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Laminar Distribution of the Sources of Ascending Spino-Supraspinal Pathways Involved in Nociceptive Transmission and Pain Modulation

Abstract: In the present study, we made an attempt to detect more exactly the laminar distribution of spinomesencephalic, spinothalamic and spinotelencephalic tract neurons and to estimate the character of axonal collateralization in these spino-supraspinal pathways in the rat by means of retrograde double-labelling of cells with Fluoro-Gold (FG) and Primuline O (Pr). We found that sources of spino-supraspinal pathways to the upper centers of the brain stem, nucleus accumbens and septal nuclei were mixed together in the marginal zone, deep dorsal horn, reticulated area, lamina X and the lateral spinal nucleus, but have as a rule their own discrete projection fields. Thus, spino-supraspinal projections could be characterized as extensive but not diffuse according to the current concept of structural and functional organization of the ascending spinal systems related with pain or motivational-affective reactions connected with pain. It was shown recently that suppression of tonic activity in an ascending spino-supraspinal pathway activates a descending supraspinal anti-nociceptive circuit with an opioid link in nucleus accumbens. We propose that the main effect of the straight spinal input into the limbic system is to facilitate nociceptive sensitivity, but not to suppress nociceptive transmission on the level of the spinal cord.

Key Words: Nucleus accumbens, Thalamus, Fluorescent double-labelling, Spinal cord, Nociception.

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Introduction

Electrophysiological studies confirm the importance of the spinothalamic tract (STT) in transmission of nociceptive information to higher brain stem sites (1). It has been demonstrated by means of multiple retrograde fluorescent tracers that a population of cells of STT origin exhibits collaterals to the lateral periaqueductal (PAG) in the rat (2, 3). A spinotelencephalic tract has also been found recently in rats (4, 5). It was shown that there is a direct projection from neurons of the spinal cord to some regions of the telencephalon, including nucleus accumbens and septal nuclei which are probably involved in motivational-affective reactions (6). The development and application of a new technique for retrograde labelling with Fluoro-Gold (FG) or Primuline O (Pr) has resulted in an enormous increase in our knowledge of the long ascending pathways originating from the spinal cord (4, 7) or descending pathways terminating on spinal neurons (8, 9) as well as information on the extent of collateral projections. In the present study, we made an attempt to detect more exactly the laminar distribution of spinomesencephalic, spinothalamic and spinotelencephalic tract neurons and to estimate the character of axonal collateralization in these spino-supraspinal pathways in the rat by means of retrograde double-labelling of cells with FG and Pr.

