THE PYRAMIDAL TRACT OF THE HEDGEHOG (ERINACEUS EUROPAEUS) AND ITS RELATIONSHIP WITH THE OLFACTORY BULB

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INTRODUCTION

The corticospinal tract varies considerably from one mammalian species to another. These variations can probably be attributed to the fact that the tract is phylogenetically young, since older tracts present a somewhat more costant pattern (13, 15, 23, 24).

Important variations are found in the location of corticospinal fibers. In marsupials, for instance, they course in the ventral part of the dorsal funiculus (17, 18, 31), while in insectivores the bulk of pyramidal fibers runs into the ventral funiculus (3), and in rodents into the dorsal funiculus (3, 9), with some exceptions, e.g. the rat (4, 10). In carnivores the main corticospinal tract runs into the dorsolateral funiculus as in primates and in man (1, 5).

Important variations are also found in the caudal extension of the corticospinal tract and its termination in spinal grey matter. A progression seems to exist from mammals with poor manual dexterity such as the pig, rabbit, goat, opossum, whose pyramidal tract extends to the first cervical or thoracic segments and whose fibers terminate in the dorsal horn, lamina IV and VI, to animals with increasing motor dexterity, such as the cat or rat whose corticospinal fibers extend throughout the spinal cord and terminate in the dorsal horn and in the intermediate zone, lamina IV, VI and VIII. In animals with the highest manual dexterity such as the baboon, chimpanzee and man the corticospinal fibers extend throughout the spinal cord and terminate in the dorsal horn, in the intermediate zone and in the ventrolateral motoneuronal part of the anterior horn, including laminae IV, VI, VIII and IX.

In the majority of mammalian species, the pyramidal tract decussates in the lower part of the medulla oblongata, prior to reaching the spinal cord. However, in a few mammals such as the mole (12, 16, 29), klipdassie (3, 29) and procavia (29) no pyramidal fiber decussation occurs in the oblongata.

The hedgehog (*Erinaceus europaeus*) is an insectivore with a unique place in phylogenesis, since it is the only existent placental mammal known to have existed in the cretaceus period (26). Some authors mostly using Marchi's degenerative

method, deny the crossing of the pyramidal tract in this species (2, 7, 14, 28, 30), while other authors, even recently, found a pyramidal crossing (3, 20). Besides, the extent of the pyramidal tract in this archaic mammal is controversial, since the pyramidal ending is described either in first cervical segments (20, 30) or in lumbar segments (3). The aim of the present paper is to re-examine the hedgehog pyramidal pathway by the combined use of a degenerative method and HRP tracing.

METHODS

Fourteen adult hedgehogs (*Erinaceus europaeus*) were used in this investigation. Eight animals served for Nauta-Gygax silver staining (21, 22) and six for horseradish peroxidase (HRP) tracing.

Animals for silver staining were anaesthetized with pentobarbital (30-40 mg/kg body weight) and put onto a stereotaxic apparatus. An appropriate craniotomy was performed and the dura mater cut and upset to expose the left hemisphere, which was immediately protected with warm mineral oil at 25-30° C.

The electrical stimulation of the left hemisphere was carried out by a Grass S4 stimulator, with silver ball electrodes (diameter 0.4 mm; interelectrode distance 2 mm). A threshold was first established at a light level of anaesthesia. The responsive motor points were mapped in the fronto-parietal cortex and in the olfactory bulb using either single shocks or trains of pulses with the following parameters: 0.1-1 msec duration; 10-100/sec 0.2-7 V.

The delimited fronto-parietal cortex in five animals and a rhinencephalic responsive point in three subjects, with the underlying white matter, were removed by suction and the operation wound was sutured. The surgical procedures were performed aseptically. All the animals recovered well showing no motor deficits at a clinical observation, even transitory, and were kept alive for 14-21 days. After this survival period, they were anaesthetized again to be perfused with saline solution and then with 10% neutral formalin through the left ventricle. The cerebral hemisphere of the operated side, the brain stem and the spinal cord were removed, appropriately subdivided and immersed in sucrose-formaline for 3-5 days. Then, transversal and longitudinal frozen sections 20 μ thick were obtained. After a prolonged bath in diluted formalin, these sections were treated with the silver degeneration technique according to Nauta and Gygax (21, 22).

