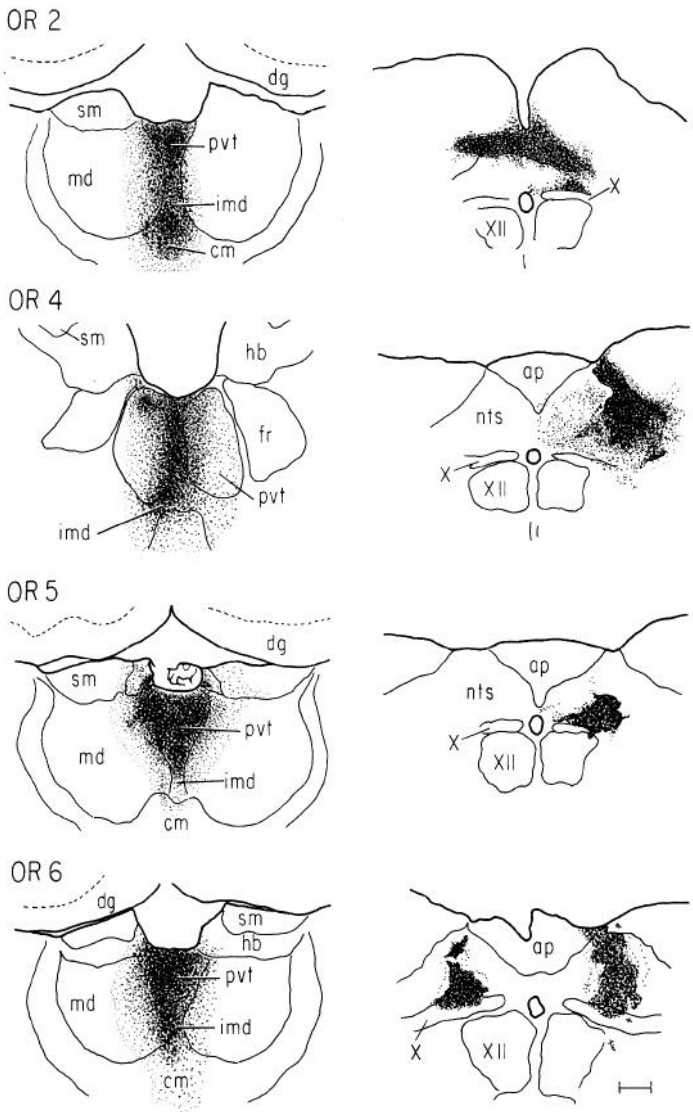

Figure 1. Camera lucida drawings of injections of FluoroGold in the midline thalamus (*left column*) and of rhodamine-microbeads in the nucleus tractus solitarii (NTS) (*right column*). In each case, the FluoroGold deposit is concentrated in the paraventricular thalamic nucleus and also encompassed the intermediodorsal, central medial, and mediodorsal nuclei. Approximate distances from bregma defined in the atlas by Paxinos and Watson (1986) are −2.30 mm (*OR2*), −3.60 mm (*OR4*), −2.12 mm (*OR5*), and −2.80 mm (*OR6*). The injection site in the NTS is centered on the commissural (case OR2) or medial (cases OR4, OR5, OR6) subnucleus and variably involves the dorsal part of the dorsal motor nucleus of the vagus. See Appendix for abbreviations used in figures. Scale bar: 500 μm for *left column*, 250 μm for *right column*.

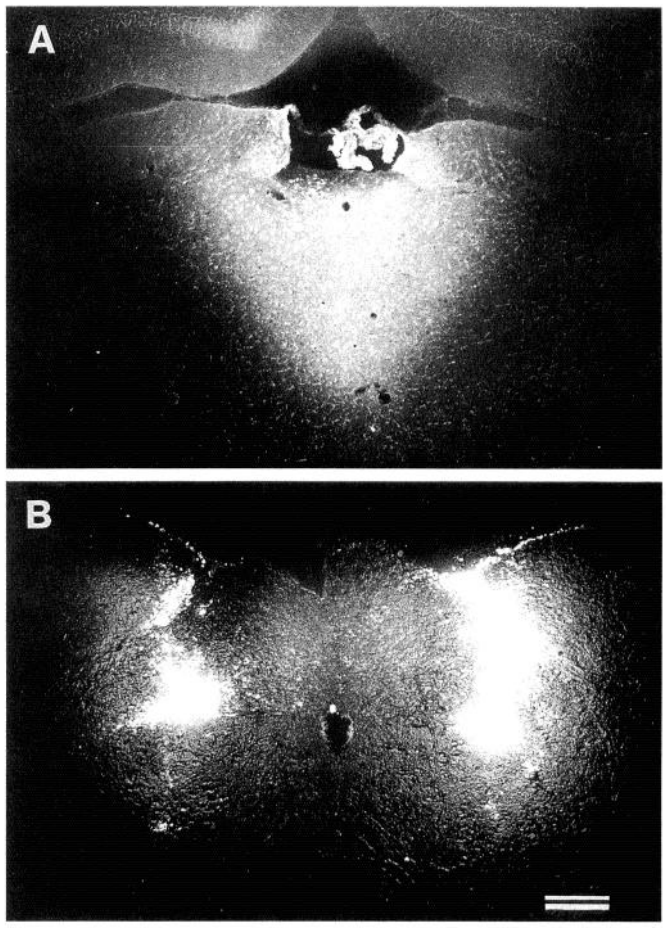

Figure 2. Photomicrographs demonstrate representative injection sites. FluoroGold was injected into the midline thalamus (*A*, case OR5) and rhodamine-microbeads into the nucleus tractus solitarii (NTS) (*B*, case OR6). Scale bar: 500 μm for *A*, 250 μm for *B*.

sources of afferent projection to the nucleus tractus solitarii (NTS), first-order recipient of primary visceral afferents (Ruggiero et al., 1994), or an area of the rostral ventrolateral medulla involved in visceral reflex control (Yasui et al., 1990). Comparisons of other projection nuclei also predict additional probable sources of shared afferents, including regions identified as members of ascending cortical activating systems (Starzl and Magoun, 1951; Shute and Lewis, 1967; Vincent et al., 1986; Jones and Cuello, 1989). Dual labeling studies, however, have yet to confirm the existence of these hypothesized projection neurons.

The present study was therefore designed to determine whether neurons in the CNS issue collaterals to cells engaged in diffuse thalamocortical sensory processing by the midline thalamus and viscerosensory reflex integration by the NTS.

A preliminary report of these observations was presented at the 23rd annual meeting of the Society for Neuroscience (Otake et al., 1993).

Materials and Methods

Data were obtained from four male Sprague–Dawley rats (200–250 gm) anesthetized with halothane (induced at 4% and maintained at 2% in 100% O_2). Two fluorescent tracers, FluoroGold (FG; 2% in distilled water; Fluorochrome Inc.) or rhodamine-impregnated latex microbeads (R-Mb; 50% in distilled water; Luma-Fluor Inc.), were used for dual retrograde-labeling experiments. Under sterile conditions, the sagittal sinus was exposed by perforating, with a dental drill, parietal bone along the sagittal suture, extending 2–3.5 mm caudal to bregma. The dural membranes were cut and reflected, and FG was injected stereotaxically into the paraventricular and intermediodorsal nuclei of the thalamus with a glass micropipette attached to a syringe. Axial muscles of the neck were next cut and reflected and an occipital craniotomy made to expose the posterior fossa. The dura were cut and reflected and the dorsum of the medulla exposed. The micropipette was inserted into stereotaxically defined loci in the NTS by using obex as stereotaxic zero. Thereafter, injections of R-Mb were made at the level of the obex or 0.5 mm caudally, at the calamus scriptorius. A total volume of 100 nl of each tracer was injected over 10 min. The micropipettes were left *in situ* for 20–30 min following each injection. Bone wax was applied and the tissues sutured. After a survival period of 5–7 d, the animals were