Material and Method

Sprague-Dawley rats (n=7) of both sexes (170-350 g) were anesthetized with nembutal (45 mg/kg, i.p) and placed in a stereotaxic frame. Three injections (200 nl each) of Pr (Reichert, Austria, 10% aqueous solution prepared in 2% dimethylsulfoxide) were made according to the stereotaxic atlas (10) unilaterally in the lateral PAG (Fr-7.0) or lateral thalamus (which includes the ventrobasal complex) and posterior thalamic nuclei (Fr-3.5). Simultaneously the same volume of FG (Fluorochrome INC, USA, 2% solution) was administered into nucleus accumbens (Acb) and medial septal nuclei (Sn) (Fr+1.2). Pressure injections of fluorochromes were made manually through a glass micropipette (tip diameter=50-80 μm) attached to the needle of a 10-μl Hamilton syringe. All rats survived 6-7 days and were then deeply anesthetized and perfused with 4% paraformaldehyde solution. After perfusion,
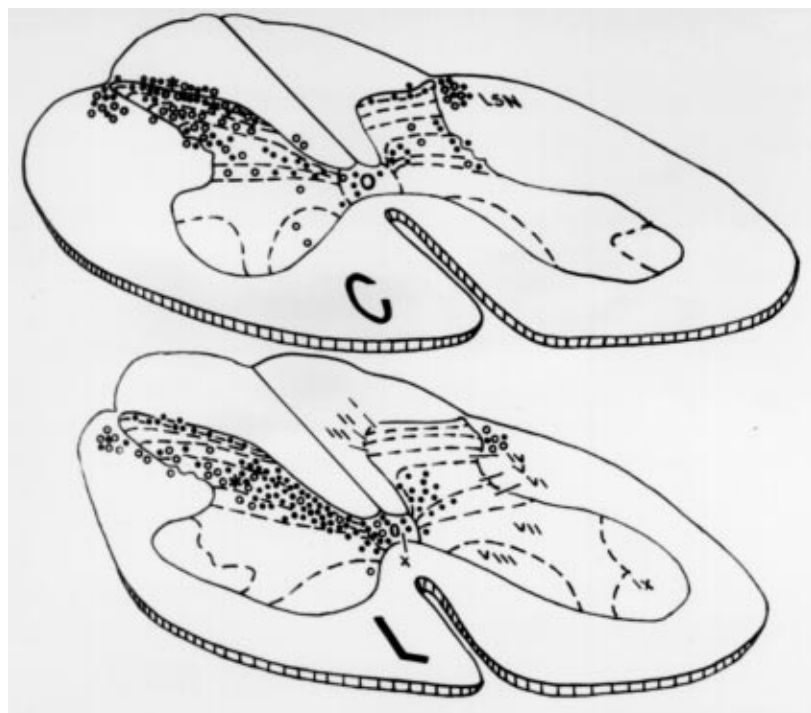
Fig. 1. Fluorescence photomicrographs of labelled neurons in lumbar (a) and sacral (b) segments. Note in (a) that large Pr-labelled spindle cell in the deep dorsal horn exhibits brightly fluoroscent golden grains in the cytoplasm and proximal dendrites (Pr injection into thalamus). Large multipolar FG-labelled neuron in lamina V in (b) exhibits blue fluorescence throughout the cytoplasm, proximal and distal dendrites (FG injection into nucleus accumbens). X 1300 (a) and X 500 (b)

the brain and identified spinal segments were very quickly embedded in soft paraffin wax. The diameter of the spinal cord was measured in the beginning and the end of the embedding procedure and the compression of tissue during the embedding in paraffin wax was found about 12.8%. Thus, the sizes of labelled neurons were recalculated according to the compression ratio. It was found that a prolonged time of embedding resulted in fading of fluorescence. Serial sections were cut on a microtome through the injection sites at 50 μm and through the cervical enlargement (C6-C8), thoracic cord (Th5-Th7), lumbar enlargement (L3-L5) and sacral spinal cord (S1-S3) levels at 20 μm. Sections were immediately mounted on glass slides from a warm (49°C) sucrose-chrom-alum-gelatine solution. Mounted sections were air-dried overnight in a refrigerator, then dehydrated in ethanol, cleared in toluene, coverslipped with a non-fluorescent medium (Entellan) and examined through a fluorescence microscope. The fluorescence was excited by ultraviolet light, wavelength 360-420 nm, filtered through BG 12 + BG 3 filters. Pr-labelled neurons were identified in the spinal cord sections by the presence of bright gold fluorescent grains (fluorochrome) in the cytoplasm and proximal dendrites and in the neurons labelled with FG, the cytoplasm and dendrites appear blue (Fig. 1). In neurons double-labelled with Pr and FG, each dye is well seen in the cytoplasm and

can be observed and photographed with the appropriate filter system. The appearance of double-labelled cells ranged from those with large amounts of Pr and small amounts of FG to those possessing intense blue fluorescence and few golden-yellow granules. Diagrams of representative spinal cord levels were made according to the stereotaxic atlas (10). Labelled cells were plotted on these diagrams according to their laminal location, combining several tissue sections per diagram (160-200 sections). For comparison of differences among populations of labelled neurons on the 4 levels of the spinal cord, a Student's t-test for independent means was used. Data were represented as mean ± SEM (standard error of the mean).

