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Descending projections to coccygeal spinal segments in the cat

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ABSTRACT

The descending projections to coccygeal spinal segments influencing tail movement were studied by retrograde wheat germ agglutinin–horseradish peroxidase (WGA–HRP) tracing methods in 12 cats. WGA–HRP solution was injected into the 3rd and 4th coccygeal segments and hemisection of the right side of the spinal cord was performed at the 3rd sacral–1st coccygeal segmental level. Labelled propriospinal neurons were distributed in laminae III–VIII and X of the spinal cord between the cervical and lumbosacral spinal segments, predominantly on the left. The greatest density of labelled cells was in the lumbar enlargement. In the brainstem a high density of labelled cells was observed mainly in the lateral vestibular, gigantocellular reticular and magnocellular reticular nuclei on the left side, and the nucleus raphe magnus, pallidus and obscurus. These findings indicate significant descending projections to the coccygeal spinal cord in the cat which could be responsible for tail movements in the cat.

INTRODUCTION

We have studied neural control of tail movement in cats (Wada et al. 1990; Wada, 1991; Wada & Tokuriki, 1991). Cats show various types of tail movement for locomotion, balance, defence, micturition, defaecation and sexual activity. These movements are accomplished by systematic activation of tail muscles and interaction between the tail and other parts of the body. To understand neural control of the cat tail movements, we investigated descending projections to coccygeal spinal segments anatomically. Masson et al. (1991) showed supraspinal and propriospinal descending pathways to sacrococcygeal spinal segments in rats but failed to limit the injection to the spinal segments innervating the tail. In this study, descending pathways to coccygeal (Co) segments influencing only tail movements (below Co2) were investigated using the retrograde wheat germ agglutinin–horseradish peroxidase method in cats.

METHODS

The experiments were performed on 12 long-tailed adults cats of both sexes. The cats were anaesthetised with pentobarbital (40 mg/kg i.p.) and a laminectomy was performed from 5th lumbar (L5) to the 2nd caudal vertebrae (Ca2). The dura mater was incised and opened to expose the coccygeal segments of the cord. A solution of 5% WGA–HRP (Sigma) was injected into the dorsal part of the 3rd and 4th coccygeal (Co3–4) spinal segments on the left using a Hamilton 1 μl syringe with a glass microelectrode (tip diameter 30–45 μm). The Co3–4 spinal segments were identified by the entry zone of the dorsal roots. The volume injected varied between 0.2 and 0.8 μl. Hemisection of the spinal cord on the right side was performed with forceps at S3–Co1 in all cats. After a survival time of 3 d, the animals were deeply anaesthetised with sodium pentobarbital and perfused transcardially with 2 l of saline (38 °C), 3 l of fixative (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4) and 1 l of 20% sucrose in phosphate buffer (pH 7.4). The brainstem, cerebellum and spinal cord were removed and stored overnight in 20% sucrose-phosphate buffer at 5 °C.

Serial frozen transverse sections of the brainstem, cerebellum and spinal cord were cut at 60 μm. The HRP preparations were treated with tetramethylbenzidine (TMB) by the method of Mesulam (1978). All sections of the brainstem and cerebellum and every 5th serial section of the spinal cord were mounted on gelatin-coated glass slides and lightly counterstained with neutral red. The locations of the

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labelled cells were transferred onto paper with a microphotodrawing system.

RESULTS

Injection site

The extent of injection is shown schematically in Figure 1. WGA–HRP diffused across the entire transverse section of the cord and spread over several segments. WGA–HRP activity was observed in spinal segments below the Co2 level in 9 cats (nos 1–4, 6–10) and was observed at the Co1 level in 1 cat (no. 5). Two cats (nos 11, 12) were excluded from this study, since ependymal cells in the central canal were found to be stained by WGA–HRP in spinal segment levels above the cordotomy.