Animals used for horseradish peroxidase (HRP) tracing were anaesthetized in the same way and placed on the stereotaxic instrument. Peroxidase (0.1 μl of 10% aqueous solution) was injected into the cerebral peduncle, the motor responsive area of the olfactory bulb and the ventral column of the spinal cord at level of C_3-C_4 or of the cervical enlargement (C_5-T_3) of different animals by using a sterile microsyringe mounted in a micromanipulator.

The animals were then perfused 24-36 hours after injection using heparin containing saline solution and a fixative composed of buffered paraformaldehyde and glutaraldehyde, pH 7.4. The ipsilateral and contralateral fronto-parietal areas and the responsive point of the ipsilateral olfactory bulb were removed, stored in the perfusion fixative for 3-5 hours and cut serially in 25-30 μ thick transversal frozen sections. The free floating sections were collected in 0.1 M phosphate buffer and immediately treated with sodium nitroprusside and tetramethilbenzidine (TMB) using perhydrol as a substrate for detecting peroxidase activity as proposed by Mesulam (19). In all the animals, the injection area was embedded in paraffin and cut. The 8 μ thick sections obtained were stained with hematoxylin-eosin in order to ascertain the precision of the HRP injection.

RESULTS

The hedgehog has a brain lacking sulci and fissures and is classified as lissencephalic. Thus the description of the cortical areas eliciting somatic motor activity was difficult. However, two regions were clearly identified: the frontoparietal area, and that in the olfactory bulb (rhinencephalic motor area) (Fig. 1).

The former motor area is constantly bordered by two parallel pial blood vessels. They originate in the medial border and show a transverse course on the dorsolateral surface of the hemisphere. Therefore, these blood vessels can be considered as landmarks. Furthermore, this area extends laterally as far as a longitudinal sulcus.

The rhinencephalic motor area occupies the inferior third of the lateral surface of the olfactory bulb and extends aborally to the bordering fronto-parietal portion of the hemisphere. It extends for about 3 mm² on the ventrolateral surface of the caudal third of the bulb.

1. *Electrophysiological experiments.* — In the fronto-parietal motor area, trains of pulses (2-7 V, 0.1 msec, 10-100/sec) induced slight contractions of the ipsilateral muscles of the face, neck, shoulder, forelimb and of the abdomen with a certain latency, while single shock stimulation was ineffective. On the contrary, stimulation of the rhinencephalic motor point (single shock 1 msec, 0.2-0.4 V) elicited prompt and evident contractions of the same groups of muscles.

We were unable to identify in the two motor areas the representation for the hindlimb musculature.

2. *Degenerative experiments.* — The ablation of fronto-parietal and rhinencephalic motor points allowed us to identify a degenerated ipsilateral bundle, extending to the first cervical segments of the spinal cord (C_3-C_4). No significant differences were found in the course, size and extension of the bundle following either fronto-parietal or rhinencephalic excision. After removal of the fronto-parietal area many degenerating axons reached the ipsilateral olfactory area as mapped electrophysiologically (Fig. 2).
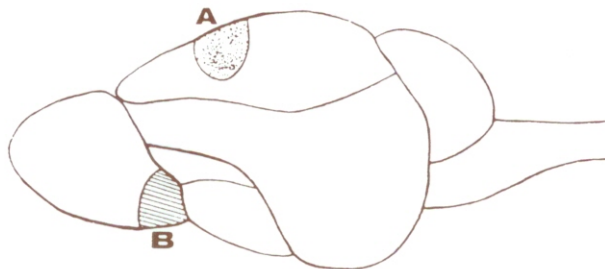

