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Differences in dopamine β-hydroxylase immunoreactivity between the brains of genetically epilepsy-prone and Sprague–Dawley rats

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Biochemical studies have indicated that norepinephrine is present in lower levels in certain brain regions of genetically epilepsy-prone rats (GEPR-9s) as compared to non-epileptic Sprague–Dawley (SD) rats. In this study, the immunocytochemical localization of dopamine β-hydroxylase (DBH), the synthesizing enzyme for norepinephrine, was compared between GEPR-9s and SD rats. Brain regions caudal to the inferior colliculus, such as the cerebellum and locus coeruleus, showed no differences in the distribution of DBH-like immunoreactive (DBH-I) neurons and fibers. In contrast, differences in the distribution of DBH-I fibers were observed in more rostral brain regions including the central nucleus of the inferior colliculus, thalamus, piriform, orbital and somatosensory cortices and hippocampus. In these areas, the number, and often the staining intensity, of DBH-I processes was lower in GEPR-9s as compared to SD rats. It was interesting to note that other cortical regions displayed no differences in DBH immunoreactivity between GEPR-9s and SD rats. These results provide anatomical data that support previously described biochemical results. Furthermore, the reduced number of fibers and their decreased staining intensity in specific brain regions provide greater details to resolve the localization of deficiencies in the noradrenergic fiber plexus of GEPR-9s.

INTRODUCTION

A well characterized genetic model of epilepsy is the genetically epilepsy-prone rat (GEPR) which exhibits motor seizures in response to intense auditory stimuli[25]. There exist 2 colonies of GEPRs, GEPR-3 and GEPR-9, which display moderate and severe seizures, respectively. Classification of rats into either of these 2 colonies is based upon specific behavioral criteria; GEPR-3s are identified as having clonic convulsions whereas GEPR-9s are described as having tonic extensor convulsions[25].

Numerous studies have indicated that biochemical abnormalities occur in neurotransmitter systems in the brains of GEPRs and some of these changes appear to be correlated with seizure severity[15]. The early pharmacological analysis of the monoaminergic systems established a reciprocal relationship of drug-induced changes in noradrenergic or serotonergic activity to alterations in seizure intensity[16]. Thus, increases in the severity of

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seizures were associated with decreases in monoaminergic activity[9]. In addition, it has been reported that GEPR progeny which were not susceptible to seizures became susceptible following drug-induced monoaminergic depletion[8]. Taken together, these data suggest that the monoamine systems play a role in both the susceptibility to seizures as well as the intensity of seizures. More recently, studies have demonstrated that lower amounts of norepinephrine and its markers (high affinity uptake and DBH activity) occur in all major brain areas except the cerebellum and striatum of GEPR-9s as compared to Sprague–Dawley (SD) rats[3,10,14,16].

Although pharmacological and biochemical results have demonstrated defects in the noradrenergic system of GEPRs, the anatomy of this system has yet to be described in GEPRs. However, anatomical studies of another neurotransmitter system that uses γ-aminobutyric acid (GABA) have revealed significant differences between the brains of GEPRs and SD rats. Specifically, increased numbers of GABAergic neurons were found in the central nucleus of the inferior colliculus of GEPR-9s as compared to SD rats[28,29]. These data were corroborated by a biochemical study that showed increased levels of GABA, glutamate, and taurine in the inferior colliculus of GEPR-9s[26]. Other data have shown a role for GABA in the inferior colliculus in that GABA has been demonstrated to reduce the incidence and severity of audiogenic seizures when either its synaptic concentration is increased or its postsynaptic effect is mimicked by drugs[5]. Furthermore, the GABA antagonist, bicuculline, has been shown to induce audiogenic seizure-like activity in rats when injected into the inferior colliculus[5,22]. These observations; as well as the pharmacological data of Faingold et al.[6], strongly suggest that an abnormal GABA system in the inferior colliculus may play a role in seizure expression.

The purpose of the present study was to determine whether anatomical differences in the noradrenergic system exist between the brains of GEPR-9s and non-epileptic SD rats. This system has been extensively described in the normal SD rat and consists of numerous cell bodies located mainly in the locus coeruleus and an associated fiber plexus that innervates virtually all brain areas[18–20,30]. Immunocytochemical methods to localize dopamine β-hydroxylase (DBH), the synthesizing enzyme that oxidizes dopamine to form norepinephrine, were utilized. This enzyme is not present in dopamine- or indolamine-containing neurons and, therefore, it is a reliable marker for the noradrenergic system. Although DBH is present in epinephrine-containing neurons, their localization to only the hypothalamus provides for an analysis of norepinephrine labeled cells and processes in virtually all of the remaining brain regions in immunocytochemical preparations obtained with an anti-DBH serum.

