Spinohypothalamic Tract Neurons in the Cervical Enlargement of Rats: Locations of Antidromically Identified Ascending Axons and Their Collateral Branches in the Contralateral Brain

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Kostarczyk, Ewa, Xijing Zhang, and Glenn J. Giesler, Jr. Spinohypothalamic tract neurons in the cervical enlargement of rats: locations of antidromically identified ascending axons and their collateral branches in the contralateral brain. J. Neurophysiol. 77: 435–451, 1997. Antidromic activation was used to determine the locations of ascending spinohypothalamic tract (SHT) axons and their collateral projections within C1, medulla, pons, midbrain, and caudal thalamus. Sixty-four neurons in the cervical enlargement were antidromically activated initially by stimulation within the contralateral hypothalamus. All but one of the examined SHT neurons responded either preferentially or specifically to noxious mechanical stimuli. A total of 239 low-threshold points was classified as originating from 64 ascending (or parent) SHT axons. Within C1, 38 ascending SHT axons were antidromically activated. These were located primarily in the dorsal half of the lateral funiculus. Within the medulla, the 29 examined ascending SHT axons were located ventrolaterally, within or adjacent to the lateral reticular nucleus or nucleus ambiguus. Within the pons, the 25 examined ascending SHT axons were located primarily surrounding the facial nucleus and the superior olivary complex. Within the caudal midbrain, 23 examined ascending SHT axons coursed dorsally in a position adjacent to the lateral lemniscus. Within the anterior midbrain, SHT axons traveled rostrally near the brachium of the inferior colliculus. Within the posterior thalamus, all 17 examined SHT axons coursed rostrally through the posterior nucleus of thalamus. A total of 114 low-threshold points was classified as collateral branch points. Sixteen collateral branches were seen in C1; these were located primarily in the deep dorsal horn. Forty-five collateral branches were located in the medulla. These were primarily in or near the medullary reticular nucleus, nucleus ambiguus, lateral reticular nucleus, parvocellular reticular nucleus, gigantocellular reticular nucleus, cuneate nucleus, and the nucleus of the solitary tract. Twenty-six collateral branches from SHT axons were located in the pons. These were in the pontine reticular nucleus caudalis, gigantocellular reticular nucleus, parvocellular reticular nucleus, and superior olivary complex. Twenty-three collateral branches were located in the midbrain. These were in or near the mesencephalic reticular nucleus, brachium of the inferior colliculus, cuneiform nucleus, superior colliculus, central gray, and substantia nigra. In the caudal thalamus, two branches were in the posterior thalamic nucleus and two were in the medial geniculate. These results indicate that SHT axons ascend toward the hypothalamus in a clearly circumscribed projection in the lateral brain stem and posterior thalamus. In addition, large numbers of collaterals from SHT axons appear to project to a variety of targets in C1, the medulla, pons, midbrain, and caudal thalamus. Through its widespread collateral projections, the SHT appears to be capable of providing nociceptive input to many areas that are involved in the production of multifaceted responses to noxious stimuli.

INTRODUCTION

Nociceptive input from the spinal cord to the hypothalamus has been traditionally thought to be transmitted exclu-
rior thalamus, where they could no longer be detected. Recently, Newman et al. (1996) have shown that injections of biotin dextran into the spinal cords of monkeys anterogradely label large numbers of fibers and terminals in many of the areas of the hypothalamus in which labeled SHT fibers were seen in the comparable studies in rats. These findings suggest that a substantial SHT exists in primates and that the course and areas of termination of SHT in primates are strikingly similar to those reported in rats.

Physiological studies in the cervical (Dado et al. 1994b) and lumbosacral enlargements (Burstein et al. 1987, 1991a) have shown that the majority of SHT neurons are activated strongly by noxious mechanical and thermal stimuli. In addition, Katter et al. (1996a,b) have recently shown that SHT neurons in sacral segments are activated by noxious stimulation of visceral as well as cutaneous structures. These findings indicate that SHT neurons at all levels of the spinal cord and spinal trigeminal nucleus (Burstein et al. 1991b) are powerfully activated by noxious stimuli. They also suggest that the transmission of nociceptive information appears to be a prominent function of the SHT.

Previously, we used the method of antidromic activation to determine the locations of SHT axons within the diencephalon and spinal cord (Burstein et al. 1987, 1991a; Dado et al. 1994a,c). We also used these methods recently to examine the locations of descending SHT axons within the ipsilateral brain and their sites of apparent termination (Zhang et al. 1995). In the present study we used these methods to determine, for the first time, the location of SHT axons as they ascend toward the hypothalamus in the contralateral brain. In addition, we attempted to determine, also for the first time, whether ascending nociceptive SHT axons give rise to collateral branches in the brain stem and, if so, to what areas such branches are likely to project.