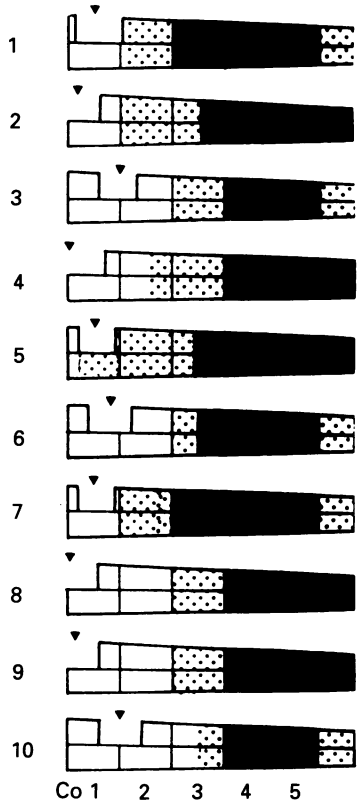

Fig. 1. Diagram showing extent of reaction product from injected WGA–HRP in 10 cats. Black areas indicate heavy WGA–HRP staining and stippled areas light WGA–HRP staining. Arrowheads indicate levels of cordotomies.

Propriospinal projection

Figure 2 shows a typical example of the distribution of the labelled cells (dots) in spinal segments C4, C8, T6, L4 and L7 (no. 10). Labelled cells were present throughout the lumbar and lower cervical spinal cord, predominantly on the left. In particular, many labelled cells were observed in the lumbar enlargement. In the cervical segments, a few labelled cells were observed in 4 cats (nos 1, 2, 7, 10). Labelled cells were identified within laminae III–VIII and X (Fig. 5*d*). Especially high cell densities were found in laminae III–V. This agrees with previous reports on propriospinal connections reported (Matsushita et al. 1979; Yezierski et al. 1980; Menetrey et al. 1985; Masson et al. 1991). Labelled cells included those of large and medium size, and multipolar, dipolar, spindle, triangular and circular cells. Labelled cells were observed in the right side of the lumbar enlargement in 2 cats (nos 6, 10).

Descending supraspinal neurons in the brainstem

Figures 3 (cat no. 10) and 4 (no. 8) show labelled cells in the brainstem in 2 cats.

Vestibular nuclei. Extensive labelling was found within the vestibular complex. The most extensive and dense area of labelling was within the left lateral vestibular nuclei. The labelled cells were medium to large spindle-shaped and oval cells (Fig. 5*a*). Leong et al. (1984) and Peterson & Coulter (1977) have reported labelling of the lateral, medial, and spinal vestibular nuclei after lumbosacral HRP injection. Pompeiano & Brodal (1957) have shown a somatotopic arrangement within the lateral vestibular nuclei. In this study, labelled cells were not observed in the

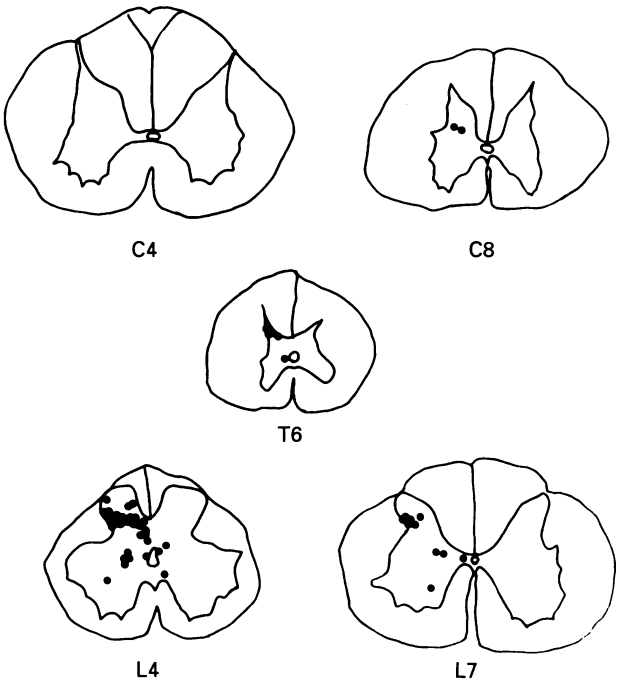

Fig. 2. Representative labelling of neurons (filled circles) in the spinal cord (cat 10). Many labelled cells were observed in the lumbar spinal segments but none at upper cervical levels.

