Location of cat brain stem neurons that drive sweating

Anthony D. Shafton and Robin M. McAllen
Florey Institute of Neuroscience and Mental Health and Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia

Submitted 22 January 2013; accepted in final form 1 March 2013

Shafton AD, McAllen RM. Location of cat brain stem neurons that drive sweating. Am J Physiol Regul Integr Comp Physiol 304: R804–R809, 2013. First published March 6, 2013; doi:10.1152/ajpregu.00040.2013.—The brain stem premotor pathways controlling most noncardiovascular sympathetic outflows are unknown. Here, we mapped the brain stem neurons that drive sweating, by microinjecting excitant amino acid (L-glutamate or d,l-homocysteate: 0.4–3 nmol) into 420 sites over the pons and medulla of eight chloralose-anesthetized cats (70 mg/kg iv). Sweating was recorded by the electrodermal potential at the ipsilateral forepaw pad. Responses were classified as immediate (<5 s latency) or delayed (>10 s latency). Immediate responses were obtained from 16 sites (1–3 per animal) and were accompanied by no change in blood pressure. Those sites were clustered between the facial nucleus and the pyramidal tract in the rostral ventromedial medulla (RVLM). Microinjections into 33 surrounding sites caused delayed electrodermal responses of lesser amplitude, while the remaining 371 sites evoked none. To retrogradely label bulbospinal neurons that may mediate electrodermal responses, fluorescent latex microspheres were injected into the region of the intermediolateral cell column in the fourth thoracic segment in an earlier preparatory procedure on six of the animals. A cluster of retrogradely labeled neurons was identified between the facial nucleus and the pyramidal tract. Neurons in this discrete region of the RVMM, thus, drive sweating in the cat’s paw and may do so via direct spinal projections.

The present study investigates the medullary representation of neurons that control a noncardiovascular sympathetic outflow—sweating. It extends findings from an earlier study in which sites on the ventral medullary surface were found to drive electrodermal responses in the cat’s paw (24), but their anatomical location was not defined.

METHODS

This study used eight cats and comprised two types of experiments. They were carried out in accordance with National Health and Medical Research Council guidelines and were approved by the Animal Experimentation Ethics Committee of the Florey Institute.

Acute functional mapping experiments. Cats (n = 8) were anesthetized with alpha chloralose (70 mg/kg iv) after premedication with ketamine hydrochloride (11 mg/kg im). Supplemental doses of alpha chloralose (10–20% of initial dose iv) or pentobarbital sodium (6–18 mg iv) were given if required. After surgery was complete, cats were paralyzed with bolus doses of pancuronium bromide (1 mg iv). Adequacy of anesthesia was tested by withdrawal reflexes before paralysis and at intervals when paralysis wore off. It was also monitored throughout by reference to blood pressure and the meiotic state of the pupil.

Animals were given a tracheostomy and ventilated artificially with oxygen-enriched air. End-tidal CO$_2$ levels were monitored and kept at 3.5–4% by adjusting ventilation. The right femoral artery and vein were cannulated: blood pressure was monitored from the artery, while drugs and fluids were administered through the vein. The animal was held supine in a stereotaxic frame, and the brain stem was approached ventrally (27), exposing an area extending 4–5 mm laterally from the midline, and from the antlanto-occipital membrane rostrally to the superior olives. After opening the dura, micropipettes with a shaft diameter of 1 mm and a tip of 10–20 μm were mounted in a micromanipulator and used to microinject 4–30 nl of either 0.1 M sodium glutamate or 0.1 M d,l-homocysteate into different brain stem sites to activate neuronal cell bodies but not axons (11). Injections were made by air pressure, and their volumes were measured from the meniscus by a dissecting microscope with a calibrated graticule. The pipette was inserted into different sites separated by 0.3–0.5 mm, following a grid pattern over the ventral medulla as far as permitted by surface vessels. Injections were made at several depths in each penetration. They were made on the left side of the medulla in two cats and on the right side in six.

Electrodermal responses (EDR) were recorded from the pad surface of the forepaw ipsilateral to the medullary amino acid injections. A small perspex cup embedded with an Ag/AgCl pellet electrode (Clarke Electromedical, Edendine, Kent, UK) and filled with electrode jelly was glued to the pad with cyanoacrylate cement. An Axoprobe 1A amplifier (Axon Instruments, Sunnyvale, CA) was used to monitor the DC potential of the pad relative to a chlorided silver wire inserted into the subcutaneous tissue of the limb. Any background potential was nulled. EDR were detected as rapid negative shifts in surface potential (16, 37). In seven out of eight animals, a pair of platinum wire electrodes was placed under the intact median nerve, insulated from underlying tissue with polyethylene sheet, and covered with petroleum jelly. Supramaximal electrical stimuli (two to five square wave pulses of 20–30 V, 0.5-ms width) were delivered there.