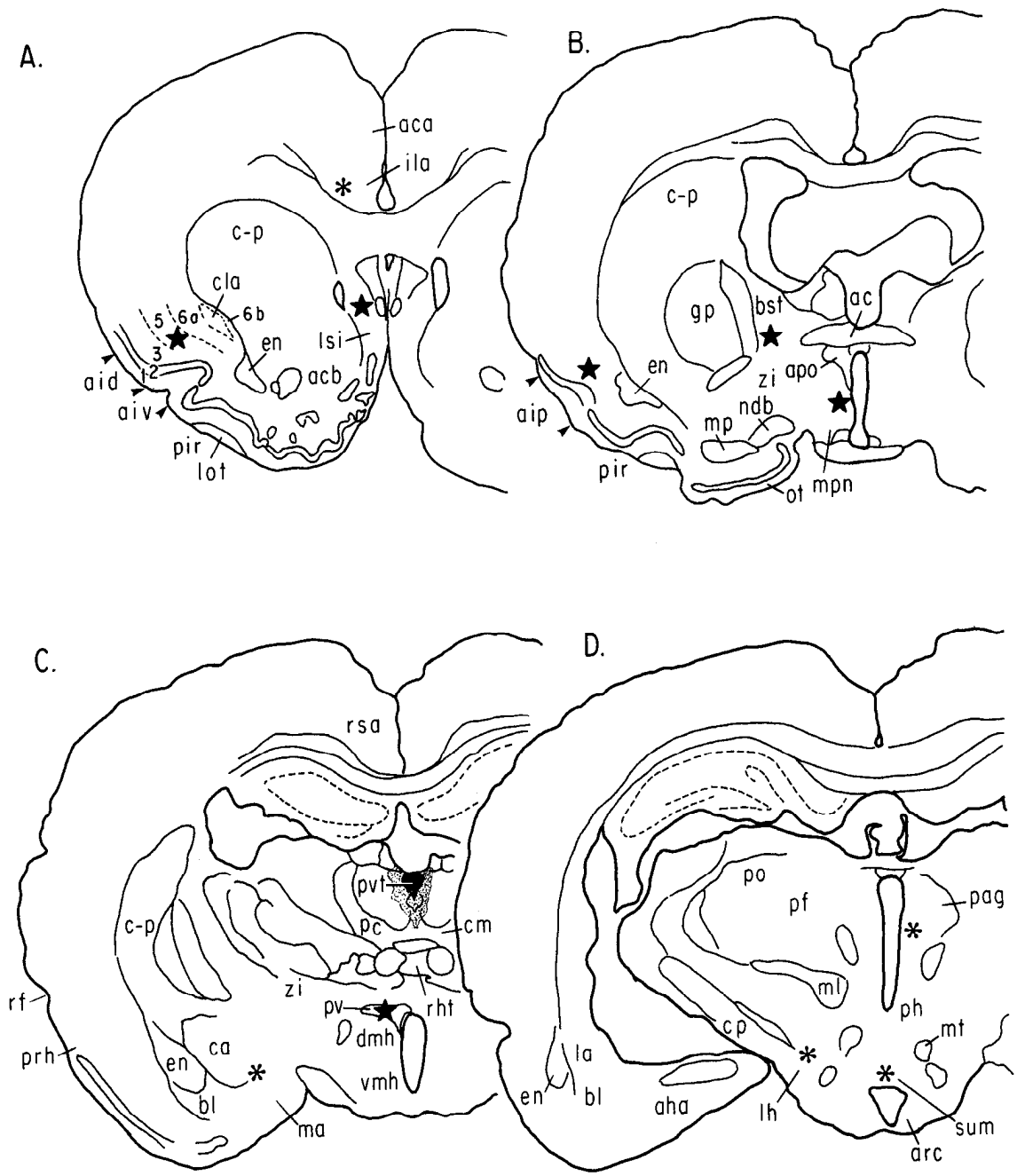

Figure 3. Diagrams summarize the locations in transverse sections of dually labeled cell bodies in the forebrain. The thalamic injection site is located in the plane depicted in *C*. *Stars* or *asterisks* indicate areas where dually labeled cells were observed. Areas marked with *stars* were photographed (Figs. 4–7); areas designated with *asterisks* are discussed in text.

deeply anesthetized with sodium pentobarbital (90–100 mg/kg, i.p.) and perfused transcardially with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Tissues were sectioned in the transverse plane at a 35 μm thickness on a freezing microtome, mounted on gelatin-coated slides, and air dried. The sections were examined for single- or dual-labeled cell bodies with an epifluorescent microscope (Nikon FX) at appropriate wavelengths and photographed on Kodak TMAX 400 ASA film.

Results

Injection sites

Injection sites are shown in Figures 1 and 2. In each animal, the FG deposit was centered in the paraventricular thalamic nucleus with some spread to adjacent structures including mediodorsal, intermediodorsal, and central medial nuclei (Figs. 1, 2*A*). Bilateral or unilateral deposits of R-Mb within the medial and/or commissural subnuclei of the NTS are shown in Figures 1 and 2*B*. In all cases, deposits of R-Mb were associated with variable spread to the subjacent dorsal motor nucleus of the vagus or nucleus parasolitarius.

FG-labeled cells were found in areas reported in a concomitant retrograde transport study of the midline thalamus (Otake et al., 1993). The patterns of retrograde transport of R-Mb were comparable to previously defined origins of NTS afferents (Ross et al., 1981; van der Kooy et al., 1984; Ruggiero et al., 1987, 1994).