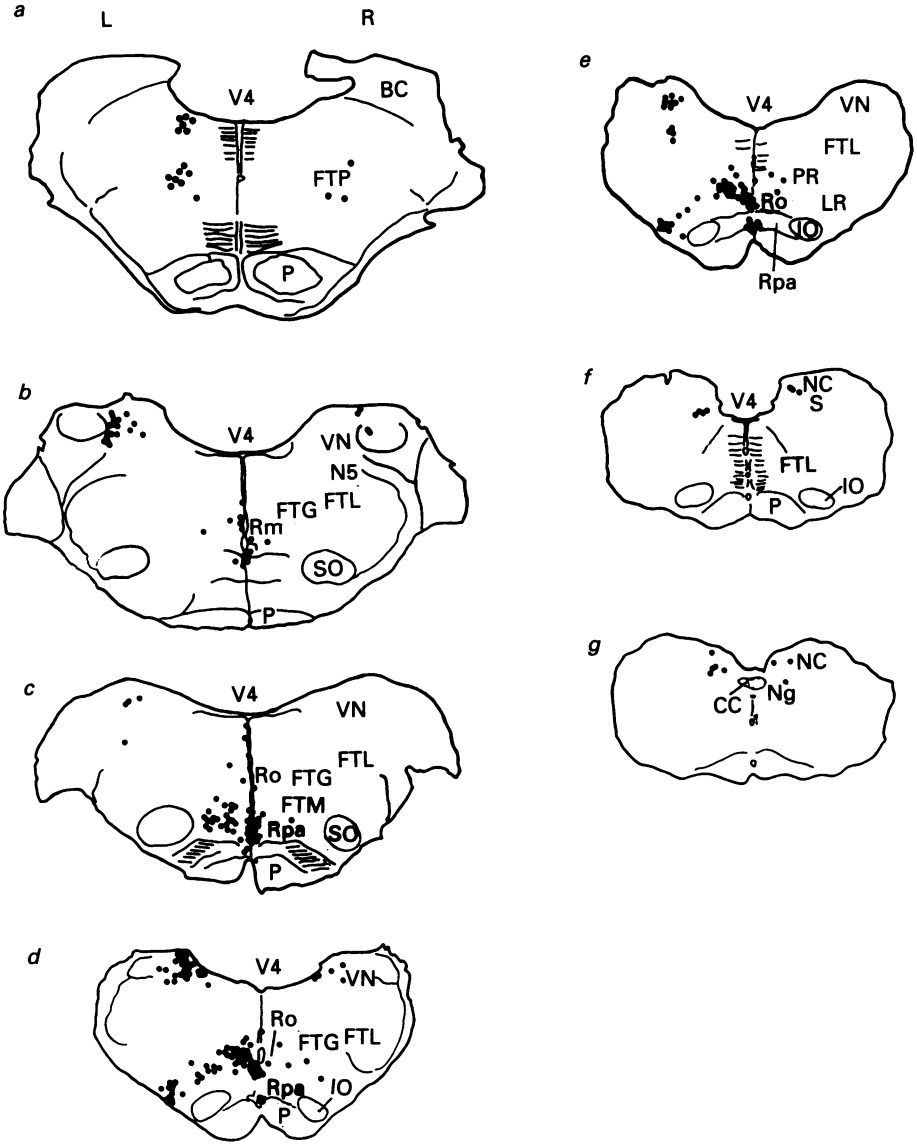

Fig. 3. Representative labelling of neurons (filled circles) in the medulla (cat 10). Labelled cells were seen in the FTP, FTG, FTM, LR, Rm, Ro, Rpa, VN, NC and Ng. In general, labelled cells were found predominantly on the left side. BC, brachium conjunctivum; CC, central canal; FTG, gigantocellular tegmental field; FTL, lateral tegmental field; FTM, magnocellular tegmental field; FTP, paralemniscal tegmental field; IO, inferior olive; LR, lateral reticular nucleus; NC, cuneate nucleus; Ng, gracile nucleus; N5, trigeminal nucleus; P, pyramidal tract; PR, paramedian reticular nucleus; Rm, raphe nucleus magnus; Ro, raphe obscurus nucleus; Rpa, raphe nucleus pallidus; SO, superior olive; VN, vestibular nuclei; V4, 4th ventricle.

restricted area of the lateral vestibular nucleus and no distinct somatotopic organisation of labelled cells was found. Some labelled cells were observed in the right vestibular nucleus in 5 cats out of 10 (nos 1, 2, 7, 5, 10).