Address for reprint requests and other correspondence: Correspondence: R. M. McAllen, Florey Institute, Univ. of Melbourne, Parkville, Vic. 3010, Australia (e-mail: rmca@florey.edu.au).
shortly before brain injections to prime the sweat ducts and optimize conditions for detecting weak responses by the EDR (3, 20). Brain injections were made only after the electrodermal potential had fully returned to baseline.

At the end of the experiment, the pipette was returned to the site from which the largest immediate EDR had been evoked. Its effectiveness was retested with amino acid injection, and its location was marked by injection of 30–50 nl of a 1% solution of pontamine blue dye through another pipette placed into the same site.

**Retrograde tracing studies.** A preparatory procedure was performed on six of the eight cats 10–14 days before the terminal mapping experiment. These animals were anesthetized with pentobarbital sodium (40 mg/kg ip) and, in a sterile procedure, surgical access was gained to the dorsal root entry zone of fourth thoracic cord segment via a burr hole in the left dorsal lamina. The dura was incised, and small quantities of 2% lidocaine were applied to the cord surface to prevent reflex responses to touching the cord. A suspension of green fluorescent latex microbeads (LumaFluor, Naples, FL) (18) was then slowly injected into the region of the intermediolateral nucleus (IML; 1.5 mm below the dorsal root entry zone) through a 30-gauge stainless-steel needle attached via a polyethylene tube to a Hamilton syringe. Approximately 1 µl was injected at five to seven sites along the spinal segment. The wound was closed in layers, packing the burr hole with oxidized cellulose gauze (Surgicel; Johnson & Johnson, Melbourne, Australia). Postoperatively, animals were given antibiotic (0.6 ml im depomycin; Intervet, V.M. Supplies, Clayton, Vic. Australia) and analgesia (buprenorphine, 30 – 60 µg im). All animals recovered uneventfully.

**Perfusion fixation and histological procedures.** At the end of the terminal mapping experiment, cats were given additional pentobarbital sodium (60 mg/kg iv) and were then perfused transcardially with 1 liter of a 1:1 mixture of normal saline and 1% sodium nitrite, followed by 2 liters of buffered 4% paraformaldehyde, and ~1 h later with 300 ml of 30% sucrose in 0.1 M phosphate buffer (pH 7.2). The medulla and the spinal injection site were then removed and stored overnight at 4°C in the sucrose/buffer solution. Coronal 40-µm frozen sections were cut from these tissues, washed in 0.1 M phosphate buffer (pH 7.2), mounted in series on gelatin-coated glass slides, and coverslipped with Glycergel (DAKO, Carpinteria, CA) while the sections were still moist. Medullary sections from cats that had received no spinal tracer injection were counterstained with cresyl violet and coverslipped with DePex (BDH, Poole, UK).

Sections were viewed by fluorescence microscopy, using the filter sets for fluorescein (to show retrogradely labeled cells) or rhodamine (to show pontamine blue-marked injection sites). Labeled cells were located with reference to the major landmarks using a video camera with computer-based mapping system and associated software (Ma-gellan; P. Halasz, University of New South Wales, NSW, Australia), and mapped onto standard transverse brain stem sections taken at 1-mm intervals from the superior to inferior olives.

**RESULTS**

**Functional mapping study.** In eight chloralose-anesthetized cats, the exposed ventral medulla was systematically mapped with microinjections of 0.4–3 nmol of either L-glutamate (three cats) or D,L-homocysteate (five cats) for sites that drove EDR in the paw pad. Injections followed a grid pattern as closely as permitted by surface vessels and covered a region extending 4 mm caudally from the caudal part of the superior olive (coronal planes P6 to P10 in Berman’s atlas), and from 0.5 to 4 mm lateral to the midline. The midline was spared to avoid the basilar artery. Microinjections were made at several depths in each track, up to 4 mm deep from the medullary ventral surface, ipsilateral to the paw pad recording. Contralateral sites were not investigated.