Our objectives were to determine whether neurons exist that

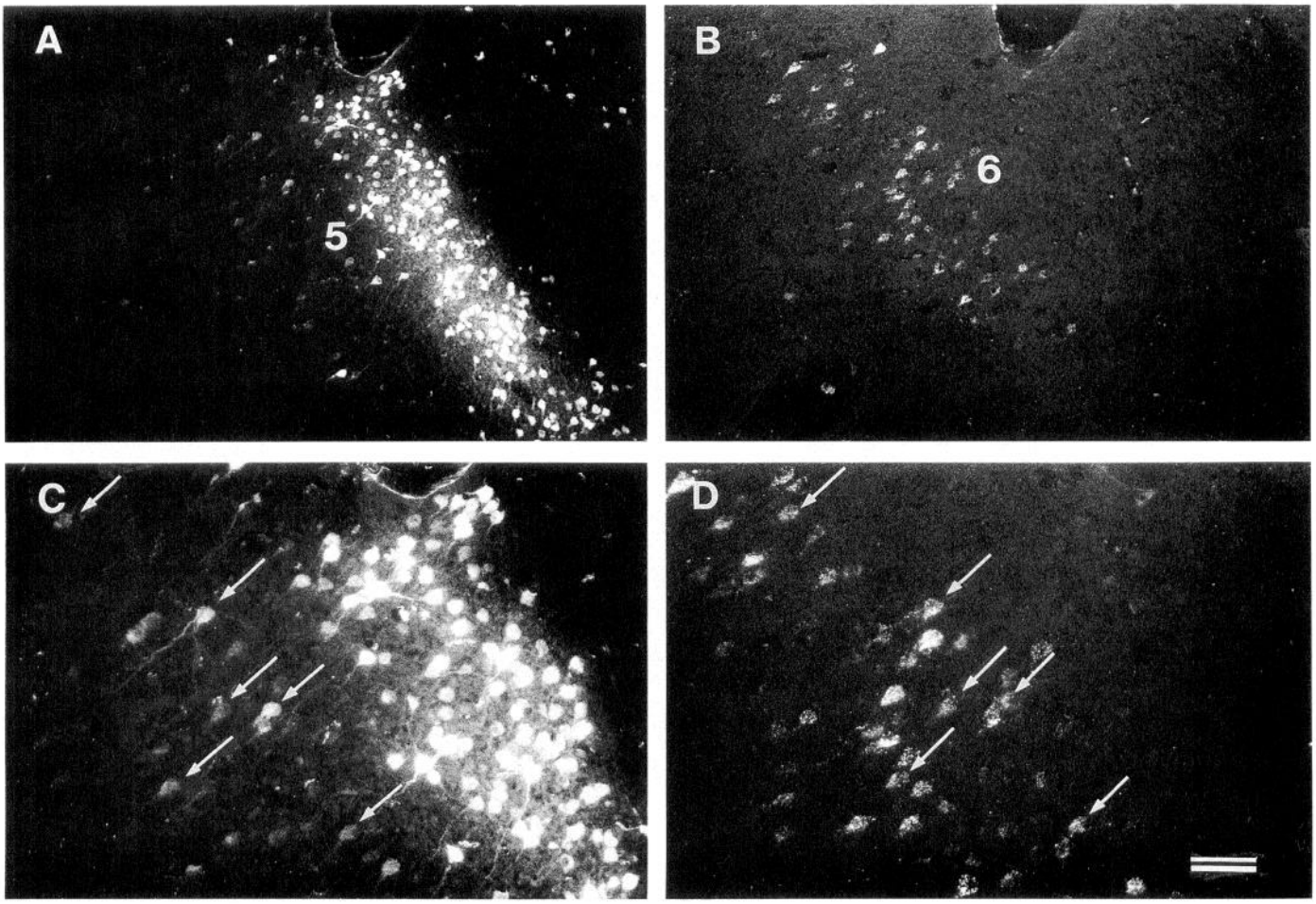

Figure 4. Photomicrographs of retrogradely labeled cells in the insular cortex from injections of FG into the thalamus (*A, C*) and R-Mb into the NTS (*B, D*). Note that the thalamic afferents are principally derived from lamina VI and NTS afferents exclusively from lamina V. Dually labeled cells were observed in lamina V and are indicated with *arrows* in higher-power photomicrographs (*C, D*). Data from case OR6. Scale bar: 100 μm for *A* and *B*, 50 μm for *C* and *D*.

issue collaterals to the MIT and the NTS. Although the majority of thalamic and medullary afferents were spatially segregated, dually labeled soma were detected at every level of the brain. The locations of collateral projection cells are summarized in Figure 3 for the forebrain and in Figure 8 for the midbrain and hindbrain.

Cerebral cortex

In the cerebral cortex, dually labeled cells were found in insular, infralimbic, and prelimbic cortices. In the insular cortex (Fig. 4), cells were retrogradely labeled from the midline thalamus in laminae VI and V (Fig. 4*A,C*). In lamina V, dually labeled cells were backfilled from the MIT and the NTS (Fig. 4*B,D*). Dually labeled cells were also found in lamina V of infra- and prelimbic cortical areas.

Subcortical forebrain

In the bed nuclei of the stria terminalis, dually labeled cell bodies were found in the anterolateral area (Fig. 5), as defined by the nomenclature of Ju and Swanson (1989). In comparison to the numbers of NTS-afferent projection neurons in the cerebral cortex and bed nuclei of the stria terminalis, those labeled in the preoptic and septal nuclei were sparse. However, double-labeled cells were clearly detected in the medial preoptic area (Fig. 6*A,B*) and the lateral septal nucleus (Fig. 6*C,D*). In the retrochiasmatic nucleus, cells were clearly dually labeled, albeit lightly, for both tracers. In the amygdala, central and medial amygdaloid subnuclei contained small number of dually labeled cell bodies. These dually labeled cells extended into the adjacent substantia innominata.

Afferents to the NTS and MIT were also labeled in the lateral hypothalamic area and organized topographically: cells of origin of thalamic afferents were skewed medially to those labeled from the NTS. Dually labeled cells in the lateral hypothalamus were detected along the border between these two territories. Supramammillary and arcuate nuclei revealed double-labeled cells although these cell groups were lightly labeled. The paraventricular hypothalamic nucleus (Fig. 7) showed a segregation of the two afferent populations. NTS afferents were heavily concentrated in the parvicellular division of the paraventricular hypothalamic nucleus (Fig. 7*B,D*); prethalamic afferents were sparsely labeled (Fig. 7*A,C*). An example of a double-labeled cell is shown in the photomicrographs of Figure 7, *C* and *D*.

Midbrain

In the periaqueductal gray, a small number of dually labeled cells was observed. Labeled cells were also found in the dorsal raphe nucleus (Fig. 9*A,B*), nucleus raphe magnus, substantia