METHODS

Six pairs of adult male GEPR-9s and Sprague–Dawley (SD) rats (Simonsen Laboratories) were used in this study. All animals were tested for audiogenic seizures prior to sacrifice in a padded sound chamber equipped with 2 electric doorbells. Seizures that were characterized by an initial wild running phase which progressed into a full tonic extensor convulsion were exhibited by all GEPR-9s[11]. SD rats showed no seizure behavior during testing. SD rats were chosen as the control animal because the GEPR-9s were originally derived from this strain of rat[16].

All animals were deeply anesthetized with sodium pentobarbital and intracardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.2 (25 °C). Following the perfusions, the brains were removed from their crania and post-fixed in 4% paraformaldehyde in PB for 2 h and then transferred to 30% sucrose in PB overnight at 4 °C. The brains were cut in either sagittal or coronal planes at 30 μm thickness using a freezing microtome, and sections were collected in cold PB. Sections from each pair of animals were processed simultaneously for the immunocytochemical localization of dopamine β-hydroxylase (DBH) with a rabbit anti-DBH serum (1:1000) (Eugene Tech. Int., Inc.) that was detected by an avidin–biotin–peroxidase complex (Vector Lab.).

Briefly, free-floating sections were washed in cold PB and then incubated in anti-DBH diluted

with PB containing 0.3% Triton X-100 and 1% normal goat serum (NGS) for 24 h at 4 °C. The sections were washed the following day in PB and incubated in biotinylated anti-rabbit IgG (1:200) in PB that included 0.3% Triton X-100 and 1% NGS for 1 h at room temperature. Following a series of washes, the tissue was then incubated in an avidin–biotin complex (1:100) diluted with PB containing 0.3% Triton X-100 for 1 h at room temperature. After a final washing, the tissue was reacted in a solution of saturated 3,3′-diaminobenzidine (Sigma) in PB containing 0.01% H_2O_2. The sections were then rinsed in PB, mounted onto gelatin-coated glass slides, dehydrated through a series of alcohols, cleared through xylene, and overlaid with Permount and coverslips. Additional sections were mounted onto slides and stained with cresyl violet to determine cytoarchitectonic and nuclear boundaries.

The anti-DBH serum was fully characterized by Joh and Ross[12]. Sections that were incubated as described above with the omission of primary antiserum showed no immunoreactivity. All brain sections were analyzed by light microscopy. The nomenclature of brain regions was based on the atlas by Paxinos and Watson[24].

RESULTS

DBH-like immunoreactive (DBH-I) staining was observed in all sections from both SD rats and GEPR-9s that were processed with anti-DBH serum. The distribution of labeled neurons in the locus coeruleus (Fig. 1) and associated subnuclei was similar to that previously described in the rat by Swanson and Hartman[31]. In addition, the DBH-I fiber plexus that emanates from the locus coeruleus and distributes itself throughout the brain was similar to that previously described[31]. In both GEPR-9 and SD rat brains, DBH-I fibers displayed numerous varicosities irregularly placed along their extent. These swellings correspond to sites of vesicle accumulation and synaptic contacts[4]. Sections from GEPR-9s and SD rats were analyzed and compared. Brain regions caudal to the inferior colliculus, such as the cerebellum and locus coeruleus, showed no differences in the staining pattern. However, differences in staining were observed for DBH-I fibers in more rostral structures. The following sections will describe selected brain regions and the differences as well as similarities in DBH-I staining observed between GEPR-9s and SD rats.

Locus coeruleus

DBH-I neurons were intensely stained in both GEPR-9s and SD rats in the locus coeruleus (LC) (Fig. 1). The number of labeled cells and the area occupied by them appeared to be similar for both types of rat. These DBH-I neurons were generally densely packed together and it was difficult to distinguish individual neurons because labeled cell bodies and dendrites overlapped in the sections. In the case when individual DBH-I neurons were observed, they were found to be either multipolar or fusiform. Immunoreactive processes were observed extending from DBH-I cell bodies. For SD rats, numerous intensely immunoreactive processes could be followed rostrally from the LC into the dorsal noradrenergic bundle through the midbrain tegmentum area (Fig. 2A). In contrast, a smaller number of lightly immunoreactive fibers were observed to extend rostrally into the midbrain of GEPR-9s (Fig. 2B). The ventral noradrenergic bundle in GEPR-9s and SD rats appeared similar.