METHODS

The methods used in this study have been described in detail previously (Dado et al. 1994a; Zhang et al. 1995). Adult male rats were anesthetized with urethan (1.3 g/kg), paralyzed with gallamine triethiodide, and artificially ventilated. End-tidal CO₂ and core temperature were maintained at normal physiological levels. Rats were placed in a stereotaxic frame, and laminectomies of the cervical enlargement. Much of the dorsal surface of the cranium was removed to allow insertion of stimulating electrodes throughout the brain. A stainless steel stimulating electrode was lowered into the area of the subdivision, where SHT axons are concentrated as they enter the hypothalamus (Burstein et al. 1991a; Cliffer et al. 1991; Dado et al. 1994a). Cathodal current pulses (500 μA, 200 μs) delivered through the tip of this electrode served as the search stimulus. Stainless steel microelectrodes were moved through the dorsal horn of the cervical enlargement contralateral to the stimulating electrode until an antidromically activated unit was isolated. The stimulating electrode was then moved from the dorsal to the ventral surface and antidromic thresholds were determined at 200-μm intervals. This procedure was repeated in different tracks in the same anterior-posterior plane until a point was located at which the antidromic threshold was ≤30 μA (such points are referred to as low-threshold points). Current pulses ≤30 μA have been shown to activate SHT axons at a distance of ≤400 μm from the stimulating electrode (Burstein et al. 1991a; Dado et al. 1994a). The criteria for antidromic activation (Lipski 1981) were constant latency of responses, ability to follow pulses delivered at high frequencies (≥333 Hz), and collision of presumed antidromic with orthodromic action potentials. After identification of an antidromically activated unit, a second stimulating electrode was inserted into the lateral funiculus of C1 on the side contralateral to the recording site. This electrode was lowered through one or more tracks (separated by 300–500 μm) in the same anterior-posterior plane until the examined unit could be antidromically activated with the use of pulses ≤30 μA. Collision of action potentials generated from the electrode in the hypothalamus and from the other in C1 was demonstrated to ensure that the action potentials generated at the two locations traveled within the same axon. In most experiments, the stimulating electrode in the hypothalamus was then withdrawn and inserted into either the posterior thalamus, midbrain, pons, or medulla, and the procedure of determining antidromic thresholds was repeated throughout multiple tracks across the medial-lateral extent of the contralateral brain. In several experiments the stimulating electrode in the hypothalamus was left at the low-threshold point and the electrode in C1 was moved into the medulla, pons, or midbrain. Collision between antidromic responses elicited at each low-threshold point in the brain with action potentials generated from either the electrode in C1 or the hypothalamus was demonstrated. Planes of stimulating tracks were separated by ≥1 mm in the anterior-posterior dimension. Antidromic latencies were measured to the nearest ±0.05 ms. Antidromic action potentials were amplified, filtered (0.1–5 kHz), sent to a computer, digitized (sampling rate 80 kHz), and stored for subsequent analysis.

Two or more low-threshold points with different antidromic latencies were frequently encountered in the same anterior-posterior level. One reason that this occurred might be that the ascending axon and one or more of its collateral branches were antidromically activated at the same level. In such cases, the point with the shortest latency was assumed to indicate the location of the ascending axon activated in passage (Lipski 1981; McMahon and Wall 1985). Low-threshold points with longer latencies at the same anterior-posterior plane were thought to reflect the position of collateral branches from the parent axon (Lipski 1981; McMahon and Wall 1985), if the latency at such points was greater than that of the point classified as the parent axon at the adjacent anterior level. Collateral branches are generally smaller in diameter and have lower conduction velocities than their parent axons (Fields et al. 1996; Lipski 1981; McMahon and Wall 1985; Shinoda et al. 1982a,b, 1986). In this study, the mean conduction velocity of the ascending axons was roughly 8 times faster than that to points classified as collateral branches (see below), supporting the idea that such points were appropriately classified. Another reason that more than one low-threshold point was found at a single anterior-posterior plane might be that an ascending axon coursed dorsolaterally or mediolaterally within the same anterior-posterior plane. We attempted to identify axons that may have shifted their location within a single anterior-posterior plane. Low-threshold points were so identified if 1) more than one was located in a single anterior-posterior plane, 2) each point had an antidromic latency that was greater than that of the ‘‘ascending axon’’ at the adjacent caudal level, and 3) each point had an antidromic latency that was less than that of the ascending axon at the adjacent rostral level (see Fig. 4, points labeled i–k). As shown below, 16 low-threshold points met these criteria. Fourteen were located in the rostral pons or caudal midbrain, in the area where anatomic studies indicate that ascending spinal axons shift their position from near the ventral surface of the brain to a more dorsal position (Mehler 1969; Zemlan et al. 1978). Low-threshold points that did not meet the criteria to be classified as ascending axons or collateral branches were designated as ‘‘not classified.’’

Conduction velocities of the presumed collateral branches were estimated. The conduction time in the collateral branch was determined by subtracting the antidromic latency of the parent axon at the anterior-posterior level of the branch point from the latency at the branch point. The conduction distance of the branches was
estimated by calculating the minimum distance between the location of the parent axon and the location of the branch point. The responses of examined neurons to innocuous and noxious mechanical stimulation of the forelimb were determined (see Dado et al. 1994b). SHT neurons were classified as high threshold (HT) if they responded only to noxious stimuli and wide dynamic range (WDR) if they responded to innocuous mechanical stimuli but at higher frequencies to noxious mechanical stimuli. SHT neurons that responded at highest frequencies to innocuous mechanical stimuli were classified as low-threshold (LT) neurons. The boundaries of the receptive fields were determined with the use of the most effective stimulus (generally pinching). At the end of each experiment, the recording site and each low-threshold point was marked with anodal current (25 μA, 10–40 s). Rats were perfused with 0.9% saline followed by 10% Formalin containing 1% potassium ferrocyanide. Transverse sections were counterstained and reconstructed with the use of a microscope equipped with a camera lucida drawing tube. The atlas of Paxinos and Watson (1986) was used to aid in identifying structures in the brain.