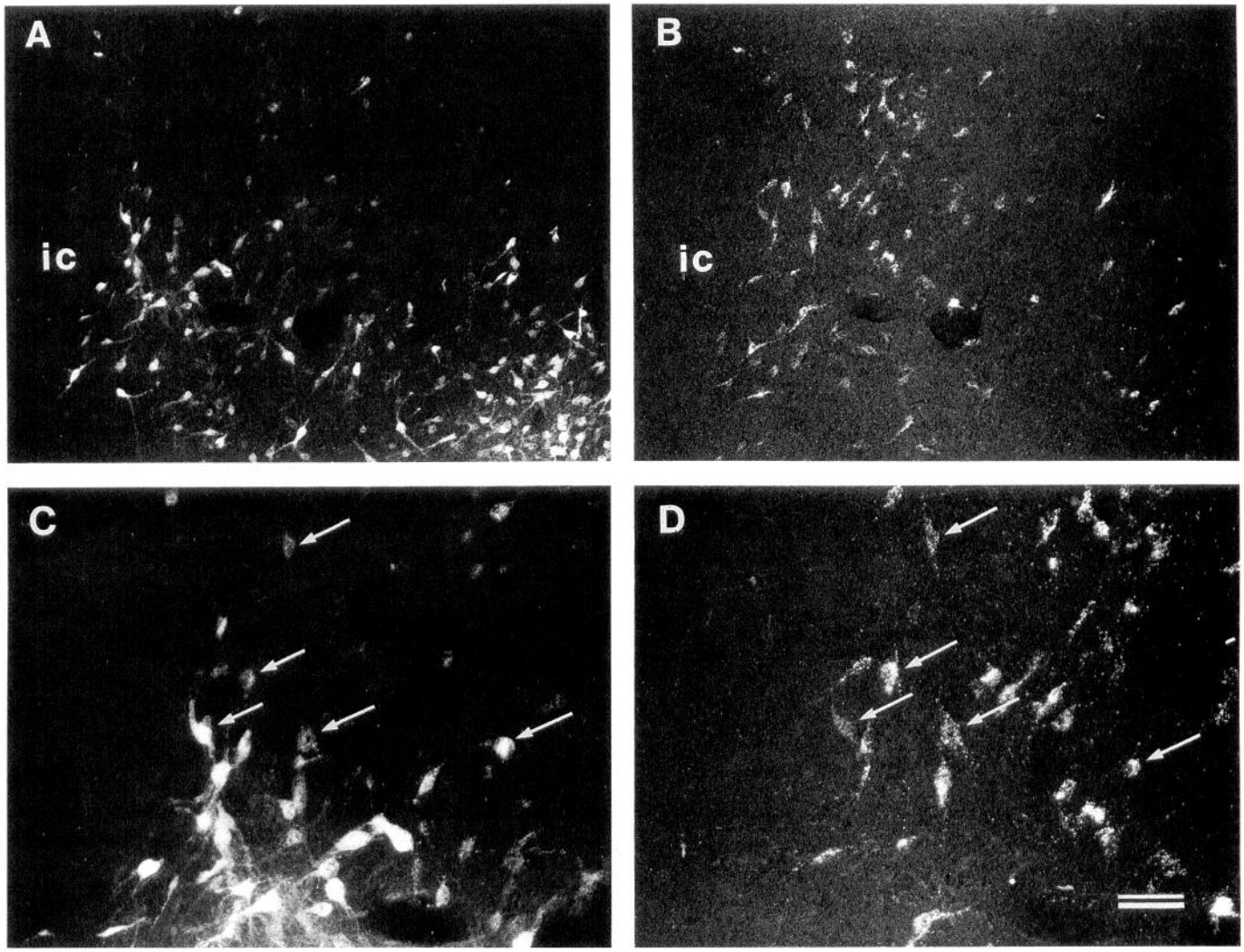

Figure 5. Photomicrographs of retrogradely labeled cells in the bed nuclei of the stria terminalis from injections of the tracers FG and R-Mb into the thalamus (*A, C*) and NTS (*B, D*), respectively. Afferents to both regions were derived mainly from the anterolateral area of the bed nuclei (Ju and Swanson, 1989), adjacent to the internal capsule (*ic*). Thalamic afferents were skewed ventrally and NTS afferents, dorsally. Dually labeled cells are indicated with *arrows* in higher-power photomicrographs below (*C, D*). Data from case OR4. Scale bar: 100 μm for *A* and *B*; 50 μm for *C* and *D*.

nigra pars compacta, and mesencephalic reticular formation (Fig. 9*C,D*).

Cerebellum

In the fastigial nucleus, dually labeled cells were detected at intermediate levels of the nucleus within its dorsolateral division (Fig. 9*E,F*). Other areas of the cerebellum were unlabeled.

Pons

In the pons, dually labeled cells were located in lateral and medial parabrachial nuclei. In the lateral parabrachial nucleus (Fig. 10*A,B*) most dually labeled cells were detected along a circumscribed lamina constituting the dorsal–lateral division. The laterodorsal tegmental nucleus (Fig. 10*C,D*) and A5 area (Fig. 10*E,F*) were other sites containing double-labeled cell bodies.

Medulla

In the medulla, collateral projection cells were labeled in the rostral ventrolateral reticular formation at a level immediately caudal to the facial nucleus (Fig. 11*A,B*). Double-labeled cells were also observed in the retroambigual area of the lateral tegmental field at the level of obex (Fig. 11*C,D*). Collateral projection neurons were also detected in the parapyramidal area contiguous with the nucleus raphe magnus (Fig. 11*E,F*). No dually labeled cells were observed in the medullary raphe pallidus or obscurus.

Discussion

This study demonstrates for the first time that each major division of the brain issues collaterals to the nondiscriminative sensory thalamus and the general visceral afferent division of the solitary nuclear complex. Dual retrograde tracing data provide strong evidence that nuclei engaged in diffuse thalamic relay function and viscerosensory processing are structurally and thus functionally integrated by shared afferents.

Methodological considerations

The specificity of our retrograde transport findings is supported by two observations. (1) The overall distributions of identified afferents were comparable to data obtained in previous retrograde tracer studies of the NTS and/or dorsal motor nucleus of

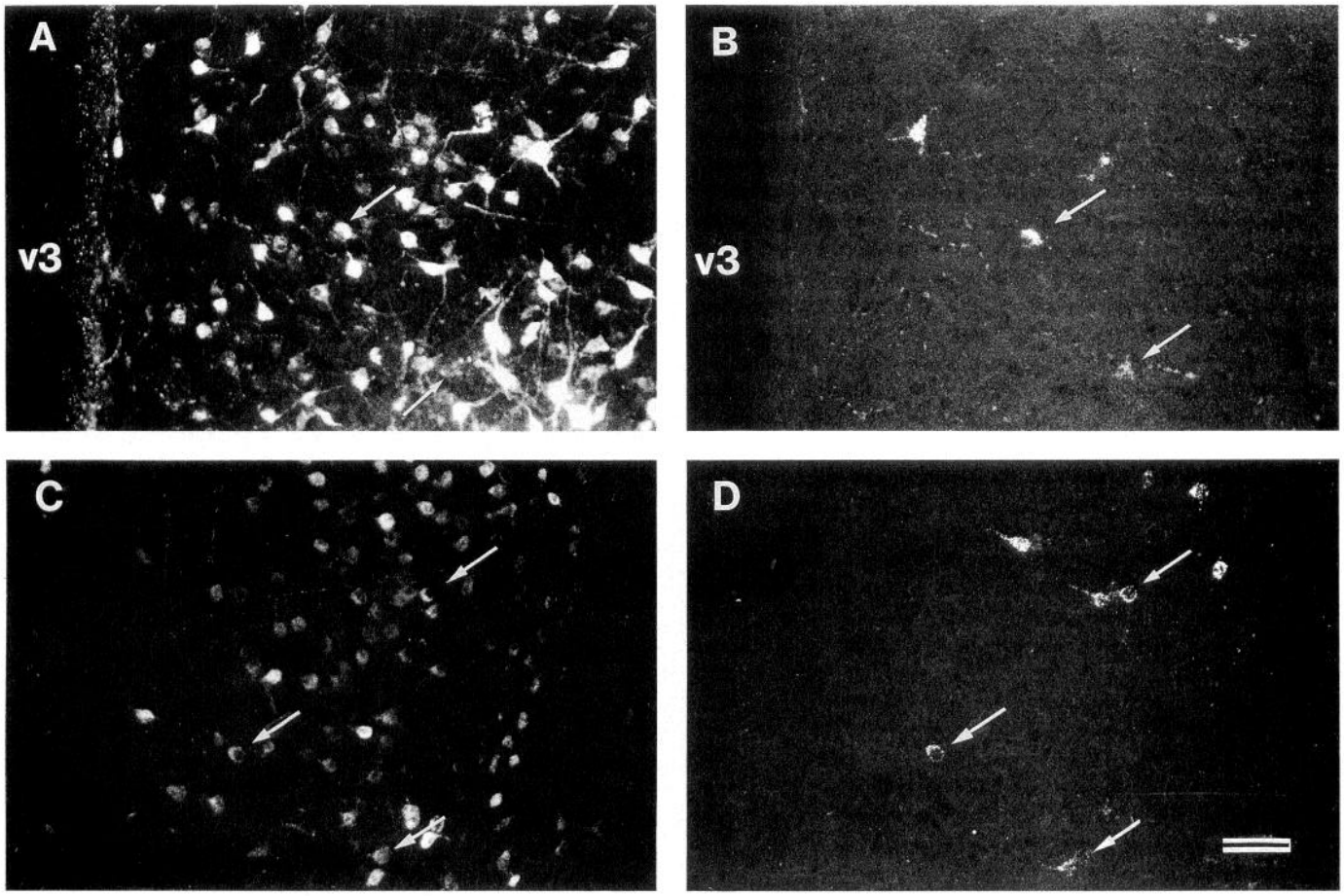

Figure 6. Photomicrographs of retrogradely labeled cells in the medial preoptic (*A, B*) and lateral septal nuclei (*C, D*) from injection of the tracers into the thalamus (*left column*) and NTS (*right column*). Dually labeled cells are indicated with *arrows*. *v3,* third ventricle. Data from case OR4. Scale bar, 50 μm.

the vagus (Ross et al., 1981; ter Horst et al., 1984; van der Kooy et al., 1984; Ruggiero et al., 1987, 1994) or the MIT (Cornwall and Phillipson, 1988; Otake et al., 1993). Based on our work, labeling patterns obtained with fluorescent dyes were identical to retrograde transport of wheat germ agglutinin–horseradish peroxidase or cholera toxin B subunit from similarly placed injection deposits (Otake et al., 1993). (2) Several of the identified sources of afferent projection have been confirmed with anterograde tracers as terminating in the NTS or MIT (e.g., Hopkins and Holstege, 1978; Ricardo and Koh, 1978; Saper et al., 1979; Saper and Loewy, 1980; Berk and Finkelstein, 1982; Eberhart et al., 1985; Holstege et al., 1985; Jones and Yang, 1985; Luiten et al., 1985; Ruggiero et al., 1987; Sesack et al., 1989).