Fig. 1 – *The pyramidal tract of the hedgehog.*

Scheme showing the two fronto-parietal (A) and rhinencephalic (B) motor areas, from which muscular contractions were elicited.
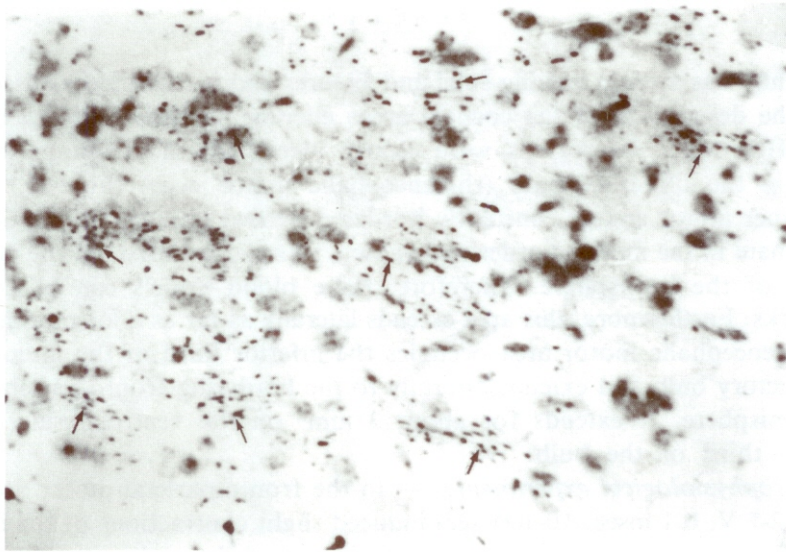
Fig. 2 – *The pyramidal tract of the hedgehog.*

Olfactory bulb, longitudinal section. Degenerating axons at level of the rhinencephalic responsive point (arrows). Nauta-Gygax's silver impregnation. x 120.

The degenerated fibers from the hemisphere reached the mesencephalon where they occupied the middle third and the bordering medial area of the lateral third of the ipsilateral cerebral peduncle (Fig. 3A, B). They then continued caudally through the basal region of the pons where the bundle became more ventral (Fig. 4). In the middle-caudal sections of the medulla oblongata, the degenerated bundle became thinner and showed a superficial paramedian topography (Fig. 5), while in the spinal cord it occupied the area of the ipsilateral ventral column which borders the ventral medial fissure (Fig. 6A). Only very few axons were present at level of the 3rd and 4th cervical segments (Fig. 6B) where the bundle terminated without crossing. The degenerated fibers were of different diameter. They were finely fragmented and the irregularly shaped particles showed a black staining (Fig. 7).

3. *HRP tracing.* — The injection sites were exactly localized by means of microscopic control of serial sections of the mesencephalon and olfactory bulb (Fig. 8).

The HRP retrograde transport always labeled ipsilateral pyramidal cells in the mapped fronto-parietal area after injections into the cerebral peduncle and into the ventral column of the first cervical spinal segments (Fig. 9A), while labeled neurons were not found in the cortex when the enzyme was injected into the cervical enlargement.

Labeled cells in the fronto-parietal cerebral cortex were more numerous after HRP injection into the cerebral peduncle, than in the olfactory responsive area (Fig. 9B).

Labeled neurons showed their perikarya and often large dendrites filled with
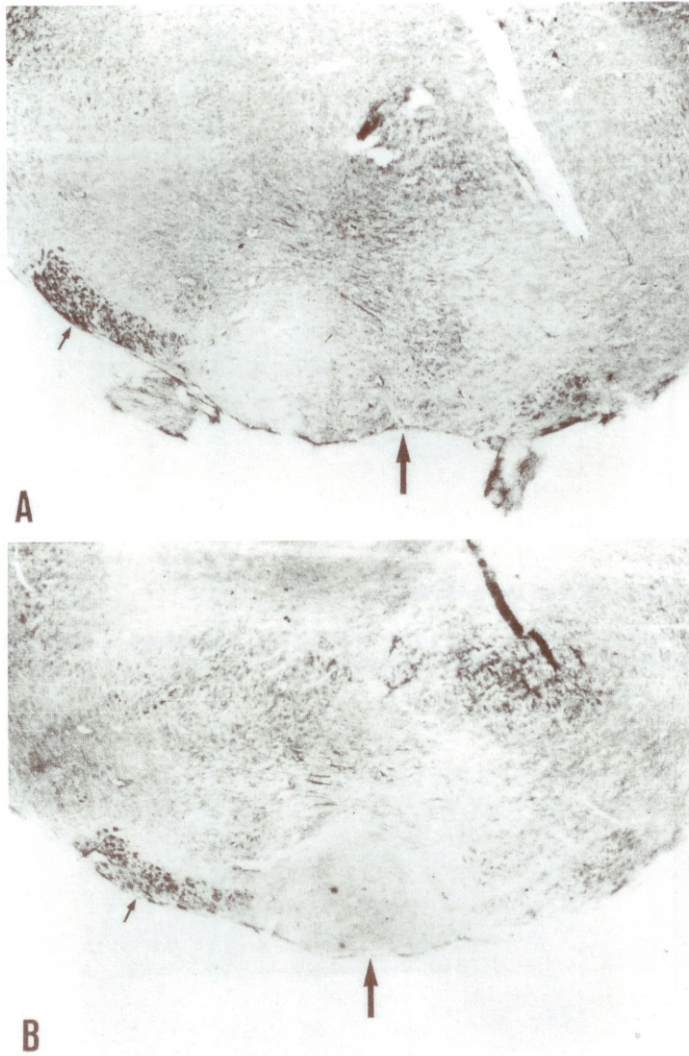
Fig. 3 – *The pyramidal tract of the hedgehog.*