Results

In the animals, the necrotic core of injection of FG was centered in Acb and an extracellular halo of dye (diffusion zone) covered septal nuclei. The spread of the dye was approximately 0.8 mm in the rostrocaudal direction. The core of the second injection of Pr included the lateral and dorsolateral divisions of the PAG or in other cases covered the middle region of the ventrobasal complex and posterior nuclear group. The spread of Pr was approximately 0.7 mm in the rostrocaudal direction. A consistent pattern of labelled neurons was found in the gray matter throughout the
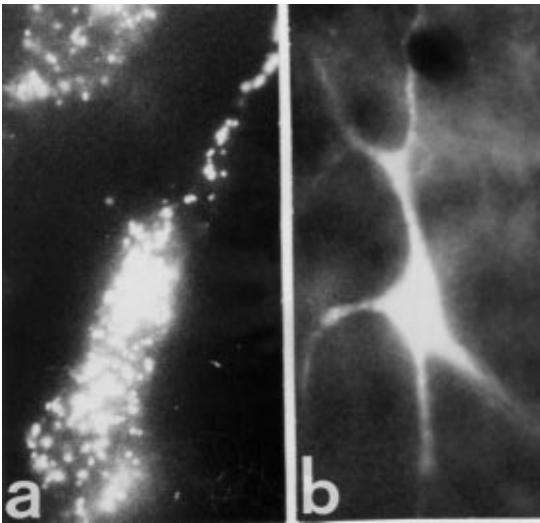
Fig. 2. Schematic representation of the distribution of retrogradely labelled neurons in the cervical (C) and lumbar (L) enlargements following an unilateral (right) primuline injection into the thalamus and Fluoro-Gold injection into nucleus accumbens.
Each dot, circle and star represent two primuline-, one Fluoro-Gold-labelled or two double-labelled cells respectively, observed in 400 frontal 20-µm-thick sections taken from one rat. Different laminae (I-X) and the lateral spinal nucleus (LSN) are indicated.

whole length of the spinal cord (C4-S3). In order to estimate the sources of spino-supraspinal pathways quantitatively, we chose the cases in which the largest number of labelled neurons were found. Most of the FG-labelled and Pr-labelled cells were located on the contralateral side to the tracer administration and mixed together, and occurred throughout the entire mediolateral extension of the marginal zone (lamina I) and nucleus proprius. Labelled cells were observed also within lamina X and the lateral spinal nucleus (LSN) in the dorsal lateral funiculus on both sides.

Cells projecting to the nucleus accumbens and/or ventrobasal thalamic complex.

In the 12 examined segments for one typical case, Acb projecting neurons (FG-labelled) and STT cells (Pr-labelled) were counted in alternate sections. The pattern of distribution for Acb projecting neurons and thalamic projecting cells on upper and lower levels was approximately the same, however the average number of sources of supraspinal projections per tissue section was higher in the cervical and lumbar enlargement than in the thoracic and sacral cord (0.23, 0.15, 0.21, 0.04 respectively for Acb projecting neurons and

0.36, 0.45, 0.05, 0.08 respectively for SST cells). Many STT cells were found in laminae I and IV, V. However, in contrast to the cervical region, the largest population of STT neurons in the lumbar enlargement was localized in the medial part of lamina V (Fig. 2). Acb projecting neurons were observed in lamina I and deep dorsal horn. This last population was laterally located. The area around the central canal contained approximately 3.9% of labelled neurons. However most labelled neurons in lamina X were found in the cervical and lumbar enlargements (Fig. 2). LSN in the dorsal lateral funiculus contained approximately 0.25% of Acb projecting neurons and SST cells about 0.19%.

Spinal neurons unequivocally fitting the criteria for double-labelling, ie. brightly fluorescent golden grains on a diffuse green luminescence of the cytoplasm, were very scarce and only identified in cervical and lumbar enlargements (Fig. 2). It was calculated that 3.9% of the Acb projecting neurons and 1.8% STT neurons were double-labelled in laminae I and V. The distribution of double-labelled cells in the lumbar enlargement (lamina V) was the reverse of that seen in the cervical enlargement (lamina I).