Cerebellum

The cerebellum showed similar immunostaining patterns for both GEPR-9s and SD rats. Numerous DBH-I fibers were observed within the granule cell layer and many processes extended into the molecular layer (Fig. 3). Often immunoreactive fibers were found apposed to Purkinje cell bodies before they entered the molecular layer where they appeared to follow their dendritic arbors. As these fibers reached the pial surface in the outer molecular layer, they would turn to lie parallel with it. The white matter of the cerebellar cortex contained only a few DBH-I fibers.

Inferior colliculus

One area that showed distinctive differences in DBH-I staining between SD and GEPR-9 brains was the inferior colliculus. The inferior colliculus in SD rats contained a moderate number of dense-

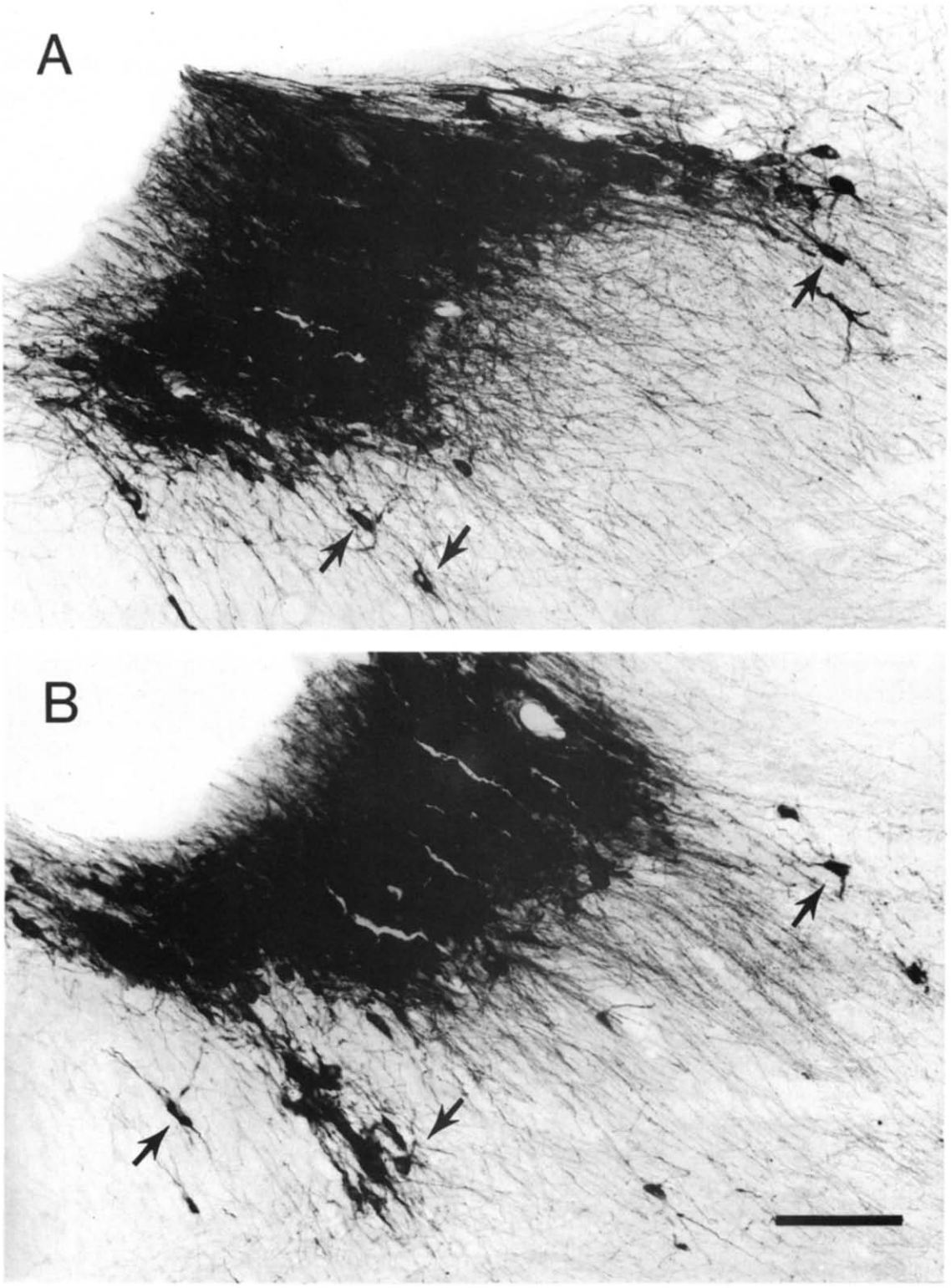

Fig. 1. Brightfield photomicrographs of DBH-I neurons in parasagittal sections through the locus coeruleus from an SD rat (A) and a GEPR-9 (B). Although intense labeling of DBH-I perikarya and processes in this nucleus tended to obscure the resolution of individual cells, a few identifiable DBH-I neurons were observed on the periphery (arrows). Calibration bar = 150 μm.

