Serotonin innervation of the cerebral cortex in lizards

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The serotonin (5-HT) innervation of the cerebral cortex in two species of lizards has been studied. Results show no differences between both species. Most of the cerebral cortex of these lizards is innervated by serotoninergic fibers, which are fine and varicose. Their density varies greatly from one cortical region to another: the areas with higher density of serotoninergic fibers and terminals are parts of the medial and dorsal cortices. There is a laminar pattern of distribution of serotoninergic fibers. In the medial cortex, 5-HT fibers are found preferentially in both plexiform layers just above and below the cellular layer. In the dorsomedial cortex, there is an immunoreactive plexus in the outermost third of the superficial plexiform layer and another in the depth of the layer, whereas 5-HT fibers are distributed evenly in the deep plexiform layer of this cortex. In the pars medialis of the dorsal cortex, serotoninergic fibers are abundant in all layers, whereas in the pars lateralis, fibers are found predominantly in the external third of the superficial plexiform layer. The lateral cortex is almost devoid of immunoreactive fibers. These results show a different organization of the cortical serotonin innervation between lizards and turtles.

INTRODUCTION

Serotonin (5-HT) acts in the CNS as a modulatory neurotransmitter and it is implicated in the control of major cerebral functions such as regulation of temperature, sleep-waking cycle and development of nervous system. Serotonin has been demonstrated in the CNS of the main vertebrate groups (see refs. 31, 32 for reviews), and in all cases serotonin neurons are located preferentially in the midline of the brainstem (thus the serotonin system is considered philogenetically old and conservative).

The serotoninergic system of reptiles has been studied using the formaldehyde-induced fluorescence (FIF) technique (see ref. 31 for a review), but this method could not demonstrate all serotonin innervated regions. More recently, with immunocytochemical techniques, using an antiserum of great specificity and selectivity for serotonin, new serotonin cell groups in the brainstem, as well as the major 5-HT pathways, of lizards and turtles have been reported. However, the cortical serotonin innervation of reptiles has been studied in turtles but not in lizards.

The present study was designed to investigate the cortical serotoninergic innervation, using an antiserotonin serum, in two closely related species of lizards: Psammordromus algirus and Podarcis hispanica, the cerebral cortex of which we have previously studied structurally and immunocytochemically.

MATERIALS AND METHODS

Twelve adult lizards, of both sexes and from two species, Psammordromus algirus (7 animals) and Podarcis hispanica (5 animals), were used. Animals were anesthetized with urethane and perfused transcardially with isotonic saline, followed by Bouin's fixative. Fixed brains were embedded in paraffin and sectioned at 10 µm in the transversal or parasagittal plane.

Sections were stained with the unlabeled antibody peroxidase-antiperoxidase technique, using rabbit anti-serotonin serum (Immuno-Nuclear) as primary antibody. All antibody dilutions were made in

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Tris-buffer, pH 7.8, which contained 0.7% lambda carrageenan and 0.5% Triton X-100 (TCT) to avoid non-specific protein binding and improve immuno-reagent penetration. After a careful wash in Tris-buffer, sections were incubated for 18–24 h in anti-serotonin serum diluted 1:1000. The sections were washed again in Tris-buffer and incubated in sheep anti-rabbit IgG diluted 1:35 for 30 min. After a wash in Tris-buffer, the sections were incubated for 30 min in PAP diluted 1:75. The sections were then incubated, for 5–10 min, in Tris-buffer containing 0.2% 3,3'-diaminobenzidine and 0.03% hydrogen peroxide. Finally, the sections were washed in distilled water, dehydrated in ethanol, cleared in xylene and coverslipped. All steps were made at room temperature.

Specificity of the immunoreactivity was ascertained by incubating sections in (1) normal rabbit serum as a substitute of the primary antibody, and (2) primary antibody preadsorbed with serotonin. Preadsorption was accomplished as follows: the primary antibody was shaken with excess amounts of the substrate, and left at 4 °C for 18–48 h. Control sections yielded no reaction product.