Results

Sixty-four neurons were antidromically activated from low-threshold points (≈30 μA) in the contralateral hypothalamus. Figure 1 shows an example of antidromic activation of an SHT neuron. The low-threshold point (15 μA) from which the neuron was antidromically activated was located in the SoD of the contralateral hypothalamus (Fig. 1, top left). The antidromic latency at this point was 2.15 ms (a1). Antidromic activation occurred at a constant latency (a1), followed trains of high-frequency pulses (a2), and collided with orthodromic action potentials (a3) and with antidromic action potentials elicited with the use of a second stimulating electrode in the upper cervical cord (a4 and a5). This SHT neuron was also antidromically activated at 10 levels in the contralateral brain stem. Each low-threshold point was surrounded medially, laterally, ventrally, and dorsally by points at which considerably more current was required to produce antidromic activation. Figure 1, bottom, depicts antidromic activation at two low-threshold points in the medulla. The antidromic latency from the point labeled b was 1.00 ms and that from the point labeled c was 1.35 ms. On the basis of the criteria listed in METHODS, point b was considered to represent the location of the ascending parent SHT axon and point c was considered to represent the location of a collateral branch from the SHT axon. Figure 1, middle, illustrates antidromic activation at one level in the rostral pons in which a single low-threshold point (labeled d, 5 μA) was located in the lateral lemniscus. The antidromic latency was 1.4 ms. Antidromic action potentials elicited from points b–d collided with antidromic action potentials elicited from the second stimulating electrode in the upper cervical cord (Fig. 1, b2 and b3, c2–c4, and d2–d4).

Recording sites

An example of a lesion at a recording site in the DDH is shown in Fig. 2A. The locations of the lesions of the recording sites of 60 SHT neurons are illustrated in Fig. 3. Lesions were not recovered in four cases. Fourteen (23%) neurons were recorded in the superficial dorsal horn; 46 (77%) were in the DDH. Fifty recording sites (78%) were located in segment C7, 13 (20%) in C8, and 1 (2%) at the border of C7 and C8.

Physiological characteristics

Twenty-nine (45%) SHT neurons were physiologically characterized. Thirteen (45%) were classified as HT neurons, 15 (52%) as WDR neurons, and 1 (3%) as an LT neuron. The lesions of 11 HT neurons were recovered; all were in the DDH. Twelve WDR neurons were recorded in the DDH and three were in the superficial dorsal horn (Fig. 3). The LT neuron was recorded in the DDH.

Three examined SHT neurons (10%) had excitatory receptive fields that covered an area of less than three toes, 18 (62%) had receptive fields that were larger but were restricted to the ipsilateral forepaw, two (7%) receptive fields extended over parts of the forepaw and limb, one (4%) covered parts of the ipsilateral forelimb and chest, and five (17%) covered the entire ipsilateral forelimb. The percentages of WDR and HT neurons and types of receptive fields were similar to those encountered in previous studies of SHT neurons in the cervical enlargement of rats (Dado et al. 1994b; Zhang et al. 1995).

Antidromic activation from the contralateral hypothalamus

A photomicrograph of an example of a lesion at an initial low-threshold point in the contralateral hypothalamus is presented in Fig. 2B. All 64 lesions at initial low-threshold points were recovered in the contralateral hypothalamus. The majority (49) was located in the SoD; others were in the optic tract (6), optic chiasm (3), lateral hypothalamus (5), and ventromedial hypothalamic nucleus (1).

Low-threshold points classified as ascending SHT axons

A total of 239 low-threshold points were classified as originating from ascending (or parent) SHT axons. Figure 4 illustrates antidromic activation of an apparent ascending SHT parent axon from low-threshold points at 13 anterior-posterior levels of the brain. In this case, no evidence was found for the presence of collateral branches from the ascending axon. Initially, the SHT neuron was antidromically activated from the SoD in the lateral hypothalamus (point a, latency = 4.70 ms). This SHT neuron was activated from four low-threshold points (points b–e; latencies = 1.60, 1.65, 1.70, and 1.80 ms) in the ventrolateral medulla. These points were located near the lateral reticular nucleus caudally and near nucleus ambiguous rostrally. The axon was also activated from ventrolateral positions within the pons (points f–i; latencies = 1.90, 2.10, 2.15, and 2.35 ms), near the facial nucleus caudally, and adjacent to or within the lateral lemniscus rostrally. The SHT axon was activated from increasingly dorsal locations in the rostral pons and caudal midbrain (points j–l; latencies = 2.50, 2.50, and 2.60 ms). In the rostral midbrain, the SHT axon was located near the brachium of the inferior colliculus (point m, latency = 2.75 ms). In the posterior thalamus the SHT axon was activated in the posterior nucleus of thalamus (points n and o, latencies = 2.90 and 3.30 ms). The unit was classified as a WDR
An example of a lesion at a low-threshold point classified as an ascending axon in the dorsal lateral funiculus in C1, is shown in Fig. 2C. Thirty-eight SHT neurons were antidromically activated from 45 low-threshold points in C1 (Fig. 5). The locations of the axons of 38 neurons were determined at one level of C1 and the locations of 7 axons were determined at two levels. With the exception of a small number of points within the ventral lateral funiculus in caudal segment C1, SHT axons ascended within the dorsal half of the lateral funiculus. In a previous study (Dado et al. 1994c), we found that in midcervical segments fewer than one third of SHT axons were located in the dorsal lateral funiculus. The remainder were in the ventral lateral funiculus. However, many SHT axons appeared to shift their locations such that within C2, 74% of all examined SHT axons were located in the dorsal lateral funiculus. The present findings indicate that the shifting of SHT axons into the dorsal lateral funiculus continues in C1 such that virtually all SHT axons are located in the dorsal lateral funiculus in rostral C1.