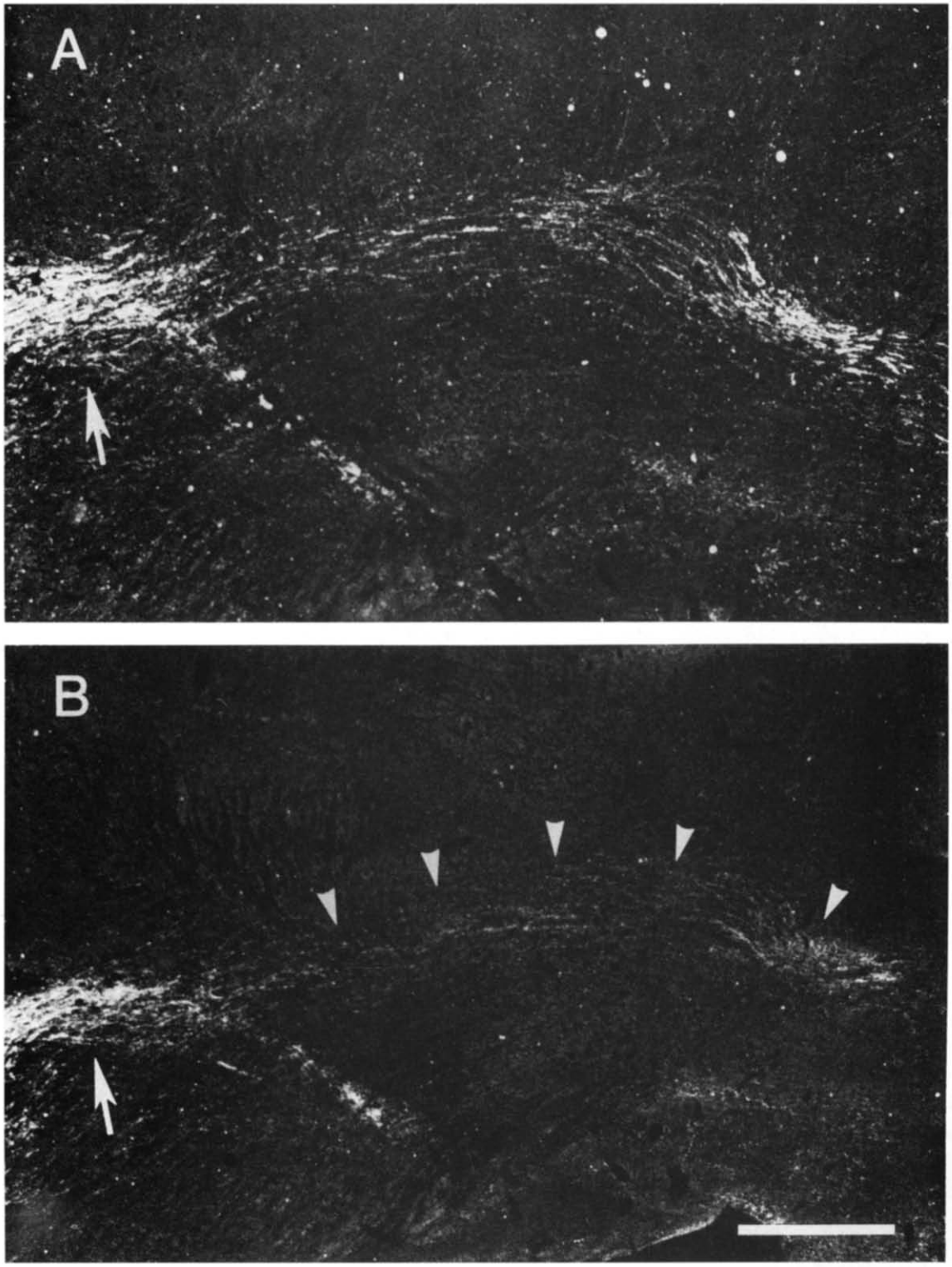

Fig. 2. Darkfield photomicrographs of parasagittal sections through the midbrain of an SD rat (A) and a GEPR-9 (B) processed for the localization of DBH immunoreactivity. Arrows indicate DBH-I fibers emerging from the locus coeruleus (not shown) that display a similar intensity of staining between animals. However, the dorsal noradrenergic bundle (arrowheads) of the GEPR-9 appeared to contain a smaller number of DBH-I fibers as compared to the SD rat. Calibration bar = 770 μm.