Mesencephalon, transverse section. In the cerebral peduncle the course of the degenerated bundle after excision of the ipsilateral fronto-parietal (A) or rhinencephalic (B) motor areas is indicated (little arrows). Large arrows indicate the midline. Nauta-Gygax's silver impregnation. x 30.

brown-stained granules. Therefore these neurons represent the origin of the axons contained in the above indicated bundle.

HRP-positive neurons were neither seen in the ipsilateral olfactory bulb nor in the contralateral fronto-parietal area.

Fig. 4 – *The pyramidal tract of the hedgehog.*

Metencephalon, transverse section. The arrow indicates the degenerated bundle after ablation of the fronto-parietal area. Nauta-Gygax's silver impregnation. x 30.
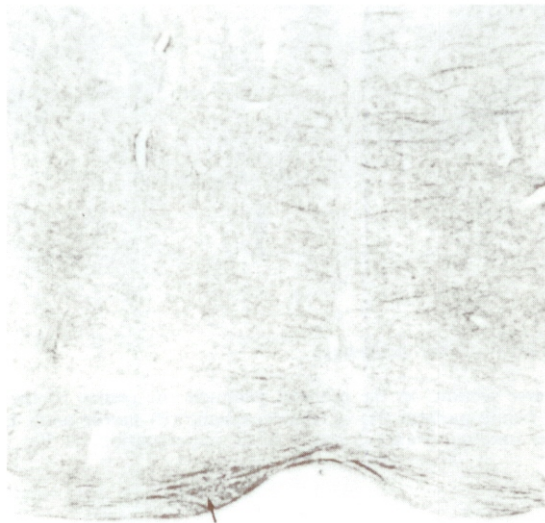