Our injection sites encompassed several subnuclei of the dorsal midline thalamus including the paraventricular thalamic nucleus. Until recently, the status of this prominent subnucleus was controversial, based on developmental studies and in particular because of the absence of an identified projection upon the cerebral cortex (see Jones, 1985, for review). Berendse and Groenewegen (1991), however, have convincingly demonstrated with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin, cortical projections, for example, onto insular and infralimbic prefrontal cortices, complementing those of other members of the midline-intralaminar complex. These observations have subsequently been confirmed and extended by K. Otake, M. Anwar, and D. A. Ruggiero (unpublished observations).

Our medullary injection deposits, although centered on the NTS, also encompassed portions of the dorsal motor nucleus of the vagus. Both subnuclei constitute members of the solitary nuclear complex (NTS-X). Branches of afferents terminating in the NTS may, therefore, also terminate within the dorsal motor nucleus of the vagus. Primary visceral afferents as well as certain centrally derived projections to the NTS form synapses on or closely invest parasympathetic motoneurons of the dorsal motor nucleus of the vagus (Loewy and Spyer, 1990). It is well established that dendrites of parasympathetic preganglionic motoneurons in the subnucleus gelatinosus of the NTS are targets of first-order gastric afferents, implicated in vagovagal mechanoreceptor reflexes (Rinaman et al., 1989). These observations suggest that retrograde tracer deposits, even if confined to the NTS, could potentially be incorporated by afferents targeting dendrites of preganglionic motoneurons.

The goal of our study was to identify specifically cell groups that issue collaterals to the NTS-X and MIT. Since other nuclei in the forebrain and lower brainstem share similar sources of afferent input, the identified cells may branch more extensively than revealed by dual-labeling studies. The reticular formation of the lateral tegmental field deep to the NTS-X, for example, is supplied by afferents derived from several of the same cell groups backfilled from the NTS or the dorsal motor nucleus of the vagus (Ross et al., 1981; Schwaber et al., 1982; van der Kooy et al., 1984; Ruggiero et al., 1987, 1989).

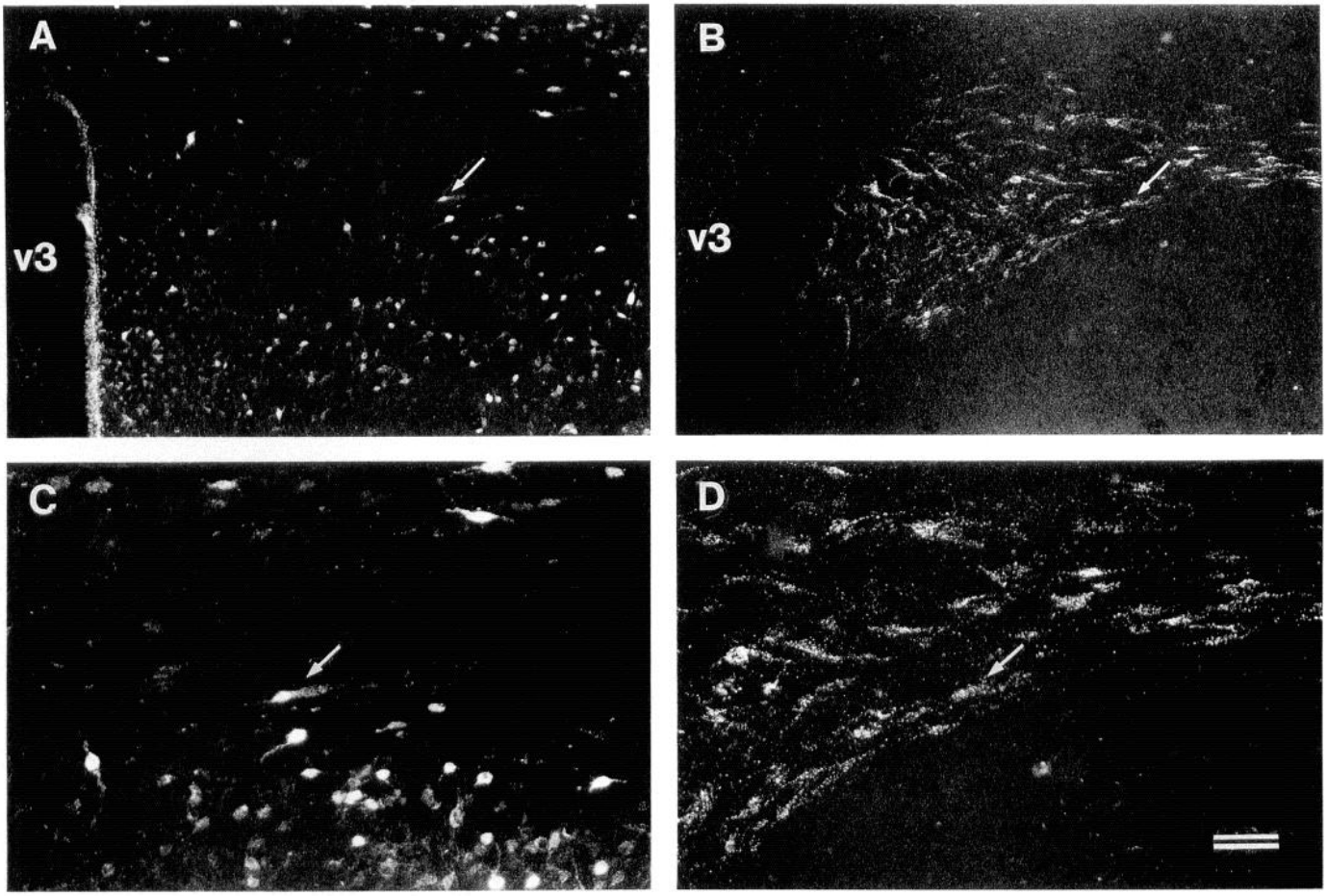

Figure 7. Photomicrographs of retrogradely labeled cells in the paraventricular hypothalamic nucleus from injections of FG and R-Mb into the thalamus (*A, C*) and NTS (*B, D*), respectively. Note the sparse labeling of thalamic afferents, compared to the dense transport from the NTS. A dually labeled cell at the marginal zone of the nucleus is indicated with *arrows* in the low- (*A, B*) and high-power photomicrographs (*C, D*). Data from case OR5. Scale bar: 100 μm for *A* and *B*, 50 μm for *C* and *D*.