Figure 2, D–F, illustrates examples of lesions at the low-threshold points of ascending axons in the medulla, pons, and midbrain. The locations of 29 SHT axons were determined at 73 low-threshold points in the medulla (Fig. 6, –14.6 through –11.8). The locations of the axons of nine neurons were determined at one level of the medulla, the locations of five axons were determined at two levels, eight axons were examined at three levels, five axons at four levels, and two axons at five levels. Ascending parent SHT axons were found ventrolaterally within or adjacent to the lateral reticular nucleus, lateral paragigantocellular nucleus, and nucleus ambiguus. In the pons, the locations of 25 ascending SHT axons were determined at 59 low-threshold points (Fig. 6, –10.5 through –8.3, ventral half). The locations of the axons of six neurons were determined at one level of the pons, the locations of eight axons were determined at two levels, seven axons were examined at three levels, and four axons at four levels. Ascending SHT axons in the pons were located primarily within or adjacent to the facial nucleus, superior olivary complex, and lateral lemniscus. The locations of 22 SHT axons were determined at 42 low-threshold points in the midbrain (Fig. 6, –8.3, dorsal half, through –5.6). The locations of the axons of eight neurons were determined at one level of the midbrain, the locations of 11 axons were determined at two levels, and 4 axons were examined at three levels. In the midbrain, the bulk of ascending axons was located laterally in or near the brachium of the inferior colliculus and the lateral part of the mesencephalic reticular nucleus. The locations of 17 ascending SHT axons were determined at 20 low-threshold points in the caudal thalamus (Fig. 6, –5.6). The locations of the axons of 14 neurons were determined at one level of the caudal thalamus, and the location of three axons were determined at two levels. Ascending axons were located in the medial part of the medial geniculate or in the posterior thalamic nucleus.

Low-threshold points classified as collateral branches of ascending SHT parent axons

A total of 114 low-threshold points were classified as collateral branches (Fig. 6). These points originated from 37 SHT parent axons that were examined at 185 anterior-posterior levels of the brain. The mean number of levels examined for each of these SHT axons was 5.1. No evidence was seen for collateral branches from 27 examined SHT neurons. However, these SHT axons were only examined at 51 levels of the brain. The mean number of levels examined for each of these 27 SHT axons was 1.8. In this group, only three SHT axons were examined at three or more levels of the brain. Therefore, because the number of levels of the brain that were examined in this group was small, it is uncertain how many axons in this group did or did not give rise to collateral branches.

An example of antidromic activation of an SHT neuron through both its parent axon and presumed collateral branches in C1 and the medulla is shown in Fig. 7. This neuron was initially antidromically activated in the SoD of the lateral hypothalamus at a latency of 2.05 ms (Fig. 7E). It was also activated through its apparent parent axon (point b; latency = 1.05 ms) in the dorsal half of the lateral funiculus (Fig. 7F) in C1. It was also activated from three points (c–e; latencies = 1.25, 1.30, and 1.45 ms) in the adjacent DDH. Note that the antidromic latencies at the presumed...
FIG. 2. Photomicrographs of lesions marking recording sites and low-threshold points. A: lesion marking recording site in the deep dorsal horn (DDH) in C7. B–F: lesions marking low-threshold points classified as parent axons (filled arrows) in the contralateral hypothalamus (B), C1 (C), medulla (D), pons (E), and midbrain (F). Open arrows in D: low-threshold points classified as collateral branches. Scale bar in A: 0.5 mm. Scale bar for B and C (in B): 0.5 mm. Scale bar for D–F (in F): 1 mm. BIC, brachium inferior colliculus; DLF, dorsal lateral funiculus; DSCP, decussation of the superior cerebellar peduncle; GR, gigantocellular reticular nucleus; G7, genu of facial nerve; IO, inferior olive; MR, median raphe nucleus; PCR, parvocellular reticular nucleus; PVN, paraventricular hypothalamic nucleus; RM, raphe magnus; RN, red nucleus; SDH, superficial dorsal horn; SpTV, spinal tract of the trigeminal nerve; SpVL, spinal trigeminal nucleus interpolaris; SpVO, spinal trigeminal nucleus oralis; VH, ventral horn; VLF, ventral lateral funiculus; VN, vestibular nucleus; VII, facial nucleus.
collateral branch points were considerably longer than those of the presumed parent axon at levels several millimeters rostrally. The locations and latencies of these latter points suggest that one or more collateral branches emanated from the SHT axon within the dorsal lateral funiculus and entered the dorsal horn. The SHT axon was activated in the ventral and lateral reticular formation in the posterior medulla (point f, latency = 1.10 ms). At this level also, the SHT axon apparently gave rise to a collateral branch (point g, latency = 1.40 ms). The SHT axon was also antidromically activated from low-threshold points at three more anterior levels of the medulla (Fig. 7F, points h–j; latencies = 1.15, 1.20, and 1.25 ms). Each of these latter points was located ventrally and laterally, within or adjacent to the lateral reticular nucleus, gigantocellular reticular nucleus, or nucleus ambiguus. No evidence for branches from this axon was found at these more rostral levels of the medulla. The neuron was recorded in the DDH, at the dorsal border of the lateral reticulated area (Fig. 7C), and was classified as a WDR neuron (Fig. 7D).

Figure 8 illustrates a different example of the path of an ascending SHT axon and its presumed collateral branches, in this case within the pons, midbrain, and posterior thalamus. This neuron was antidromically activated initially from the contralateral SoD (Fig. 8E, point a, latency = 2.00 ms). In the rostral pons, the neuron was activated via its presumed parent axon (Fig. 8F, point b, latency = 1.20 ms) and from an apparent branch (point c, latency = 1.45 ms) located medially adjacent to the ascending axon. In the caudal midbrain, the axon was also activated from what was likely its parent axon (point d, latency = 1.35 ms) and from a point classified as a daughter branch (point e, latency = 1.55 ms). The ascending axon was also located laterally in the rostral midbrain near the brachium of the inferior colliculus (point f, latency = 1.40 ms) and in the midbrain reticular nucleus (point g, latency = 1.55 ms). The parent axon continued rostrally, passing through the posterior nucleus (point h, latency = 1.60 ms), where it appeared to give rise to a branch in posterior thalamic nucleus, near the lateral border of the anterior pretectal nucleus (point i, latency = 2.40 ms). The neuron was recorded in the DDH (Fig. 8C) and was classified as a WDR neuron (Fig. 8D).