Electrodermal responses to brain microinjections were detected as surface-negative deflections of up to 20 mV in amplitude with an early peak followed by a jagged profile that declined over a period that could last several minutes. They were classified as “immediate” (1–5-s onset latency) or “delayed” (10–40-s latency). Figure 1 shows examples of both types, recorded in the same experiment. Both were preceded by priming stimuli to the median nerve. Sixteen out of 420 injection sites produced immediate EDR, which reached peak amplitudes of 10.4 ± 2.5 mV and lasted for 0.5–7 min. They were evoked from between 1 and 3 sites per animal, and those sites were always adjacent (~0.5 mm separation). In every animal, the optimal site evoking the largest immediate EDR was retested with amino acid later in the experiment, when it resulted in an immediate EDR with an amplitude ~60% of the original response (data not shown). All immediate EDR were accompanied by an unchanging arterial pressure (Fig. 1).

Delayed EDR (as in Fig. 1, right) were evoked by amino acid microinjections at 33 sites in the surrounding region. Their peak amplitudes were 3.8 ± 1.7 mV. In 24 cases, they were accompanied by little or no change in blood pressure, while it fell by 25–50 mmHg in five cases and rose by 20–40 mmHg in four other cases. The decreases in blood pressure were from microinjections medial to the immediate EDR site(s), and the increases were from sites more caudal. As reported previously (24), large pressor responses, unaccompanied by any EDR, were evoked from the more caudolaterally located subretrofacial nucleus of the rostral ventrolateral medulla (not shown).

The sites evoking immediate and delayed EDR are plotted on the sections shown in Fig. 2. Sites evoking immediate EDR
were found clustered in the RVMM between the facial nucleus and the pyramidal tract (Fig. 2). Delayed EDR were evoked from the surrounding sites.

**Retrograde tracing study.** To identify potential premotor neurons of the sudomotor pathway, retrograde tracer (fluorescent latex beads) was injected into the region of the IML of the fourth thoracic segment (T4) in six cats. That segment contains the highest number of IML cells immunoreactive to CRF (19), a selective label for preganglionic sudomotor neurons (38). Injections included the dorsolateral funiculus, the IML, and parts of adjacent spinal laminae (Fig. 3, bottom), but were confined to the T4 segment.

Almost all retrogradely labeled cells were found in the brain stem ipsilateral to the injection (left side): contralateral neurons were not investigated. Figure 3 (top) shows a fluorescence photomicrograph of a dense cluster of retrogradely labeled neurons in the RVMM from a field ventromedial to the facial nucleus, as indicated. Labeled juxtafacial neurons were medium-sized and either spindle-shaped or multipolar. Their predominant alignment was parallel to the brain surface. The distribution of 111 neurons retrogradely labeled from T4 spinal injections is illustrated in Fig. 4.

**DISCUSSION**

Following exploration of an area encompassing the caudal pons and rostral medulla, these experiments found cell bodies that drive sweating only in a small region of RVMM, between the facial nucleus and the pyramidal tract. These experiments extend previous work that showed that sweating responses could be obtained when excitatory amino acids were injected into a restricted region of the ventral medullary surface but did not localize the spot histologically (24). They also follow an extensive exploration of the cat brain stem and diencephalon for sites where electrical stimulation drove sweating (9). The results of the three studies are anatomically consistent. Electrical stimulation activates fibers as well as cell bodies, so Davison and Koss (9) mapped a fiber tract with unknown synaptic relays. The RVMM site identified here with cell body-selective stimuli (11) falls within that tract and thus represents a discrete synaptic relay in this descending sudomotor pathway.

Asahina et al. (2) investigated the effects of electrical stimulation of the medullary raphé, which they found to cause
sweat secretion in combination with vasodilatation of the paw pad, but those workers did not explore the RVMM region identified here. Davison and Koss (9), however, evoked no sweating response when they stimulated the medullary raphé of anesthetized cats: they, as we, localized the responsive region more laterally in the RVMM. Two methodological differences in the Asahina study may explain the discrepancy. First, Asahina et al. (2) used decerebrate, unanesthetized cats; second, they used 10–20-s trains of electrical stimuli and measured prolonged sweating responses. The unanesthetized preparation is likely to be far more responsive to reflex activation, and this is all the more probable with prolonged stimulation: there was no evidence to distinguish whether the sweating responses to raphé stimulus trains were due to activating descending, ascending, or collateral pathways. Davison and Koss, however, measured discrete sweating responses that were time-locked to brief stimulus volleys (0.5–2 s) in anesthetized cats (9). In that way, they were able to map a narrow responsive tract from the hypothalamus to the spinal cord, most probably a descending pathway. Our results with cell body-specific stimuli support that interpretation. While our data cannot exclude the possibility that raphé neurons, as well as RVMM neurons, contribute to the sudomotor pathway, we think it unlikely. This is firstly because the sudomotor responses to our most medial injections were clearly in decline compared with those from the optimal site, and secondly because Davison and Koss (9), who used similar recording methods to us and whose data are fully compatible with ours, found no sudomotor response from the raphé.