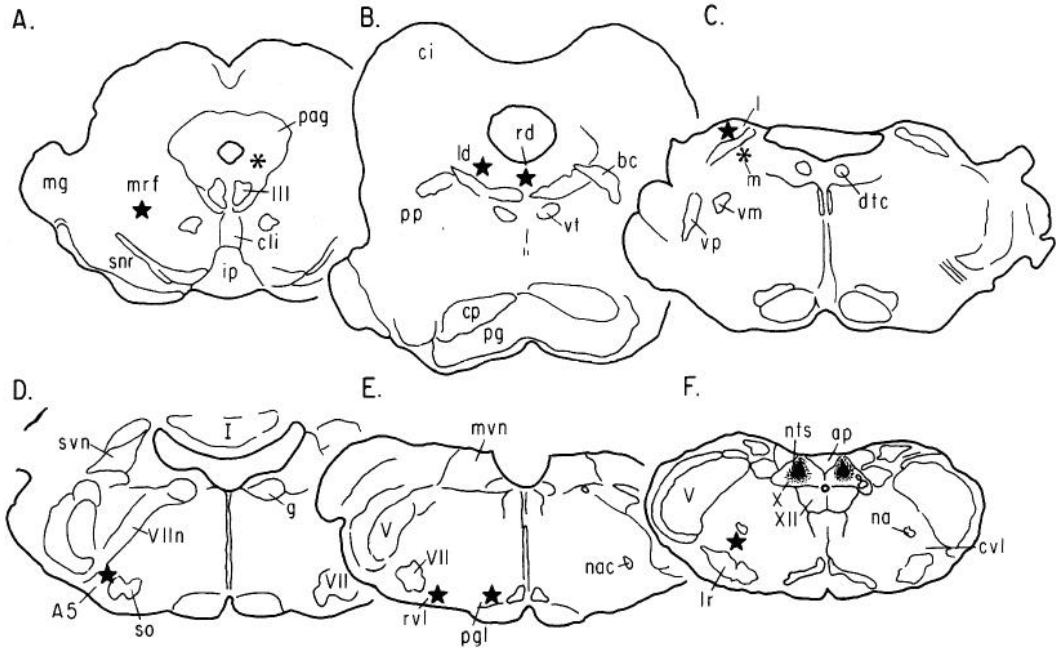

Figure 8. Diagrams summarize the locations in transverse sections of dually labeled cell bodies in the midbrain and hindbrain. The NTS injection site is located in plane *F. Stars* or *asterisks* indicate areas where dually labeled cells were observed. The areas marked with *stars* appear in the photomicrographs of Figures 9–11. Transport to areas indicated by *asterisks* are discussed in text. Cerebellum is not illustrated.

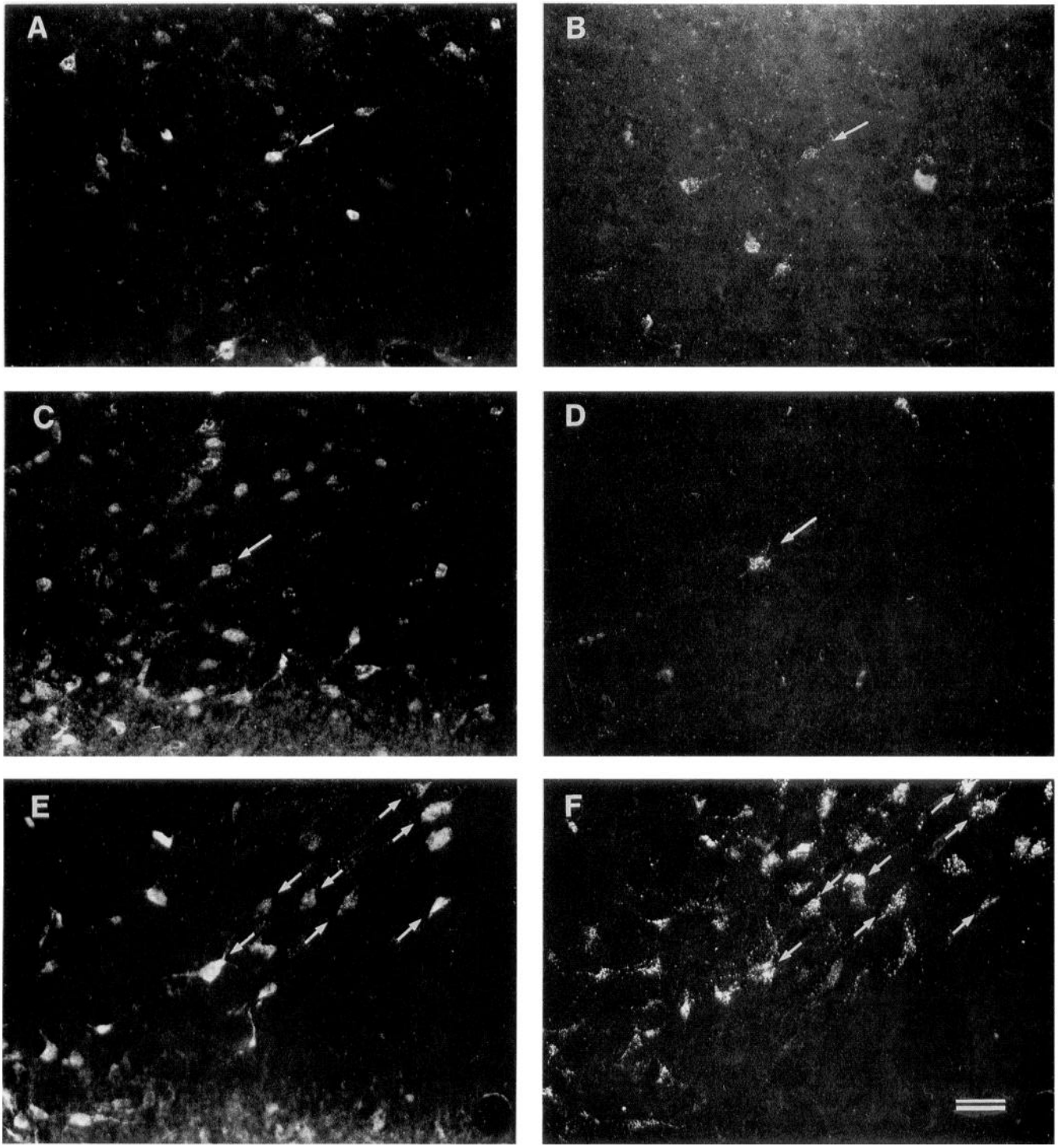

Figure 9. Photomicrographs of retrogradely labeled cells in the dorsal raphe (*A, B*) (case OR4), mesencephalic reticular formation (*C, D*) (case OR2), and fastigial nucleus (*E, F*) (case OR4) from the injections of FG into the thalamus (*left column*) and R-Mb into the NTS (*right column*), respectively. Dually labeled cells are indicated with *arrows*. Scale bar, 50 μm.