Fig. 5 – *The pyramidal tract of the hedgehog.*

Myelencephalon, transverse section. The more superficial course and the thinning of the degenerated bundle after fronto-parietal motor area excision are indicated by the arrow. Nauta-Gygax's silver impregnation. x 30.
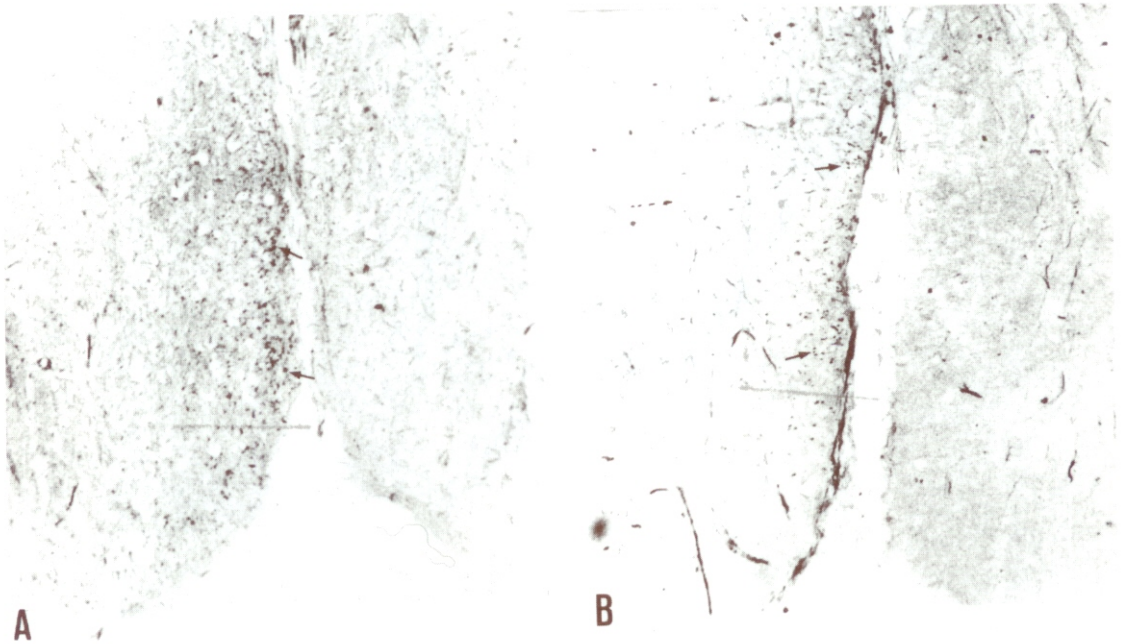
Fig. 6 – *The pyramidal tract of the hedgehog.*

Spinal cord, transverse section. The arrows indicate the location, at level of the first (A) or 3 and 4 cervical segments, of the degenerated axons after ablation of the ipsilateral rhinencephalic motor area. Nauta-Gygax's silver impregnation. x 120.
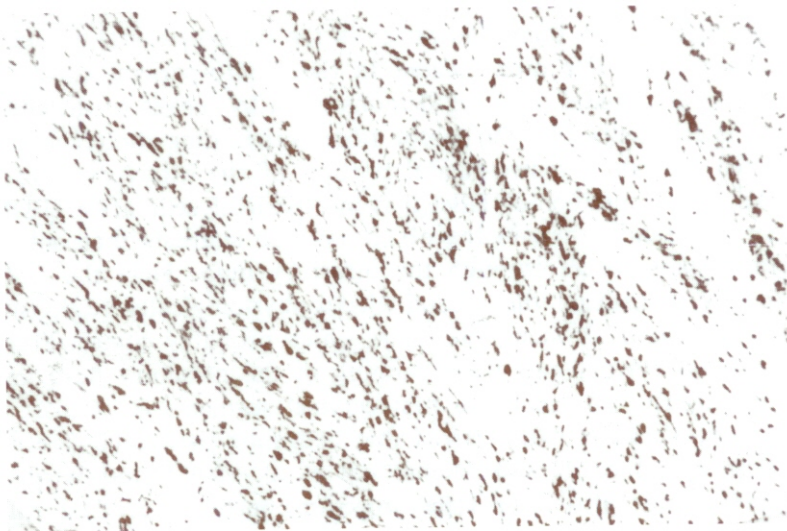

Fig. 7 – *The pyramidal tract of the hedgehog.*

Mesencephalon, longitudinal section. Structural features of the degenerated bundle. Nauta-Gygax's silver impregnation. x 120.
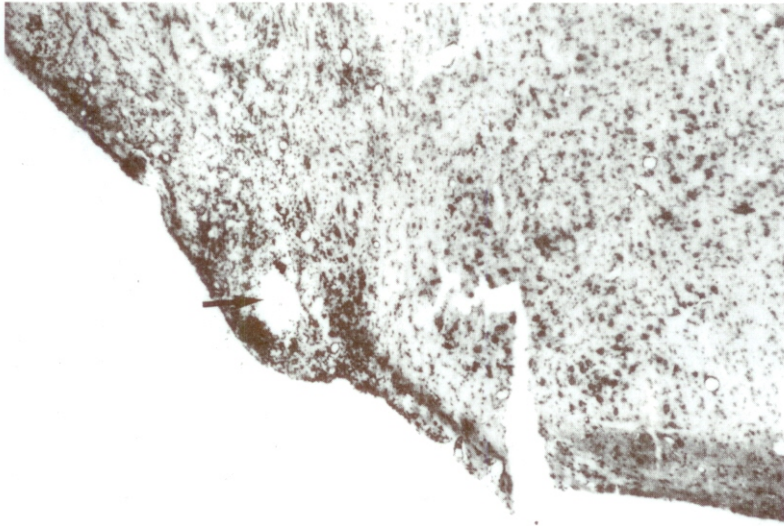
Fig. 8 – *The pyramidal tract of the hedgehog.*