The locations of 16 low-threshold points that were classified as collateral branches within C1 are shown in Fig. 5. Fourteen were located in or near the lateral reticulated area of the DDH; two branches were found in the dorsal lateral funiculus.

Figure 2D illustrates an example of lesions at the sites (open arrows) of two low-threshold points that were classified as collateral branches in the medulla. Forty-five low-threshold points that were classified as originating from collateral branches were found in the medulla (Fig. 6, –14.6 through –11.80). Fourteen low-threshold points were found in the medullary reticular nucleus caudally. Twelve low-threshold points were found within the reticular formation surrounding nucleus ambiguus. Seven were in or near the lateral reticular nucleus. Other low-threshold points that appeared to indicate the presence of collateral branches were found in parvocellular reticular nucleus (4), cuneate nucleus (3), nucleus of the solitary tract (3), and gigantocellular reticular nucleus (2).

In the pons, 26 low-threshold points were found that were classified as collateral branches (Fig. 6, –10.5 through –8.3 ventral half). These were located mainly in pontine reticular nucleus caudalis (8). Others were found in gigantocellular reticular nucleus (6), parvocellular reticular nucleus (4), superior olivary complex (4), pontine reticular nucleus oralis (1), lateral lemniscus (1), trigeminal nucleus oralis (1), and medial parabrachial nucleus (1).

Twenty-three collateral branches were found in the midbrain (Fig. 6, –8.3, dorsal part, through –5.6). These were located primarily in the mesencephalic reticular nucleus (11), brachium inferior colliculus (4), cuneiform nucleus (2), intermediate layers of the superior colliculus (2), central gray (2), and substantia nigra (2).

In the caudal posterior thalamus, four SHT axons appeared to give rise to four collateral branches (Fig. 6, –5.6). Two were located in the posterior thalamic nucleus and two in the medial geniculate nucleus.

Sixteen of the SHT neurons that appeared to give rise to collateral branches were physiologically classified; seven were HT and nine were WDR neurons. The locations of 60 low-threshold points representing presumptive branches from physiologically classified SHT neurons are illustrated in Fig. 9. Forty anterior-posterior planes were examined for possible branches from HT neurons; 34 were found. Fifty-one different anterior-posterior planes were examined for possible branches from WDR neurons; 26 were found. The ratios of branches detected to planes searched was significantly greater for axons classified as HT than for those classified as WDR ($\chi^2$, $P < 0.002$). The mean number of low-threshold points representing branches was 4.9 for each HT
neuron and 2.9 for each WDR neuron. These findings indicate that SHT cells classified as HT may give rise to more collateral branches than those classified as WDR.

SHT neurons with axons that appeared to give rise to collaterals were recorded throughout the dorsal horn. The distribution of such neurons was not obviously different from that of those SHT neurons for which branches could not be found.

Conduction velocities

The mean conduction velocity of the 64 examined SHT axons from the initial low-threshold points in the contralateral hypothalamus to the recording points in the cervical enlargement was 16.7 ± 0.7 (SE) m/s (Fig. 10). The mean conduction velocity for ascending SHT axons classified as WDR was 17.3 ± 1.3 m/s and 15.9 ± 1.4 m/s for HT axons; these conduction velocities did not differ significantly (Student’s t-test, P > 0.1). The mean conduction velocity of collateral branches in C1 was 2.1 ± 0.4 m/s, in the medulla it was 2.3 ± 0.2 m/s, in the pons it was 2.4 ± 0.3 m/s, in the midbrain it was 2.2 ± 0.3 m/s, and in the posterior thalamus it was 1.0 ± 0.2 m/s. The range of conduction velocities for all collateral branches was 0.2–6.9 m/s. The conduction velocity of 60 (53%) branches was calculated to be <2 m/s, indicating that these were likely to be unmyelinated axons. The mean conduction velocity of ascending SHT axons was roughly 8 times that of the mean conduction velocity from the parent axon to sites classified as collateral branches (Fig. 10), a significant difference (P < 0.0001, Student’s t-test). The mean conduction velocities of branches at the various examined levels did not differ from each other significantly (P > 0.05). The mean conduction velocities of branches classified as HT and WDR were 2.6 ± 0.3 m/s and 2.6 ± 0.4 m/s (not significantly different).

Discussion

Technical considerations

Throughout the last 100 years, the locations of ascending spinal axons in the brain have been studied many times with the use of anatomic techniques. Such studies have provided a detailed picture of ascending spinal axons at many levels of the brain in a variety of species (Hazlett et al. 1972; Le Gros Clark 1936; Lund and Webster 1967; Mehler 1969; Mehler et al. 1960; Mott 1895; Weaver and Walker 1941; Yamada and Otani 1977; Zemlan et al. 1978) including humans (Bowsher 1957; Gardner and Cuneo 1945; Rasmus-sen and Peyton 1941; Walker 1940). Such studies serve as the bases for much of the current understanding of the organization of ascending spinal axons, including spinothalamic tract and many other types of axons. However, we believe that the precise identity of labeled or degenerating fibers in these studies is not always entirely clear. In fact, it is almost impossible to determine whether individual labeled or degenerating fibers in the medulla ultimately reach the level of the thalamus or terminate in, for example, the pons or midbrain. This shortcoming makes it all but impossible to identify unequivocally individual spinothalamic tract fibers and branches at any level below the thalamus with the use of conventional anatomic methods.