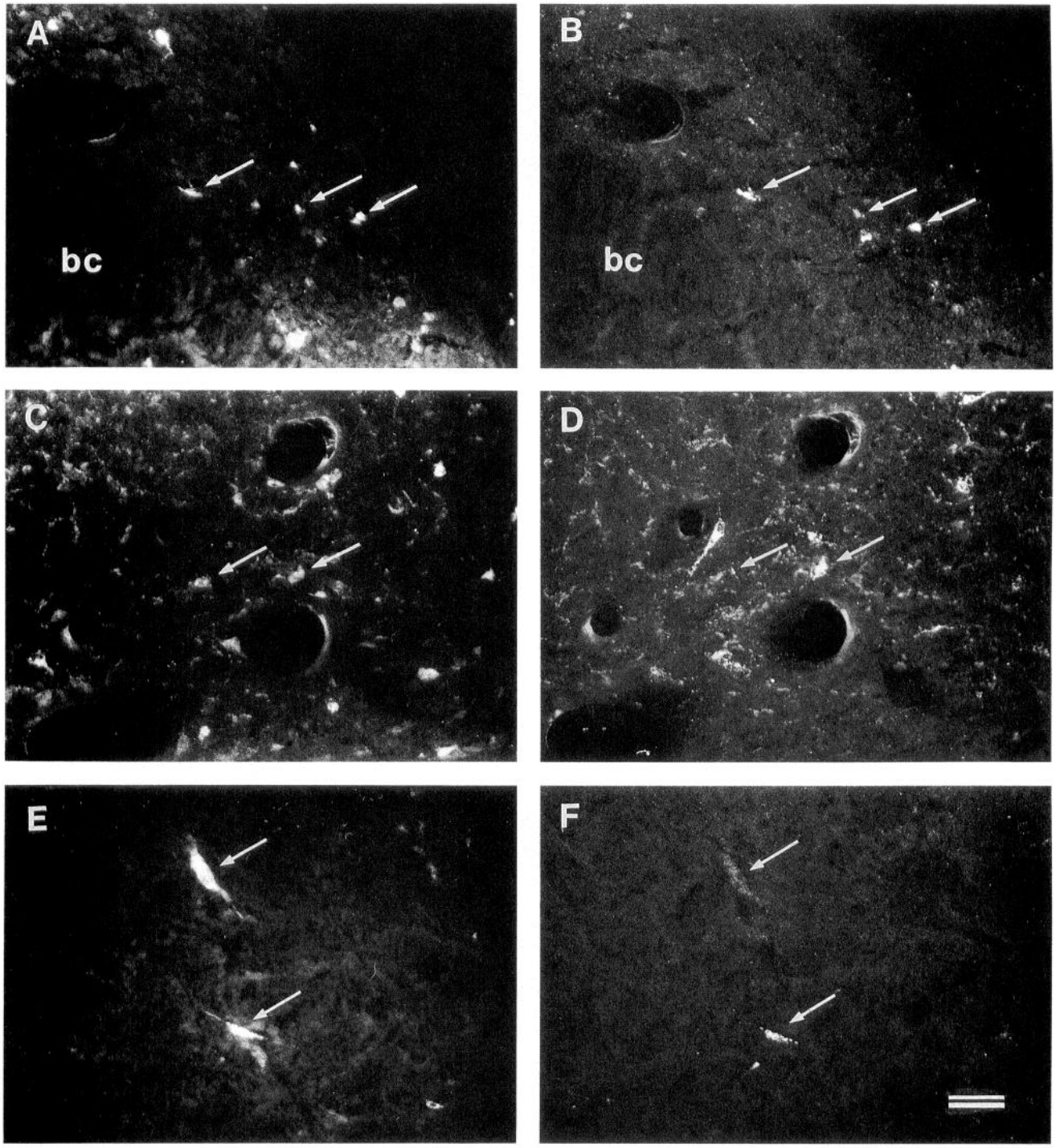

Figure 10. Photomicrographs of retrogradely labeled cells in the lateral parabrachial nucleus (*A, B*) (case OR4), the laterodorsal tegmental nucleus (*C, D*) (case OR2), and the A5 area (*E, F*) (case OR5) from injections of FG into the thalamus (*left column*) and R-Mb into the NTS (*right column*). Dually labeled cells are indicated with arrows. *bc,* brachium conjunctivum. Scale bar, 50 μm.

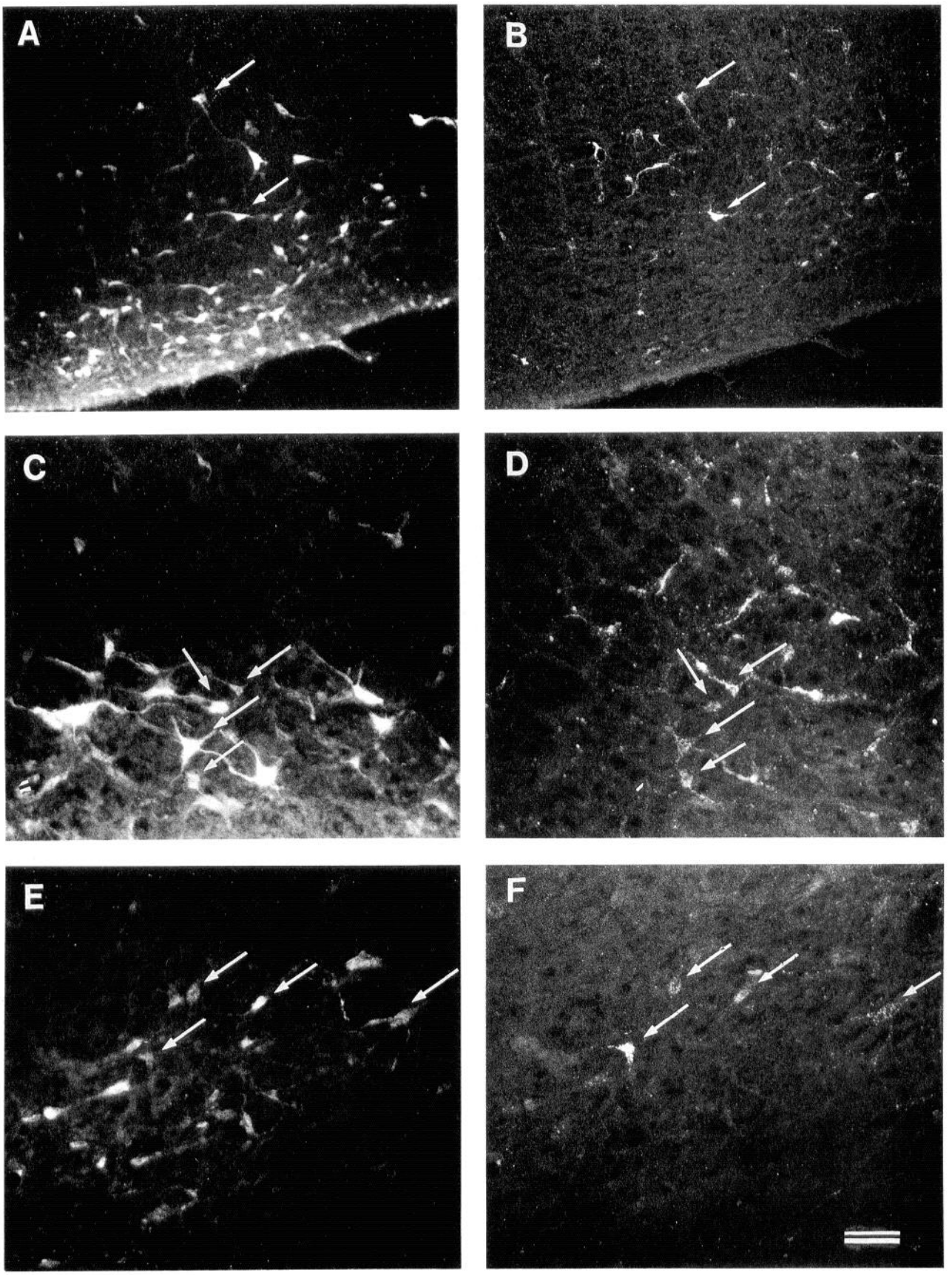

Figure 11. Photomicrographs of retrogradely labeled cells in the rostral ventrolateral reticular nucleus (*A, B*) (case OR4), the retroambigual area (*C, D*) (case OR4), and the parapyramidal area (*E, F*) (case OR5) from injections of FG into the thalamus (*left column*) and R-Mb into the NTS (*right column*). Dually labeled cells are indicated with *arrows*. Scale bar: 100 μm for *A* and *B*, 50 μm for *C–F*.

Functional considerations

Of functional importance are the neural networks through which afferents from the internal and external milieux are gated by the CNS to modify selectively forebrain and somatovisceral reflex function. The precise functions of each of the collateral projections cannot be determined from the structural data. It is conceivable that collaterals may serve as integrators by distributing signals to nuclei subserving functionally differentiated components of afferent processing and integrative reflex control. Stimuli leading to the defense/arousal response (pain, e.g.), may be distributed, in part, by the branched afferents identified here.

The significance of multiple sources of afferents to the dorsal thalamus and NTS-X arising from different levels of the neuraxis may be related to the functional specificity of each source of afferent projection. Hierarchical levels of sensory processing have been ascribed to each of the major divisions of the nervous system (for reviews, see Gebhart, 1986; Ruggiero et al., 1994).