The size and morphology of the Acb projecting neurons and STT cells were found to vary depending upon their laminar location and segmental distribution. It should be noted that fluorochromes were accumulated solely in the cytoplasm and extended to the proximal dendrites. Only FG was occasionally extending to the distal dendrites also (Fig. 1b). In the coronal sections labelled neurons in lamina I were only small spindle cells measuring 11.02 ± 0.31 µm in their average diametre (mean between long and short axes). The neurons labelled by different dyes in the deep dorsal horn and lateral reticulated area (laminae IV-VI) were multipolar, elliptic or fusiform in shape. It should be noted that in all cases of dye injections into the thalamus STT neurons located in medial part of lamina V and lamina X were elliptic or spindle in shape. STT cells have an average diameter of 19.98 ± 0.04 µm. Acb projecting cells were found to be mostly multipolar with an average diameter of 17.91 ± 0.18 µm. The average diameter of Acb projecting cells differed significantly from the average diameter of STT cells (t=4.95, df (degree of freedom)=180, p<0.001). In addition, the average diameter of Acb projecting neurons in the lumbar enlargement (18.74 ± 0.2 µm) was slightly higher than calculated (15.99 ± 0.12 µm) for the cells in the cervical enlargement (t=2.14, df=66, p<0.05).
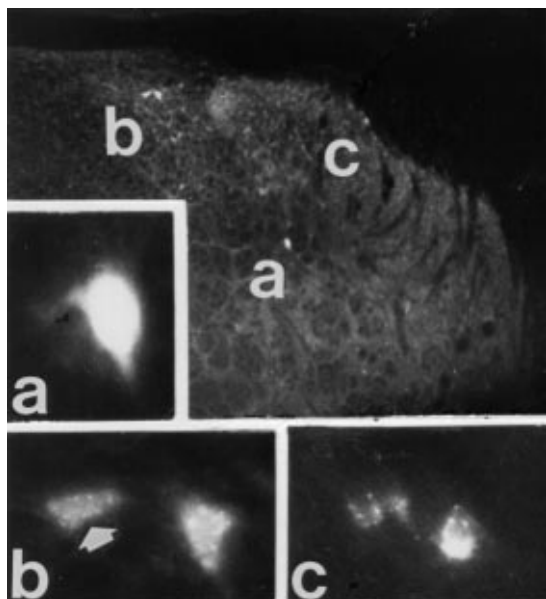
Fig. 3. Fluorescence photomicrographs of Fluoro-Gold-labelled and primuline-labelled neurons in dorsal horn in cervical segment and magnified view of these labelled cells (a-c). Note the large multipolar Fluoro-Gold-labelled neuron in the lateral reticulated area (a), medium-sized ovoid primuline-labelled and double-labelled (arrow) neurons in the lateral spinal nucleus (b), small ovoid primuline-labelled cells located in lamina I. X 100 and X 900 (a-c).

Cells projecting to the lateral PAG.

Cells of origin of PAG projections (Pr-labelled) in the cervical enlargement were predominantly distributed in laminae I (21% of all in lamina I) and IV. Cells that were labelled after injection of Pr into lateral PAG were also scattered in the medial parts of laminae V, VI, and also in lamina X or the lateral spinal nucleus. There was an average of 2.63 PAG cells per tissue section (20 µm-thick) on the cervical level of spinal cord. The pattern of distibution of PAG projecting cells in thoracic, lumbar and sacral levels was approximately the same as in the cervical segment. However, in contrast to the cervical segments, the largest population of labelled neurons in these levels was observed in lamina V. Many labelled cells in the lumbar enlargement were scattered in the medial parts of laminae VI, VII and in lamina VIII. The average number of sources of PAG projections per tissue section was lower in the thoracic and sacral segments (0.48 and 0.26 respectively) than in the cervical and in the lumbar enlargements (2.63 and 1.12 respectively). As mentioned earlier, STT cells and Acb

projecting neurons located in lamina I often displayed a spindle-shaped morphology. This is in contrast to lamina I labelled cells projecting to the PAG. These cells tended to have a triangular or ovoid shape, as shown in Fig. 3 and were only small cells measuring 9.26 ± 3.95 µm in their average diameter. In contrast, PAG projecting neurons in the deep dorsal horn were fusiform or multipolar in shape, measuring 14.73 ± 0.17 µm in their average diameter.