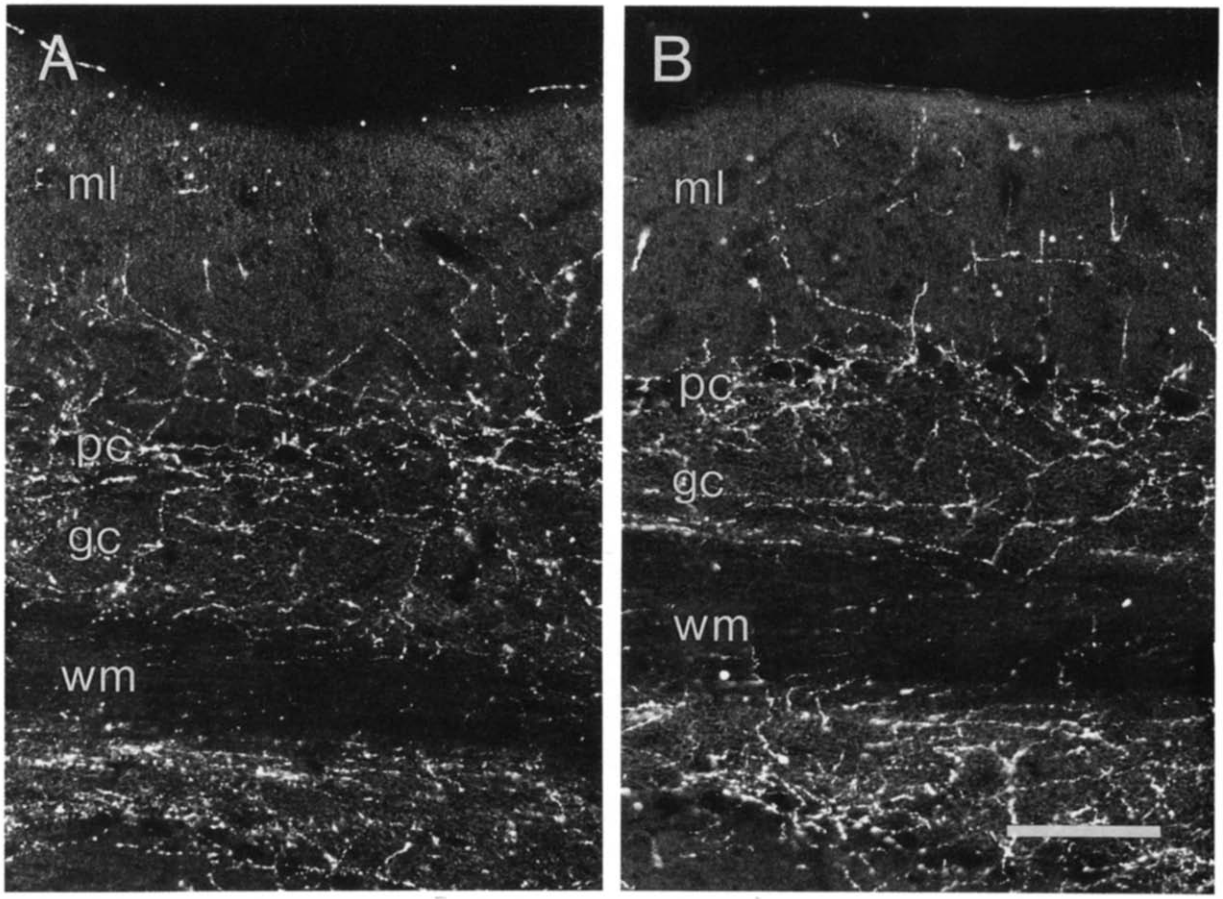

Fig. 3. Darkfield photomicrographs illustrating DBH-I fibers in parasagittal sections of the cerebellum from an SD rat (A) and a GEPR-9 (B). The distribution and intensity of DBH-I fibers in the cerebellar cortex appeared to be similar between the SD rat and GEPR-9. Abbreviations: wm, white matter; gc, granule cell layer; pc, Purkinje cell layer; ml, molecular layer. Calibration bar = 125 μm.

ly stained immunoreactive fibers in all subregions. However, the dorsal and external cortices contained relatively more DBH-I processes than the central nucleus (Fig. 4A and C).