Collaterals from the medullary reticular formation stem from an area of the lateral tegmental field (LTF) long recognized as a sensory convergent center (Valverde, 1961). The LTF has been implicated as an outlet for lower brainstem reflex circuits involved in generating sympathetic nerve discharge, respiratory patterning, and somatovisceral reflex integration (Gebber and Barman, 1985; Reis and Ruggiero, 1991; Yates, 1992). One circumscribed source of collaterals from LTF was observed in the rostral ventrolateral medulla (rVLM)—a region of reticular formation integrated in bidirectional feedback circuits with the NTS-X (cardiovascular, Ciriello and Caverson, 1986; respiratory, Ellenberger and Feldman, 1990). This observation is intriguing in view of the importance of this region in afferent convergence and somatoautonomic reflex control. Reflexes ascribed to the rVLM include nociceptive and exercise pressor responses (Iwamoto et al., 1982; Stornetta et al., 1989), the baroreceptor reflex (Granata et al., 1985), and chemoreceptor reflex control of autonomic and respiratory motor neurons (Bennaroch et al., 1986; Millhorn and Eldridge, 1986; Ruggiero et al., 1991). Interestingly, prethalamic afferents arise from sites in rVLM implicated in oxygen-conserving reflexes such as the cerebral ischemic response (Kumada et al., 1979; Guyenet and Brown, 1986) or the global cerebrovascular vasodilation to hypoxia (Underwood et al., 1986).

Collaterals to the MIT and NTS-X also derive from the laterodorsal tegmental nucleus in the mesopontine tegmentum. The functional significance of these collaterals is unknown. Perhaps related is evidence that this region, together with the pedunculopontine tegmental nucleus, has been implicated in ascending cholinergic regulation of sensory gating functions, attributed to the Ch6 and Ch5 groups, respectively (Steriade and Llinás, 1988). The Ch5 and Ch6 areas also project to the basal ganglia and are thought to play a key integrative role in extrapyramidal motor function (Woolf and Butcher, 1986; Beninato and Spencer, 1987; Rye et al., 1987). The NTS-X and rVLM are enriched in cholinergic afferents involved in central autonomic regulation (Spencer and Talman, 1986; Giuliano et al., 1989; Ruggiero et al., 1990). Collateral projections to the NTS-X and, conceivably, the aforementioned visceral relay center in ventrolateral medulla (Yasui et al., 1991) predict that neurons in the mesopontine tegmentum may also influence visceral reflex function and integrate these two levels of afferent processing. Evidence of collaterals from the periaqueductal gray may be related to functions ascribed to this region, including nociceptive control (Basbaum and Fields, 1984; Pechura and Liu, 1986) and an outlet for behavioral expression (Magoun et al., 1937; Bandler, 1982).

Forebrain afferents derive from areas of cerebral cortex and subcortical telencephalon implicated in affective processing of multimodal afferents (LeDoux, 1987, for review).

Prominent sources of collaterals are the insular and infralimbic areas of cerebral cortex. Functions ascribed to these cortical areas are related to their role in conveying olfactory, gustatory, and general visceral signals from the periphery, by way of specific sensory thalamic relay neurons or other sensory relay nuclei in brainstem, for example, the parabrachial complex (Cechetto and Saper, 1987; Allen et al., 1991). The insular cortex is thought to couple behavioral and autonomic components of emotional expression through connections with the infralimbic prefrontal cortex, dorsal thalamus, hypothalamus, and amygdala (Krushel and van der Kooy, 1988).

Collaterals to the dorsal thalamus and NTS-X also arise from areas of the amygdala or bed nuclei of the stria terminalis implicated in autonomic or behavioral components of conditioned defense or fear responses. The amygdala is thought to lie in a pivotal position by serving as a convergence center for complex multimodal affective afferent processing and an outlet for behavioral expression by the telencephalon. Amygdaloid neurons appear to trigger "fixed-action" patterns of behavior by way of anatomically divergent pathways that engage somatomotor, autonomic, neuroendocrine, and affective components of behavioral responses. It is conceivable that these branched afferents may contribute to some observed effects attributed to the amygdala or its efferent pathways. Examples include visceral responses to emotionally charged stimuli or other of the experiential phenomena associated with temporal lobe epilepsy (Hilton and Zbrozyna, 1963; Igic et al., 1970; Smith et al., 1980; Bandler, 1982; Gloor et al., 1982; Bandler and McCulloch, 1984; Mondlock and Davis, 1985; Iwata et al., 1986a,b). The lateral hypothalamus, another source of collaterals, is a mandatory diencephalic relay for cortical-sympathetic responsivity (Cechetto and Chen, 1990).

In conclusion, the collateral projections identified in this study may coordinate alterations in electrocortical activity and visceral reflex excitability associated with recurring phases of the sleep–wake cycle or modes of behavioral expression.

Appendix

Abbreviations used in figures

ac	anterior commissure
aca	anterior cingulate area
acb	nucleus accumbens
aha	amygdalohippocampal area
aid	agranular insular area, dorsal part
aip	agranular insular area, posterior part
aiv	agranular insular area, ventral part
ap	area postrema
apo	anterior preoptic nucleus of Loo
arc	arcuate nucleus
A5	A5 noradrenergic cell group
bc	brachium conjunctivum
bl	basolateral amygdaloid nucleus
ca	central amygdaloid nucleus
ci	inferior colliculus
cli	caudal linear nucleus of raphe
cm	central medial thalamic nucleus
cp	cerebral peduncle
c-p	caudate-putamen

cvl	caudal ventrolateral reticular nucleus
dg	dentate gyrus
dmh	dorsomedial hypothalamic nucleus
dtc	dorsal tegmental nucleus, central (compact) division
en	endopiriform nucleus
fr	fasciculus retroflexus
g	genu of the facial nerve
gp	globus pallidus
ila	infralimbic area
hb	medial and lateral habenular nuclei
I	lingula
III	oculomotor nucleus
imd	intermediodorsal thalamic nucleus
ip	interpeduncular nucleus
l	lateral parabrachial nucleus
la	lateral amygdaloid nucleus
ld	laterodorsal tegmental nucleus
lh	lateral hypothalamic area
lot	lateral olfactory tract
lr	lateral reticular nucleus
lsi	lateral septal nucleus, intermediate part
m	medial parabrachial nucleus
ma	medial amygdaloid nucleus
md	mediodorsal thalamic nucleus
mg	medial geniculate complex
ml	medial lemniscus
mp	magnocellular preoptic nucleus
mpn	medial preoptic nucleus
mrf	mesencephalic reticular formation
mt	mammillothalamic tract
mvn	medial vestibular nucleus
nac	nucleus ambiguus (compact formation)
ndb	nucleus of the diagonal band
nts	nucleus of the solitary tract
ot	olfactory tubercle
pag	periaqueductal gray
pc	paracentral thalamic nucleus
pf	parafascicular thalamic nucleus
pg	pontine gray
pgl	paragigantocellular reticular nucleus
ph	posterior hypothalamic area
pir	piriform cortex
po	posterior thalamic nucleus
pp	pedunculopontine tegmental nucleus
prh	perirhinal cortex
pv	paraventricular hypothalamic nucleus
pvt	paraventricular thalamic nucleus
rd	dorsal raphe nucleus
rf	rhinal fissure
rht	rhomboid thalamic nucleus
rsa	retrosplenial agranular cortex
rvl	rostral ventrolateral reticular nucleus
sm	stria medullaris
snr	substantia nigra, pars reticulata
so	superior olivary complex
sum	supramammillary nucleus
svn	superior vestibular nucleus
V	spinal trigeminal nucleus
VII	facial nucleus
VIIn	facial nerve
Vm	motor nucleus of the trigeminal nerve
vmh	ventromedial hypothalamic nucleus
Vp	principal sensory nucleus of the trigeminal nerve
vt	ventral tegmental nucleus
X	dorsal motor nucleus of the vagus
XII	hypoglossal nucleus
zi	zona incerta
1–6	cerebral cortex, layers I–VI

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