Mesencephalon, transverse section. The arrow indicates the site of HRP injection. Hematoxylin-eosin. x 160.

DISCUSSION

In the present study two motor areas were electrophysiologically identified in the hedgehog hemisphere. The former localized in the fronto-parietal cortex, the latter in the olfactory bulb. The area located in the fronto-parietal cortex represents the origin of the pyramidal tract, as demonstrated by means of degenerative and HRP tracing experiments. All the motor fibers originating there, or a large part of them, reach the latero-ventro-caudal portion of the olfactory bulb (Fig. 10). However, the higher number of labeled fronto-parietal cells after HRP injection in the cerebral peduncle than in the olfactory bulb suggests that only a part of the pyramidal fibers passes through the olfactory bulb. Here the fibers, which run superficially, constitute the second electrophysiologically responsive point.

At the level of the brain stem, the fibers which probably come «directly» from the fronto-parietal cortex, and these running in the olfactory bulb and turning caudally, join together to reach the first cervical segments (C_3-C_4) of the spinal cord. Previously, Verhaart (30) referred that hedgehog pyramidal fibers terminate in the cervical spinal cord, and Michaloudi et al. (20) found labeled neurons in the cerebral cortex also after HRP injections into the cervical enlargement. Therefore, the hedgehog, like opossum, rabbit, guinea pig, pangolin, bat, mole and ungulates (6, 15, 25), is provided with a short pyramidal tract. Moreover, during its whole course the bundle is always ipsilateral and shows no crossing.

This special arrangement of the pyramidal tract may explain some findings of
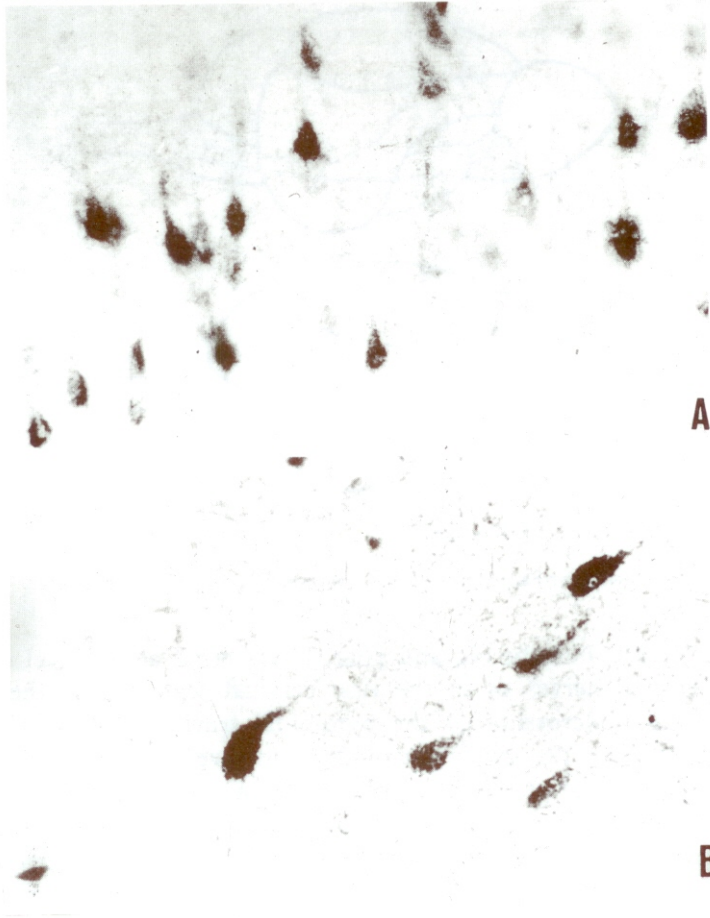
Fig. 9 – *The pyramidal tract of the hedgehog.*