In general, the DBH-I staining pattern in the GEPR-9 inferior colliculus appeared to contain fewer DBH-I fibers than that found in SD rats. The subregion of the inferior colliculus that showed the largest reduction in both the number and staining intensity of DBH-I fibers was the central nucleus (Fig. 4B and D). This finding was observed in 5 out of 6 paired cases. In 3 out of 6 cases, the intensity of immunoreactivity as well as the numbers of DBH-I fibers in the external cortex of the GEPR-9 appeared to be slightly lower than in the SD rat.

Superior colliculus

A large number of DBH-I fibers were observed in the superficial gray and zonal layers of the superior colliculus in both GEPR-9s and SD rats. The number of fibers and intensity of immunostaining appeared similar for the 2 types of rat in these layers. In comparison, a reduced number of DBH-I fibers was observed in the deeper layers of the superior colliculus in both GEPR-9s and SD rats. Although the staining intensity of fibers in the deeper layers was similar for GEPR-9s and SD rats, the number of fibers appeared to be slightly reduced in GEPR-9s in 2 out of 6 cases.

Thalamus

In the non-epileptic SD rat, DBH-I processes

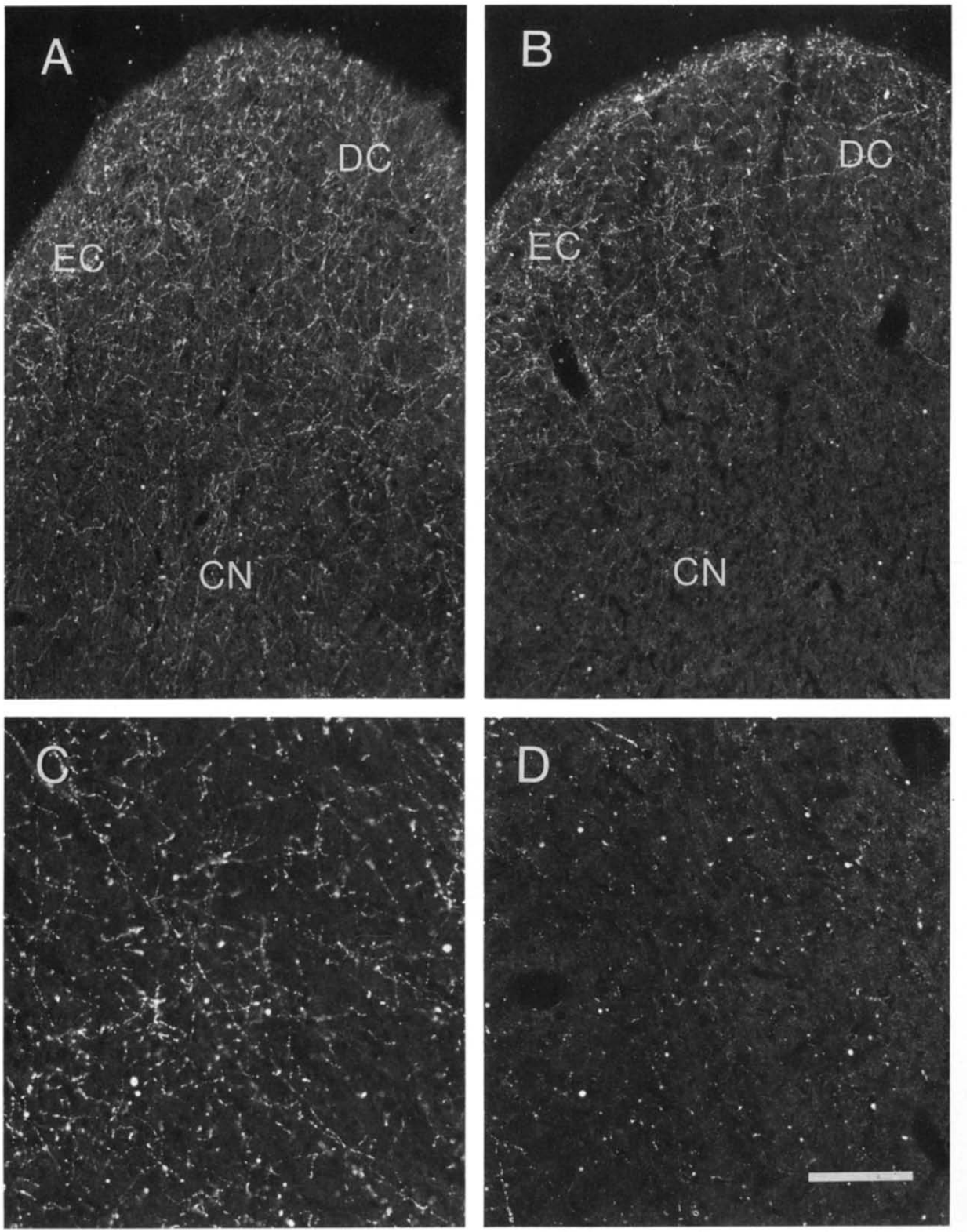

Fig. 4. Darkfield photomicrographs showing the distribution of DBH-I in the inferior colliculus (A and B) and, at higher magnification, the central nucleus (CN) of the inferior colliculus (C and D) in an SD rat (A and C) and GEPR-9 (B and D). In both the SD rat (A) and the GEPR-9 (B), a moderate number of DBH-I fibers were observed within the dorsal and external cortices (DC and EC, respectively). However, a striking difference was observed in the CN where DBH-I processes were virtually absent in the GEPR-9 (D) as compared to the SD rat (C). Calibration bar = 150 and 100 μm for A and B, and C and D, respectively.

were observed to be moderately distributed throughout the thalamus (for a complete description, see Swanson and Hartman[31]). In comparison, the thalamus of GEPR-9s in 5 out of the 6 paired cases displayed a reduced number of immunoreactive processes. For example, a large reduction of immunoreactive fibers was observed in the thalamic reticular and ventral posterior lateral nuclei of GEPR-9s as compared to SD rats (Fig. 5). In addition, reduced numbers of DBH-I fibers were also observed within the ventral posterior medial, medial geniculate (particularly the ventral aspect), dorsal lateral geniculate, and anterior thalamic nuclei.

Striatum

Fibers immunoreactive for DBH were seldom observed in the striatum of either GEPR-9 or SD rats. In fact, this region was the most sparsely innervated area examined in the present study. Occasionally, a small number of DBH-I fibers were found to course through the internal capsule fascicles as well as the striatal gray matter. The number of DBH-I fibers did not differ between GEPR-9s and SD rats.

Cerebral cortex