In this study, the method of antidromic activation was used to identify each examined neuron by initially antidromically activating it from a low-threshold point in the hypothalamus. Attempts were then made to establish the position of the ascending axon and collateral branches of the same SHT neuron at one or more levels of the brain. An advantage of this approach over anatomic methods is that it allows the response properties of the examined axons to be determined. This method has been used previously to locate ascending SHT axons in the spinal cord (Burstein et al. 1991a; Dado et al. 1994c), diencephalon (Burstein et al. 1991a; Dado et al. 1994a; Zhang et al. 1995), and descending SHT axons in the midbrain, pons, and medulla (Zhang et al. 1995).

In a few previous studies, antidromic activation has been used to determine the locations of a small number of apparent branches from spinothalamic tract axons (Fields et al. 1977; Giesler et al. 1981; Price et al. 1978). McMahon and Wall (1985) used antidromic activation to examine ascending axons of neurons in the marginal zone that projected to the midbrain. Collateral branches were found from these axons within the parvocellular reticular and cuneate nuclei, dorsal to locus coeruleus, the cuneiform nucleus, and the central gray.

In the course of this study, two or more low-threshold points for antidromic activation were frequently encountered within the same anterior-posterior plane. These low-threshold points often had differing antidromic latencies. We have used criteria that we believe help indicate whether such points reflect the presence of parent axons or collateral branches. For example, at a plane having several low-thresh-

FIG. 4. Example of the course of an SHT axon in the contralateral medulla, pons, midbrain, and posterior thalamus. A: representation of a dorsal view of the area of the brain outlined by the box in the diagram in B. Penetrations with a stimulating electrode were made at multiple anterior-posterior levels in the contralateral brain. The minimum antidromic threshold in each penetration is represented by a symbol (inset). C: location of the recording site in C. D: responses of this neuron to cutaneous stimuli. E: locations of lesions made at low-threshold points a–o and antidromic action potentials elicited at each low-threshold point. The axon was activated at progressively longer latencies at each level in the contralateral brain stem (points b–o). Note that this SHT axon was examined at 1-mm intervals from the caudal medulla to the posterior thalamus. In the medulla the parent axon was located near the lateral reticular nucleus. Note also that at the level of the junction of the pons and midbrain (~9 from the bregma), there were 3 low-threshold points in the lateral lemniscus. The latencies from ventral point i to dorsal point k increased, suggesting that the axon shifted dorsally in the lateral lemniscus and then ascended to the brachium of inferior colliculus in the midbrain. The parent axon ascended through the posterior nucleus of thalamus. AH, anterior hypothalamus area; APT, anterior pretectal nucleus; AQ, aqueduct; CN, cuneiform nucleus; CP, cerebral peduncle; CST, corticospinal tract; DLG, dorsal lateral geniculate nucleus; FR, fasciculus retroflexus; GN, gracile nucleus; IC, internal capsule; InC, inferior colliculus; LP, lateral posterior thalamic nucleus; MG, medial geniculate nucleus; ML, medial lemniscus; MT, mammillothalamic tract; NTB, nucleus of the trapezoid body; PC, posterior commissure; PN, pontine nucleus; PRNC, pontine reticular nucleus caudalis; PO, posterior thalamic nucleus; PSV, trigeminal sensory nucleus principalis; RT, reticular thalamic nucleus; SNC, substantia nigra, compact part; SNR, substantia nigra, reticular part; SOC, superior olivary complex; V, trigeminal motor nucleus; VGC, ventrobasal complex; VMH, ventromedial hypothalamic nucleus; ZI, zona incerta; 7N, facial nerve.
of these cases occurred in the anterior pons and posterior midbrain, where ascending spinal axons are known to shift position from near the ventral surface of the brain stem to a more dorsal position near the brachium of the inferior colliculus (Mehler 1969; Zemlan et al. 1978). Therefore our criteria for classifying multiple low-threshold points reflecting the position of the same parent axon in one anterior-posterior plane appear to be reasonably effective. However, it should also be pointed out these criteria are not unequivocal. For example, a short-length daughter branch with a conduction velocity that was similar to that of the parent axon could have been misclassified as a parent axon. We believe that the criteria we used are logical, and that they help make reasonable interpretations of these complex results. In addition, the interpretations that they produce fit with many of the known anatomic arrangements of ascending spinal axons. However, these interpretations should be considered tentative; it will be important to attempt to evaluate their accuracy with the use of other techniques including intra-axonal dye filling methods.

Although our results indicate that a large number of collateral branches leave the SHT in a number of areas extending from C1 to the posterior thalamus, antidromic methods often do not provide an accurate picture of the total number of branches that emanate from a parent axon. Antidromic activation has been used to examine a number of projection systems in the brain and spinal cord, including cortico-, vestibulo-, and rubrospinal projections (Abzug et al. 1974; Shinoda et al. 1976, 1977). Intra-axonal injections of horseradish peroxidase have also been used to reexamine these same systems (Shinoda et al. 1982a,b, 1986). Comparison of the results of these two methods indicates that the antidromic activation method can reveal the course of parents axons and at least some of the daughter branches that emanate from them. However, such comparisons also indicate that antidromic activation provides only an incomplete picture and that large numbers of daughter branches, particularly those that either have a small diameter or a short length, are often not revealed. Therefore, although we have seen many examples of what were apparently branches from SHT axons, we may have been unable to detect many other branches from SHT axons. For this reason also, it would be useful to label individual ascending SHT axons intra-axonally.

Locations of presumed parent SHT axons and collateral branches from them

Within the medulla, ascending parent SHT axons were located ventrolaterally, in or near the lateral reticular nucleus and nucleus ambiguus. Within the medulla, evidence was found for the presence of 45 branches from SHT axons. Nearly 90% of these low-threshold points were located in one of the nuclei that comprise the medullary reticular formation. These included the medullary reticular nucleus, the reticular formation surrounding nucleus ambiguus, the lateral reticular nucleus, and the parvocellular and gigantocellular reticular nuclei. Smaller numbers of branches were found in the cuneate nucleus and the solitary nucleus. Our findings suggest that large numbers of branches emanate from SHT axons in the medulla and that their primary target is the nuclei of the medullary reticular formation.