Labelled pyramidal cells of the fronto-parietal motor area after HRP injection into the cerebral peduncle (A) and olfactory bulb (B). HRP method. x 300 (A, B); x 750 (C).

the present research. The olfactory bulb responsive area cannot be considered, of course, as a true motor area, since it is made up of pyramidal fibers coming from the fronto-parietal area. The olfactory region responds to single shocks with a low threshold, since it is constituted by fibers which, in addition, run rather superficially in a restricted area. In contrast, the fronto-parietal area responds to electrical stimulation with a longer latency and only to higher intensity trains of pulses, being constituted by a large pool of neurons distributed in several layers. Finally, the absence of labeled neurons in the contralateral fronto-parietal area after HRP injection into the cerebral peduncle and into the ventral column of the first cervical spinal segments, is due to the total noncrossing of the hedgehog pyramidal tracts.

The lack of a pyramidal crossing is remarkable since also in animals with a
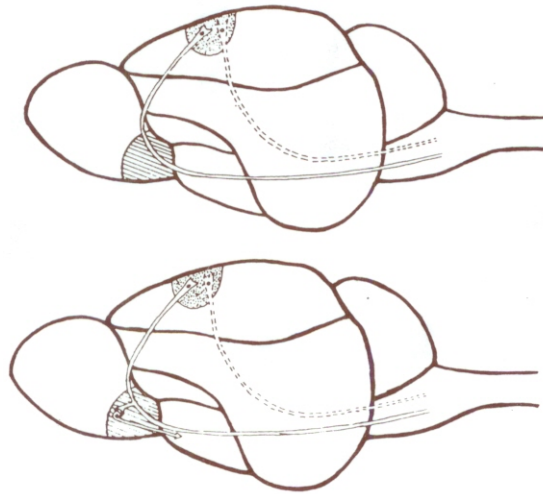
Fig. 10 – *The pyramidal tract of the hedgehog.*

The scheme shows possible arrangements of the pyramidal tract in the hedgehog.

short pyramidal tract, like the pig and goat, fibers cross at the level of the motor nuclei of the cranial nerves or at the first cervical segments of the spinal cord (11, 25). Essential noncrossing of the pyramidal tract is described in the mole (12, 16), Myrmecophaga (8) and Procavia (29). However, in these animals a pyramidal crossing is absent at the level of the medulla, but is described in different segments of the spinal cord. In the hedgehog, on the contrary, the pyramidal bundle does not cross at any level before its termination in the first cervical segments of the spinal cord. This finding is in agreement with the ipsilateral motor response that is elicited by electrical stimulation. On the other hand, the absence of a pyramidal crossing in this mammal was admitted by Kotzenberg (14), Bischoff (2), Draeseke (7), Van der Vloet (27) and more recently by Verhaart (30), while a slight crossing was observed by Broere (3) and Michaloudi et al. (20).

Finally, the unusual presence of pyramidal fibers in the olfactory bulb remains to be clarified. Do they have synaptic relations with the olfactory neurons? If so, the function of motor fibers in the olfactory bulb could be similar to that of the pyramidal fibers ending in nuclei which contribute to ascending systems in other animal species.

SUMMARY

The pyramidal tract of the hedgehog has been investigated. The motor cerebral cortex was first located electrophysiologically and subsequently ablated. Ablation of the motor cortex did not apparently cause motor deficits.

The cerebral hemisphere of the operated side, brain stem and spinal cord were

removed for histological examination. The Nauta-Gygax and the horseradish peroxidase methods were employed to study the course and origin of the above mentioned tract.

The pyramidal tract runs ipsilaterally in the cerebral peduncle, and does not go beyond the first cervical segments of the spinal cord (C_3-C_4); moreover, their fibers do not cross at spinal level. The close relation between this anatomical pathway and the olfactory bulb of the same side is also reported.

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