DBH-I fibers were found distributed throughout all areas of the cerebral cortex in both GEPR-9s and SD rats. The cortical distribution of noradrenaline-containing fibers has been previously described in the rat[17,23] and will not be detailed here. Numerous regions showed no obvious differences in the distribution of DBH-immunoreactivity between GEPR-9s and SD rats. However, several cortical areas exhibited striking differences. The following description will focus on these latter regions.

The SD piriform cortex contained more DBH-I fibers as compared to that of GEPR-9s (Fig. 6). In the SD rats, a dense network of fibers was observed within layer 3 and many fibers appeared to course through layer 2 toward the pial surface where they typically curved to lie parallel to it. Thus, many DBH-I fibers oriented parallel to the pial surface were observed in the outer half of layer 1. In addition, numerous lightly labeled fibers were found within the lateral olfactory tract (Fig. 6A). In contrast, the GEPR-9 piriform cortex displayed a smaller number of DBH-I processes, and they often appeared to have reduced staining intensity as compared to SD rats (Fig. 6B). Layer 1 displayed the most dramatic difference. A reduction in the number of DBH-I fibers was also observed within the deeper layers of piriform cortex.

Two other areas of cortex, orbital and somatosensory, displayed significant differences in the distribution of DBH-I fibers. The ventral lateral orbital and lateral orbital cortices of GEPR-9s contained reduced numbers of DBH-I fibers in comparison to SD rats in all layers except layer 1 where DBH-I fibers appeared relatively similar in number (4 out of 6 cases) (Fig. 7A and B). Somatosensory cortex also displayed differences between GEPR-9s and SD rats. Fig. 7C and D illustrate the reduced number and staining intensity of DBH-I fibers within somatosensory cortex of GEPR-9s as compared to SD rats. It should be noted that this cortical region in both SD rats and GEPR-9s appeared to contain smaller numbers of DBH-I fibers than adjacent areas of neocortex.

In summary, cortical regions that showed differences in the distribution and staining of DBH-I fibers included the piriform, orbital and somatosensory cortices. In contrast, other regions (occipital and temporal cortices) displayed no observable differences.

Hippocampus

Most regions of the hippocampus showed dramatic differences in the number and staining intensity of DBH-I fibers between GEPR-9s and SD rats in all cases (Fig. 8). In general, DBH-I processes were observed in all hippocampal regions in both GEPR-9s and SD rats. The complete distribution of noradrenergic fibers in the SD hippocampus has been previously described[21,31]. Consistent with these findings, the SD rat displayed the largest number of immunoreactive axons within stratum lacunosum-moleculare of the CA1 pyramidal cell layer (terminal site of perforant path and Schaffer collateral fibers), stratum lucidum of the CA3 pyramidal cell layer (terminal site of mossy fibers), and the molecular layer and hilus of the dentate gyrus. The other layers of these hippocampal subregions had smaller numbers of DBH-I fibers.