Paraffin embedded brains, sectioned at 10 µm and stained with the hematoxylin-eosin method, were used for cytoarchitectural studies.

RESULTS

General cytoarchitecture (Fig. 1)

The cerebral cortex of Psammodromus and Podarcis has been described elsewhere. Briefly, it comprises 4 regions referred to as medial, dorsomedial, dorsal and lateral. Each of these is formed by 3 layers: superficial plexiform layer, cellular layer and deep plexiform layer, from the cortical surface to the ependymal line. The cellular layer of the dorsal cortex overlaps medially the cellular layer of the dorsomedial cortex (superpositio medialis) and laterally the cellular layer of the lateral cortex (superpositio lateralis).

Cortical distribution of 5-HT fibers

5-HT fibers innervating the cerebral cortex of Psammodromus and Podarcis are predominantly fine, with small and spheric varicosities. Cortical 5-HT immunolabeling is similar, in density and distribution, in the different specimens of the two species.

Most of the cerebral cortex of these lizards is innervated by 5-HT fibers, although their density varies greatly from one cortical region to another (Fig. 2). There is a slight increase in the number of 5-HT fibers and terminals at caudal levels, but regional differences subsist from rostral to caudal levels of the cortex.

Medial cortex. (Fig. 3a–d). The medial cortex show a characteristic pattern of innervation. Medial most regions have few 5-HT terminals compared with lateral zones of this cortex (Fig. 2); the 5-HT innervation reaches its maximum at the border with the dorsomedial cortex (Fig. 3d). The laminar distribution of immunoreactive fibers is also heterogeneous. In the superficial plexiform layer, 5-HT fibers are found at all levels, although there is a heavier innervation of the outer third and the zone just above the cellular layer (Fig. 3b and 4). These fibers do not course in a well-defined direction within the layer, but sometimes a preferred orientation parallel to the cortical surface can be observed. The cellular layer of the medial cortex is largely devoid of 5-HT fibers. A few cross through this layer without branching and divide in the superficial plexiform layer (Fig. 3c). In the deep plexiform layer, 5-HT fibers are mainly found just below the cellular layer, whereas the innermost zones of this layer lack immunoreactivity (Fig. 3b,c). Within the deep plexiform layer, immunopositive fibers course in every direction.

Dorsomedial cortex. The 5-HT innervation in the dorsomedial cortex is less intense than in the adjacent regions of the medial cortex (Figs. 2 and 4). The 5-HT fibers are located mainly within both plexiform layers and almost totally lacking in the cellular layer. There is a 5-HT plexus in the outermost third of the superficial plexiform layer and another in the depth of this layer, above the somata of the cellular layer (Fig. 4). Immunoreactive fibers spread homogeneously within the deep plexiform layer.

Dorsal cortex. The dorsal cortex shows marked heterogeneity of its serotonin innervation. Its medial portion has the densest terminal field of all cortex, whereas its lateral zones are provided with only scarce fibers (Figs. 2, 5). In the pars medialis of the dorsal cortex, 5-HT fibers are abundant in all layers,
even the cellular layer, which here is formed by loosely packed cell somata. In the deep plexiform layer, a few fibers may be seen around cell clusters (Fig. 6), but 5-HT fibers are distributed rather evenly throughout this layer. In the pars lateralis of the dorsal cortex, innervation is much less dense; 5-HT terminals are found in both plexiform layers but predominantly in the external third of the superficial plexiform layer (Fig. 5).

Lateral cortex. The lateral cortex is the region with the least 5-HT innervation in the cerebral cortex (Figs. 2 and 7); very few 5-HT fibers are found in its superficial plexiform layer, preferentially at caudal levels of the cortex (Fig. 7).

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**Fig. 1.** Detail of a transverse section of the telencephalon showing the cerebral cortex and two subcortical areas: anterior dorsal ventricular ridge (ADVR) and septum (S). The cerebral cortex consists of 4 regions: medial (M), dorsomedial (DM), dorsal (D) and lateral (L) cortices. Each of them consists of 3 layers: superficial plexiform layer (spl), cellular layer (cl) and deep plexiform layer (dpl). Arrow indicates the superpositio medialis. Hematoxylin-eosin. ×100.