Cell projecting both to the Acb and the lateral PAG were very scarce and scattered throughout laminae I, V and also the lateral spinal nucleus (Fig. 3). An average of 4.1% of all PAG and 10.6% of all Acb projecting cells were double-labelled.

Discussion

The STT is regarded as one of the most important ascending no ciceptive pathways. The cells of origin of the spinothalamic tract in rats have been found by several investigators using retrograde labelling with horseradish peroxidase (11) and cholera toxin (12). The most effective labelling of STT neurons as far as PAG and Acb projecting cells has been obtained with fluorescent dyes (3, 4).

The pattern of distribution of Acb-, thalamic- and PAG-projecting neurons in the rat spinal cord revealed in the present study is consistent with previous reports (3, 4). The largest populations of sources of long spino-supraspinal pathways to analgesic zones of PAG, thalamus and Acb were located on the contralateral side of the spinal cord within the marginal zone, deep dorsal horn, lateral reticulated area and the area around the central canal. These sources were also scattered in the lateral spinal nucleus over the entire extent of the spinal cord.

We found that sources of spino-supraspinal pathways to the upper centers of the brain stem and telencephalon were mixed together in the dorsal horn and the lateral spinal nucleus, but have as a rule their own discrete projection fields. Thus, spino-supraspinal projections could be characterized as extensive but not diffuse according to the current concept of structural and functional organization of the ascending spinal systems related with pain or motivational-affective reactions connected with pain (13).

An interesting finding concerned the distribution of sources with divergent axon collaterals in the cervical and lumbar cord only. This scarce population of double-labelled neurons was localized within regions where

the largest number of cells of origin of supraspinal projections were observed (lamina I, V and the lateral spinal nucleus). The present study provides anatomical evidence that nociceptive signals relayed over the spinotelencephalic pathway has access via collaterals to the dorsolateral PAG or the lateral thalamus, also.

It is known that STT neurons send collaterals to several regions of the CNS, including the medullary reticular formation (14), parabrachial area (15) and the PAG (3). Spinothalamic neurons have descending propriospinal collaterals (16). However, our experiments failed to detect a significant number of collaterals of STT cells to the telencephalon.

We observed the cases in which the core of the injection sites (where the main uptake of fluorochrome presumably occurred) included ventrobasal nuclei. Localized in this part of thalamus, nociceptive neurons are responsible for sensory and discriminative aspects of the painful stimulus (17), whereas neurons in the medial nonspecific intralaminar nuclei are responsible for motivational-affective reactions connected with pain (18).

The Acb and septal nuclei are thought to play an important role in the initiation of several types of motivational or affective behaviours (6, 19, 20). Our findings indicate that several neurons in the spinal cord as a rule form discrete straight projections in the telencephalon. These projections may provide a direct afferent input to limbic areas that are involved in the motivational-affective responses to somatosensory stimulation.

In this study, we wish to emphasize the role of Acb and septal nuclei in pain perception and pain modulation. Thus, it has been shown that many cells in the septal nuclei can be excited by noxious somatosensory stimulation (21), and electrical stimulation in the ventromedial septal area can inhibit nociceptive responses of dorsal horn neurons (22). In support of a role for Acb in pain control, some investigators have reported that Acb contains opioid receptors (23, 24) and microinjection of morphine into Acb produces antinociception (25). A lot of nociceptive neurons in the spinal cord also have ascending exitatory inputs to the brain stem. Excited cells, mainly in PAG, which in turn excite descending controlled systems that have been proposed to play a role in modulation of nocicepiton (26, 27). However, inhibition of tonic activity in ascending spino-supraspinal pathways in some cases evokes antinociception. Recently Gear et al. (28) have shown that suppression of tonic activity in an ascending spino-supraspinal pathways disinhibits or activates a descending supraspinal antinociceptive circuit with an opioid link in Acb. Thus, permanent inhibition of supraspinal opioid-dependent antinociceptive mechanism by ascending tonic activity in the spino-supraspinal pathway implies that the main effect of the spinal input into the limbic system is to facilitate nociceptive sensitivity, but not to suppress nociceptive transmission at the level of the spinal cord (28).

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