Anatomic studies have described the course of ascending
FIG. 6. Summary of the locations of lesions in the contralateral medulla, pons, and midbrain marking 313 low-threshold points for antidromic activation of 54 SHT neurons. The distance from bregma of each plane is indicated to the right of each section. Note that parent SHT axons in the medulla were located in and around the lateral reticular nucleus. In the pons, axons were concentrated in or around the facial nucleus and superior olivary complex, then shifted dorsally in the lateral lemniscus to the area medial to the brachium of inferior colliculus in the midbrain. At the junction of the midbrain and the thalamus, parent SHT axons ascended through the posterior nucleus of thalamus. Note also that evidence for many branches from SHT axons was found throughout the brain stem, particularly within the medulla. DLL, dorsal nucleus of lateral lemniscus; IpN, interpeduncular nucleus; LPGi, lateral paragigantocellular; PbN, parabrachial nucleus; RP, raphe pallidus; Rs, rubrospinal tract; TB, trapezoid body.
FIG. 7. Example of an SHT axon that appeared to issue branches in C1 and in the caudal medulla. A: dorsal view of the area of the brain outlined by the box in the diagram in B. C: location of the recording site in C7. D: responses of this WDR neuron to cutaneous stimuli. E and F: locations of lesions made at low-threshold points a–j and antidromic action potentials elicited at each low-threshold point. The initial low-threshold point a was in the SoD; the latency was 2.05 ms. The axon was also activated in the contralateral upper cervical cord and medulla at low-threshold points b–j. The shortest-latency low-threshold points, b and f, were located in the dorsal lateral funiculus and ventral medullary reticular nucleus and appear to represent the locations of the parent axon. Latencies at points c–e and g were longer than those at low-threshold points at the next rostral levels, suggesting that slowly conducting daughter branches emanated from the parent axon at each of these levels. These branches were located dorsally and medially from the parent axon, mainly in the reticular formation. The parent axon in the medulla was located in the ventrolateral medullary reticular formation. ECu, external cuneate nucleus; Hb, habenular nucleus; ICP, interior cerebellar peduncle; PyD, pyramidal tract decussation.
Example of an SHT axon that issued several branches in the pons, midbrain, and posterior thalamus. A: representation of a dorsal view of the area of the brain outlined by the box in the diagram in B. C: location of the recording site in C. D: responses of this WDR neuron to cutaneous stimuli. E and F: locations of lesions made at low-threshold points a–i and antidromic action potentials elicited at each low-threshold point. The initial low-threshold point a was in the contralateral hypothalamus. The axon was also activated at progressively longer latencies at each level in the contralateral pons, midbrain, and posterior thalamus (points b–i). Note that in some levels there were 2 low-threshold points with different latencies. Latencies in points c, e, and i were longer than those at low-threshold points in the next rostral levels, suggesting that slowly conducting daughter branches emanated from the parent axon in these levels. Point c was located in the pontine reticular nucleus; points e and i were in the mesencephalic reticular nucleus and posterior thalamic nucleus. In the pons, the axon ascended in the lateral lemniscus, then shifted dorsally to the brachium of inferior colliculus in the midbrain. The parent axon ascended through the posterior nucleus of thalamus. BSC, brachium superior colliculus.
spinal fibers within the medulla of rats. These studies have examined degenerating fibers produced by cordotomies (Mehler 1969) or fibers that were anterogradely labeled by applying horseradish peroxidase within the spinal cord white matter (Zemlan et al. 1978). Within the medulla, large numbers of ascending fibers were noted in the same regions of the ventrolateral medulla. Fibers were described leaving this region and coursing dorsally and medially into the reticular formation. Zemlan et al. (1978) also noted labeled spinal fibers within the cuneate nucleus. Therefore these previous and the present findings suggest that collateral branches of SHT axons contribute heavily to spinoreticular tract projections and to a lesser extent to the spinosolitary tract (Mentrey and Basbaum 1987) and to the projection from the spinal dorsal horn to the cuneate nucleus (Cliffer and Giesler 1989; Giesler et al. 1984).

In a previous study of SHT axons (Zhang et al. 1995), we noted that after crossing the midline in the posterior part of the optic chiasm, several SHT axons descended as far as the ipsilateral medulla. In that study, descending SHT axons were antidromically activated from five low-threshold points in the reticular formation of the ventral medial medulla. In that study we also found evidence in several cases for the presence of branches that emanated from the parent SHT axons in the contralateral medulla and crossed into the ipsilateral medulla (see Fig. 7 in Zhang et al. 1995). Therefore both branches from ascending and descending SHT axons appear to project to a number of areas of the medullary reticular formation. These findings suggest that SHT projections to the medullary reticular formation are bilateral.

Within the posterior pons, the majority of ascending SHT axons was antidromically located ventrolaterally, primarily within or near the facial nucleus, superior olivary complex, and lateral lemniscus. Apparent branches were activated in pontine reticular nucleus caudalis, gigantocellular reticular nucleus, lateral lemniscus, superior olivary complex, parvo-cellular reticular nucleus, pontine reticular nucleus oralis, trigeminal nucleus oralis, and the medial parabrachial nucleus. In the pons, the majority (69%) of SHT branches was activated in the reticular formation. Therefore it appears that in the pons also, collateral branches from SHT axons provide a prominent projection to the reticular formation. In previous anatomic studies in rats, ascending spinal axons were also seen primarily in the ventrolateral part of the pons. However, in contrast to our findings, ascending spinal axons were located lateral and ventral to the facial nucleus, not within it. In these previous studies (Mehler 1969; Zemlan et al. 1978), terminal degenerating fibers were seen in all of the areas in which we antidromically activated apparent SHT branches including several reticular nuclei.