**Fig. 2.** Immunostained section at a similar level to Fig. 1. A differential innervation of the cerebral cortex can be observed. M, medial cortex; DM, dorsomedial cortex; D, dorsal cortex; L, lateral cortex; S, septum; ADVR, anterior dorsal ventricular ridge. ×100.
DISCUSSION

Specificity

Specificity of anti-serotonin serum (Immuno Nuclear) is well documented. In order to prove its applicability in the lacertilian nervous system, we carried out the controls mentioned in Materials and Methods. However, we cannot rule out the possibility of cross-reaction of the antiserum with unknown tissular antigens.

Cortical distribution

Our results demonstrate a wide serotoninergic innervation in the lacertilian cerebral cortex. This innervation is not uniform but presents an important regional variation; parts of the medial and dorsal cortices are the areas with higher density of 5-HT fibers. Pioneer studies in reptiles5-30,33 using the FIF technique could not demonstrate 5-HT fibers in cortex. With the development of immunocytochemical methods and the use of anti-serotonin serum38, serotoninergic fibers have been demonstrated in the cerebral cortex of turtles40. This is the only study carried out in reptiles where a cortical 5-HT innervation is reported, thus we compare our results in lizards with that of turtles, although the cortical organization of these two groups of reptiles may be different.

The 5-HT innervation of the medial cortex of Psammodromus and Podarcis is clearly different to that of Clemmys japonica40. The main difference affects the cellular layer which, in lizards, lacks immunoreactive fibers — except a few ones crossing the layer —, whereas in Clemmys the cellular layer present a dense innervation.

The reptilian medial wall, including medial and dorsomedical cortices, is generally considered homologous to the mammalian hippocampal formation in basis of its medial cortical position and its connections with other cortical and thalamic regions2,6,25,26. However, there is controversy about which, if any, of the zones of the medial wall is homologous to particular regions of the hippocampal formation4,22,27. The mammalian hippocampal formation has a distribution of 5-HT fibers that closely resembles that of the medial wall of Psammodromus and Podarcis. Thus, in rats24, using an antiserum against serotonin, a dense band of 5-HT fibers and terminals is observed just underlying the granular layer of the dentate gyrus, whereas much less immunoreactive fibers are found in the rest of the polymorphic layer, in the cellular layer and in the molecular layer. In the dentate gyrus of monkeys1, 5-HT fibers are preferentially observed in the external half of the molecular layer, whereas the internal half and the cellular layer show very few radially oriented fibers. The hilus of the dentate gyrus contains a dense plexus of 5-HT fibers, especially just below the cellular layer.

In Psammodromus and Podarcis, the 5-HT innervation of the medial cortex presents surprising similarities with the dentate gyrus of rats24 and monkeys1. Whether both regions are homologous awaits demonstration and further investigations are needed in these and other reptiles. Besides, the 5-HT innervation of the lacertilian medial cortex shows regional differences along its medial-to-lateral extension: 5-HT fibers are denser in the boundary between the medial and dorsomedical cortices. This could be related to a functional subdivision of the medial cortex, as has been suggested in other lizards6.

The origin of 5-HT fibers innervating the medial cortex may be different from those innervating the dorsomedical and dorsal cortices, since, using the HRP method6, afferences to the medial cortex of lizards are not observed caudally to the diencepha-

Fig. 3. a: detail of the medial (M) and dorsomedical (DM) cortices. Similar regions to each one of the squared areas are immunostained in b–d. S, septum. Hematoxylin-eosin. b: most of the 5-HT fibers are located in the superficial plexiform layer (spl) and in the outer third of the deep plexiform layer (dpl). Some varicose fibers (arrows) cross the cellular layer (cl). c: a prominent plexus is confined to the external half of the deep plexiform layer (dpl). Varicose fibers (arrows) cross the cellular layer (cl) and branch in the superficial plexiform layer (spl). d: boundary between the medial (M) and dorsomedical (DM) cortices, which presents a dense 5-HT innervation. cl, cellular layer, a: ×106; b–d: ×198.