Reticular formation. Labelled neurons were identified within the reticular formation throughout the medulla and, to a lesser extent, the pons. Most labelled neurons were observed in the magnocellular and gigantocellular reticular nuclei, the dorsal and ventral portions of the paramedian reticular nucleus, and the left lateral reticular nucleus. Leong et al. (1984) and Masson et al. (1991) observed that these areas of the reticular formation are related to descending pathways in the rat. Kausz (1991) also showed that these areas contain descending pathways to the thoracic and sacral spinal cord in the cat. The labelled cells were generally large or medium-sized multipolar neurons, with variable dendritic ramifications (Fig. 5*c*). In all cats, some labelled cells were observed on the right side, the side of the cordotomy.

Raphe nucleus. A high density of labelled cells was observed in the raphe nucleus, the raphe nuclei magnus (Fig. 5*b*), pallidus and obscurus. This corresponded to the findings of Kausz in cats (1991) and Masson et al. (1991) in rats. The size of labelled cells varied considerably. Multipolar and bipolar cells were observed. In general, the larger cells had multiple

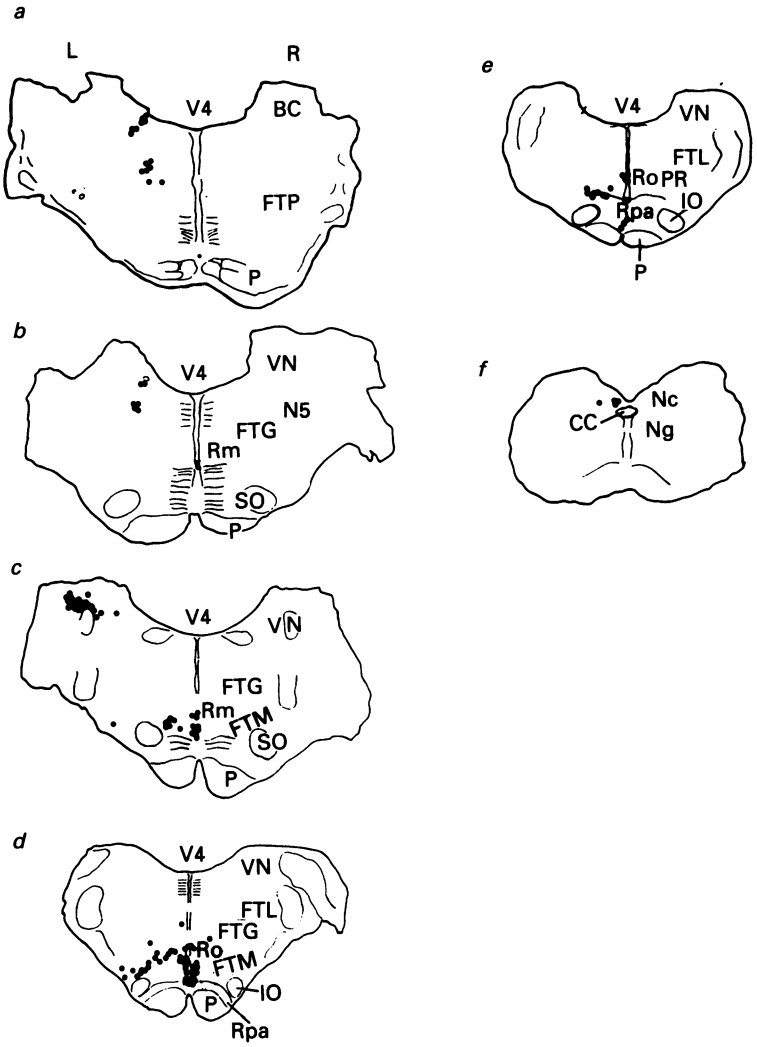

Fig. 4. Representative labelling of neurons (filled circles) in the medulla (cat 8). Abbreviations as in Figure 3.

axonal extensions while the smaller cell had few dendrites. Small labelled cells were numerous in the raphe nucleus pallidus.