Within the rostral pons and midbrain, ascending SHT axons were antidromically located laterally, as they coursed...
dorsally from near the ventral surface within the lateral lemniscus. Within the same anterior-posterior plane at this level, the same SHT axon was sometimes activated at several points. The points near the ventral surface had the shortest latencies and those located dorsally, near the brachium of the inferior colliculus, had longer latencies. Because these SHT neurons frequently could not be antidromically activated from areas immediately anterior to the most ventral points, it appeared that the SHT axons coursed dorsally at this level. Here again, our results are similar to previous anatomic studies in which labeled spinal axons were seen coursing dorsally within the same region of the lateral midbrain (Mehler 1969; Zemlan et al. 1978). In the present study, apparent branches were antidromically activated in several areas of the midbrain, including the mesencephalic reticular nucleus, the cuneiform nucleus, the brachium of the inferior colliculus, the intermediate layers of the superior colliculus, the central gray, and the substantia nigra. Many of these same areas have been shown to receive direct spinal input in anatomic studies (Mehler 1969; Zemlan et al. 1978). Our findings suggest that branches from SHT axons contribute to spinomesencephalic tract projections, here again, particularly strongly to nuclei in the reticular formation.

In the posterior diencephalon, all SHT axons were antidromically activated laterally; these axons apparently passed through the medial part of the medial geniculate and the posterior nucleus, an area in which several studies in rats have shown that large numbers of spinothalamic tract and SHT axons enter the diencephalon (Cliffer et al. 1991; Lund and Webster, 1967; Mehler 1969). A small number of apparent branches from SHT axons was detected in the posterior diencephalon; these were in the posterior nucleus of thalamus and medial geniculate.

![Diagram](http://jn.physiology.org/DownloadedFrom http://jn.physiology.org/)

**FIG. 11.** Schematic diagram illustrating the bilateral course of SHT axons and collateral branches from them. Bracket: area of the contralateral brain that was examined in the present study. Note the large number of collateral branches from SHT axons in this region. The data supporting the locations of SHT axons and collateral branches in the remainder of the figure have been reported previously (Burstein et al. 1991a; Cliffer et al. 1991; Dado et al. 1994a; Zhang et al. 1995).

**Functional implications**

Several of the areas that appear to receive input from branches of SHT axons are thought to play roles in somatic
sensory processing. The gray matter of C1 contains large numbers of neurons that respond to noxious stimuli delivered within large receptive fields that can extend over the entire body (Carstens and Trevino 1978; Smith et al. 1991; Yezierski and Broton 1991; Zhang et al. 1996). Many neurons in the upper cervical spinal cord send axonal projections that reach the midbrain, thalamus, and hypothalamus (Burstein et al. 1990a,b; Carstens and Trevino 1978; Yezierski and Mendez 1991; Zhang et al. 1996). Fourteen low-threshold points that were classified as arising from collateral branches of SHT axons were located in the gray matter of C1. Three SHT neurons that gave rise to apparent axonal branches in C1 were physiologically classified; each was a WDR neuron. In a previous study (Dado et al. 1994c), we noted that several examined spinthalamic tract/SHT axons gave off branches that appeared to terminate in the dorsal horn of C2. The present findings and our previous findings suggest that neurons in C1–C2 may receive part of their nociceptive input via collateral branches of spinal axons that reach the hypothalamus.

SHT axons appear to provide a substantial number of collateral branches into the reticular formation at all levels from the caudal medulla to the rostral mesencephalon. Several areas of the reticular formation are involved in production of arousal and cortical desynchronization (Magonu 1963) that can be produced by noxious stimuli. Other areas, including ventral medial (Basaum and Fields 1984) and lateral medullary reticular formation (Janss and Gebhart 1987; Lovick 1985; Morton et al. 1983), have been strongly implicated in the production of descending inhibitory control of nociceptive processing. Branches from ascending SHT axons may provide part of the nociceptive input from the spinal cord to these areas of the brain stem.

In a previous study (Dado et al. 1994a), we noted that >80% of axons initially antidromically activated from the posterior nucleus of thalamus in rats could also be antidromically activated from low-threshold points in the hypothalamus. In that study we referred to such cells as spinthalamic tract/SHT neurons because their axons were located in both the thalamus and hypothalamus. In this study, in all 17 cases in which the locations of SHT axons were determined in the posterior diencephalon, the examined SHT axons were activated at shorter antidromic latencies from low-threshold points in or near the posterior nucleus. Therefore it appears that a high percentage of the axons examined in this study also passed through the thalamus as they ascended toward the hypothalamus. Thus a large percentage of the neurons referred to simply as SHT neurons in this study could rightly be considered spinthalamic tract/SHT neurons.

Figure 11 illustrates a schematic representation of the course of SHT axons. The present studies indicate that identified SHT axons ascend in the lateral parts of the brain stem, midbrain, and caudal thalamus. These studies have also found evidence suggesting that ascending SHT axons give rise to numerous branches and that such branches appear to be capable of supplying a number of nuclei, particularly those in the reticular formation, with nociceptive information.

Through their widespread, complex axonal projections, monaminergic neurons in the brain stem are thought to influence activity levels of neurons throughout much of the forebrain and limbic system. In a similar manner, SHT neurons, through their widespread bilateral axonal projections, may alter the firing of neurons in many areas of the brain stem, midbrain, and diencephalon. Thus activation of SHT axons may directly affect the activity of many areas of the CNS that are known to be involved in the production of autonomic, neuroendocrine, and affective responses to noxious stimuli.

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