Fig. 4. Parasagittal section of the telencephalon showing the 5-HT innervation of the medial (M) and dorsomedical (DM) cortices. spl, superficial plexiform layer; cl, cellular layer; dpl, deep plexiform layer; D, dorsal cortex.
Ion, whereas projections to the dorsomedial and dorsal cortices from the nucleus raphe superioris can be distinguished. However, the same authors find afferences to the medial cortex from the paraventricular hypothalamic organ, which, in reptiles, has serotoninergic cells. Direct projections from 5-HT cells in the paraventricular hypothalamic organ to the telencephalon have already been observed in lampreys although not in turtles. The existence of these monoaminergic afferences from the paraventricular hypothalamic organ to certain telencephalic regions could trigger or modulate activity of these regions according to different functional states of the hypothalamus, or even with respect to concentration of diverse hormones or chemical substances in the CSF.

Studies in turtles show a wide innervation of the dorsal cortex, the molecular layer presenting the densest innervation. This contrasts with our results in Psammodesmus and Podarcis, in which the medial-most part of dorsal cortex receives a heavy 5-HT innervation in all its layers, whereas the lateral

![Fig. 5. Detail of the dorsal cortex (D). Its medial-most regions present a dense 5-HT innervation in all layers. DM, dorsomedial cortex; cl, cellular layer. ×160.](image)

![Fig. 6. Immunoreactive fibers surround cell clusters (triangles) in the deep plexiform layer (dpl) of the dorsal cortex. cl, cellular layer. ×243.](image)

![Fig. 7. General view of the lateral cortex (L). Few 5-HT fibers (arrows) can be observed in the superficial layer of the cortex. cl, cellular layer; ADVR, anterior dorsal ventricular ridge. ×179.](image)
part is loosely innervated. These differences in the 5-HT innervation of the dorsal cortex between turtles and lizards add new arguments in favor of a different organization of the cerebral cortex in these groups of reptiles as it has been previously suggested.\(^6,16,26,27\)

Regarding a possible homology of the lacertilian dorsal cortex with cortical regions of mammals, debate is still open. In turtles it seems clear that the dorsal cortex or part of it may be homologous to visual cortical areas of mammals, basically due to its affinences from the lateral geniculate nucleus\(^15,16,28\), but in lizards, in which such geniculate-cortical projection is not demonstrated\(^6,19,26\), a similar homology cannot be stated. It has been suggested that the lacertilian dorsal cortex could have a limbic function\(^27,19,26,35\), although this term is very imprecise as it does not make reference to any determined cerebral region. Furthermore, it seems clear that within the dorsal cortex a subdivision exists, either structural\(^8,14\), of connections\(^6\), or functional (this study shows a differential 5-HT innervation of medial and lateral parts of the lacertilian dorsal cortex). The 5-HT fibers in the dorsal cortex may originate in the nucleus raphe superioris, since this nucleus has serotoninergic cells\(^5,41\) and projects to the entire dorsal cortex\(^6\).

The lateral cortex of *Psammomus* and *Podarcis* is almost devoid of 5-HT fibers, whereas the 5-HT innervation of the turtle lateral cortex is very dense\(^40\). Lack of immunoreactivity in the lacertilian lateral cortex agrees with the lack of projections from the nucleus raphe superioris in lizards\(^6\).

A series of conclusions may be extracted from the present study. First, the existence of a differential 5-HT innervation within the medial and dorsal cortices agrees with a suggested functional subdivision of these areas. Second, new arguments are added in favor of the possible homology between reptilian medial cortex and parts of mammalian hippocampal formation (dentate gyrus?). And lastly, we share the view concerning the probability of the existence of variations in organization and function between the cortices of lizards and turtles.

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