Dorsal column nuclei. In the medulla oblongata, labelled cells were observed in the rostral and caudal divisions of the cuneate and gracile nuclei. Most of labelled cells were on the left side. Burton and Loewy (1977) reported neurons in the gracile and cuneate nuclei with projections to the lumbosacral spinal cord. Masson et al. (1991) and Burton and Loewy (1977) observed no labelling in the lateral cuneate nucleus, but Leong et al. (1984) recorded a significant number of labelled cells in this area. We failed to find any labelled cells in the lateral cuneate nucleus.

Labelled terminals in the cerebellum

In 2 cats (data not shown), labelled terminals were observed in lobule II. Xu and Grant (1990) noted a bilateral distribution of labelled mossy fibre terminals in lobules I–V, but we failed to find any labelled fibres in lobules I and III–V.

DISCUSSION

Our main interest is in the neural control of tail movement. Sacrococcygeal spinal segments innervate the tail, but sacral and Co1 spinal segments influence other regions of the cat body. Although the injection site in this study was at Co3–Co4, the distribution of WGA–HRP solution was over several spinal segments. In 9 out of 12 cats, the area in which WGA–HRP positive cells was restricted was in the spinal segments below Co2, as shown in Figure 1. Reid (1970) showed that afferent nerves which conduct only cutaneous sensory information from the tail enter the spinal cord through the dorsal roots below Co2. We recorded electromyographic activity induced by electrical stimulation of the sacrococcygeal ventral roots in cats and found that electromyographic