Our findings additionally confirm the observation that focal activation of this RVMM cell group to drive sweating has no measurable effect on cardiovascular variables (24), showing that these presudomotor neurons are distinct from the subretrofacial (RVLM) prevasomotor cell group (8, 26). Finally, we show that many neurons in this same RVMM region send axonal projections to the IML region of the T₄ segment, where sudomotor preganglionic neurons are prevalent (37). These observations are consistent with the possibility that sympathetic premotor neurons for sweating are located in the juxtafacial region of the RVMM. Fluorescent latex beads are taken up selectively by axon terminals (18), so all retrogradely labeled cells presumably projected to the spinal segment injected. But the spinal injection sites included not only the IML but also adjacent areas, so bulbospinal neurons of unknown function could have been labeled alongside sympathetic premotor neurons.

Sweating in cats is restricted to hairless skin such as the paw pads, and its functional role is controversial. Hasama (14) produced reproducible sweating from the paw pads when the base of the hypothalamus was heated in conscious cats, indicating a thermoregulatory role. In anesthetized cats, Magoun et al. (23) sometimes found sweating from the paw pads when the anterior hypothalamus/preoptic area was heated with a thermode, but Grewe et al. (12) found none when the hypothalamus and spinal cord were heated sufficiently to cause cutaneous vasodilatation; yet vibration and noxious stimulation did cause sweating in this preparation. A role in improving grip was postulated (12). It is possible that sweating on the cat’s paw is analogous with sweating on the human hand, which is involved in both thermoregulatory and stress responses; it also serves to improve grip (41). In line with that view, recent evidence shows that both thermal and psychogenic sweating (measured at the hand) are associated with activation of a region in human rostral medulla that is homologous with the RVMM site identified here in cats (10).

Among sympathetic premotor cell groups, the RVMM has been something of an enigma. This contrasts with the well-known functional roles of the RVLM in cardiovascular control and of the medullary raphé in thermoregulation (30, 32, 42). Moderate pressor responses were obtained when caudal parts of the RVMM region were activated with glutamate in anesthetized rats, an action that was attributed to a spinal action by parapyramidal serotonergic neurons (28). Activating more rostral RVMM neurons, those near the level of the facial nucleus, had little effect on renal or tail sympathetic nerve activity or blood pressure in rats (33). Nevertheless, this same RVMM region showed abundant transsynaptic labeling with pseudorabies virus following injections into the rat stellate ganglion (17). The logical inference is that those cells were sympathetic premotor neurons with noncardiovascular functions. Rats also sweat from their paw pads (21, 34), and the sympathetic nerve supply to the forepaw traverses the stellate ganglion (1). The RVMM premotor population that projects disynaptically to the stellate ganglion could, therefore, include the neurons that drive sweating in the rat’s paw.

Fig. 4. Map showing the distribution of 111 neurons that were retrogradely labeled by fluorescent latex microspheres following injections into the T₄ spinal segment. Sections and abbreviations are the same as in Fig. 2.
Perspectives and Significance

The present experiments have localized a group of neurons in the RVMM of the cat brain stem that drive sweating without causing any cardiovascular response. This identifies an autonomic control function for RVMM neurons, and confirms that these cells are anatomically distinct from the vasomotor cell group of the RVLM (subretrofacial nucleus). Neurons in this same RVMM region send axons to the T4 spinal segment, where preganglionic sudomotor neurons are prevalent. Taken together, these results, therefore, support the hypothesis that sympathetic premotor neurons for sweating reside in the juxtafugal region of the RVMM.

The principle is established that different sympathetic functions are represented within and between the premotor cell groups of the RVLM and medullary raphé (29, 33). The present findings add a further candidate cell group in the RVMM to the same RVMM region send axons to the T4 spinal segment, within localized regions of the central nervous system. J Neurosci Methods 6: 351–363, 1982.