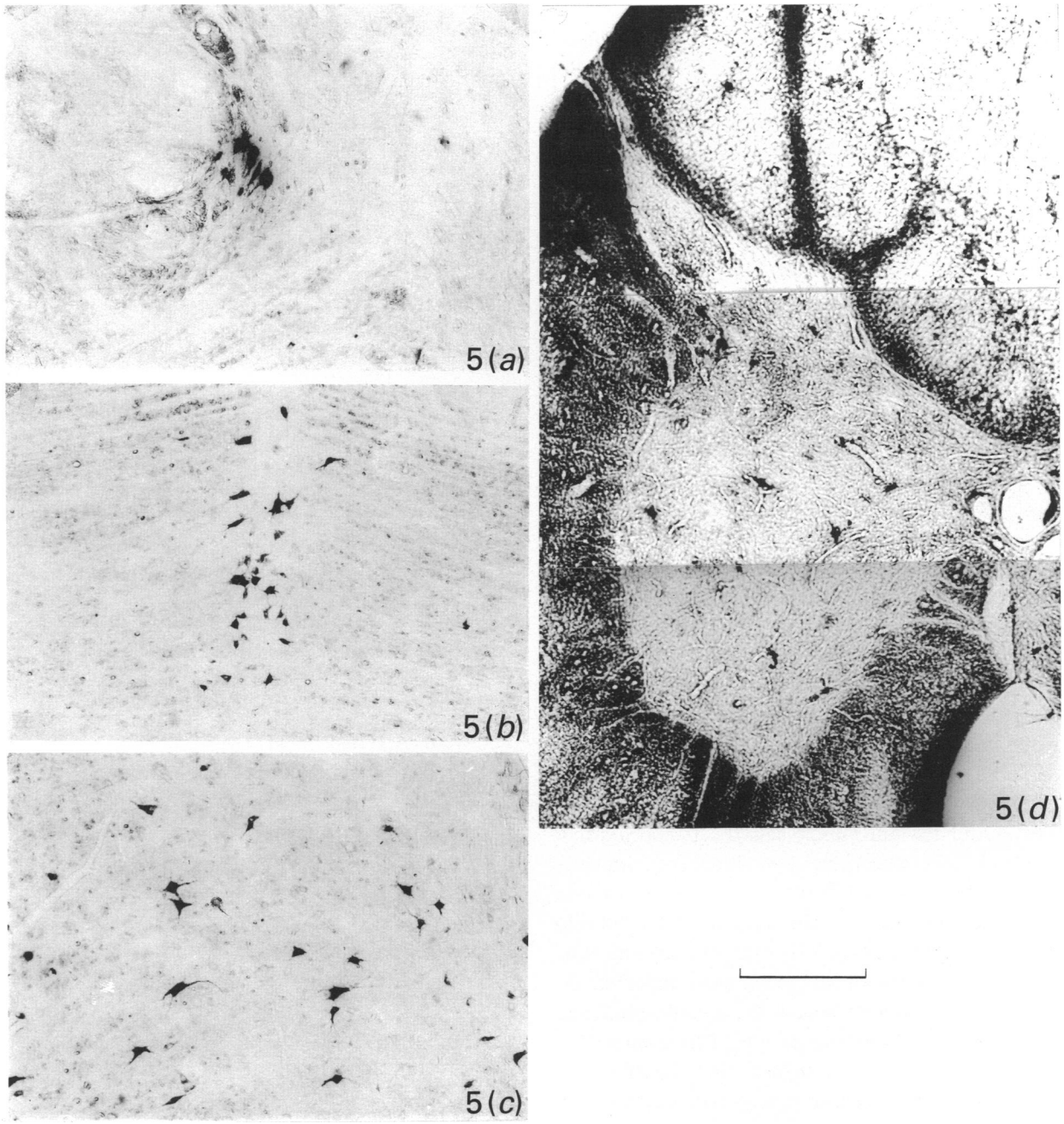

Fig. 5. (*a*) Lateral vestibular nucleus; (*b*) nucleus raphe magnus; (*c*) nucleus reticularis gigantocellularis; (*d*) labelled cells in lumbar enlargement (L4). Scale bar: (*a*) 45 μm; (*b–d*) 50 μm.

activity induced by the stimulation of the ventral roots below Co2 could be recorded only from tail muscles (unpublished results). These findings show that spinal segments below Co2 influence tail movement. They suggest that the labelled cells detected in this study influence tail movements. Three primary descending pathways from the brainstem to the coccygeal spinal segments were demonstrated: vestibulospinal, reticulospinal and raphe-spinal.

Some investigators have shown that motoneuronal activity is modulated by raphe-spinal descending pathways (Proudfit and Anderson, 1973; Fung & Barnes, 1981; Kaneko et al. 1987; Wada et al. 1989). Our results suggest that activity of tail muscle motoneurons is modulated by descending pathways from the raphe nucleus. The caudal raphe nucleus and reticular formation receive inputs from the hypothalamus and periaqueductal gray (Hosoya & Matsushita, 1981; Holstege, 1987). The connections identified by this study for the modulation of cat tail behaviour may be related to limbic circuits for the control of affective state.

Cats use delicate control of tail movement to maintain body balance. When cats negotiate a narrow

pathway, their tails are extended and constantly move. It was reported that the lateral vestibulospinal tract (LVST) is a primary descending projection from the vestibular complex to the lumbosacral spinal cord (Kuypers, 1981) and that extensor motoneurons are excited by LVST stimulation (Grillner et al. 1970). Our results suggest that the vestibulospinal descending pathways control tail movement to maintain body balance.

Mori et al. (1982) reported that the reticulospinal pathway is the primary descending pathway influencing locomotion and posture. During locomotion by 4-legged animals with long tails, such as cats and dogs, rhythmic movements of the tail muscles were observed (Necker, 1970). The reticulospinal descending pathways may influence the rhythmic tail movement during locomotion.

In the medulla oblongata, labelled cells were observed in the gracile and cuneate nuclei. Burton & Loewy (1977) and Leong et al. (1984) suggested that axons from these neurons in the lower cord segments were capable of taking up injected HRP.

Labelled propriospinal neurons were distributed between the cervical and lumbosacral spinal segments. Labelled cells were numerous in laminae III–VI. These labelled cells in the dorsal horn receive peripheral afferent inputs (Wall, 1960, 1967). Wada (1991) reported that afferent inputs from the hindlimbs modulated tail muscle activity. It appears that these neuronal connections in the spinal cord influence tail movement and coordinate movements between the tail and other parts of the cat body. Labelled cells were observed in laminae VII–VIII. Matsuyama et al. (1988) and Shinoda et al. (1988) have reported the distribution of the terminals of the reticulospinal and vestibulospinal descending pathways to laminae VII and VIII. These findings suggest that the effects of reticulospinal and vestibulospinal pathways on tail muscle motoneurons occur via neurons in lamina VII and VIII of the lumbar enlargement, in addition to the direct descending pathway to the coccygeal spinal segments.

In conclusions, this study has demonstrated various neuronal connections which could be responsible for tail movements in the cat.

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