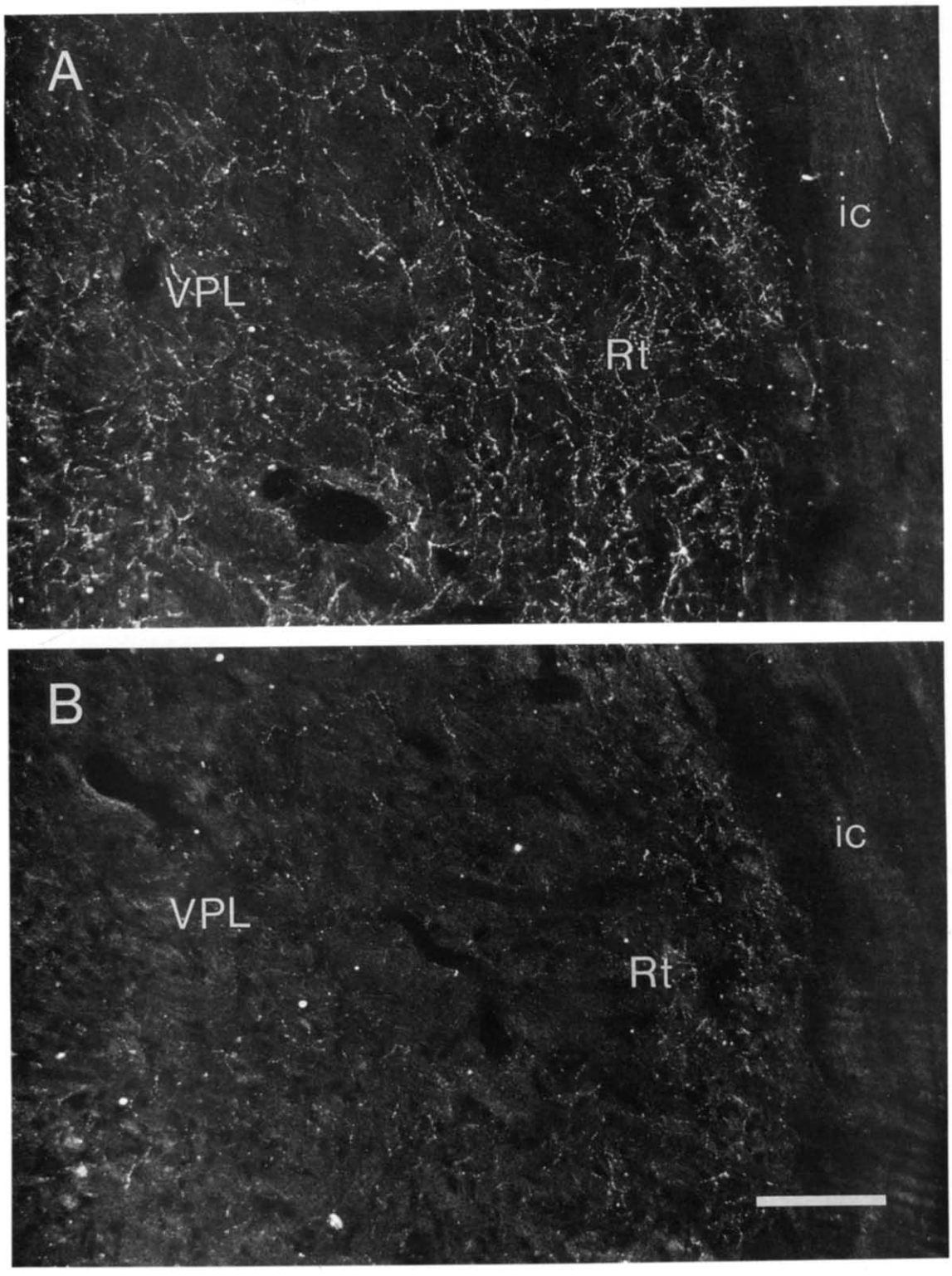

Fig. 5. Darkfield photomicrographs from 2 thalamic nuclei illustrating differences in the distribution of DBH-I between an SD rat (A) and a GEPR-9 (B). Although DBH-I processes were observed throughout the thalamus of SD rats, only occasional immunoreactive processes were seen within the thalamus of the GEPR-9s. Abbreviations: Rt, reticular nucleus; VPL, ventral posterior lateral nucleus; ic, internal capsule. Calibration bar = 150 μm.

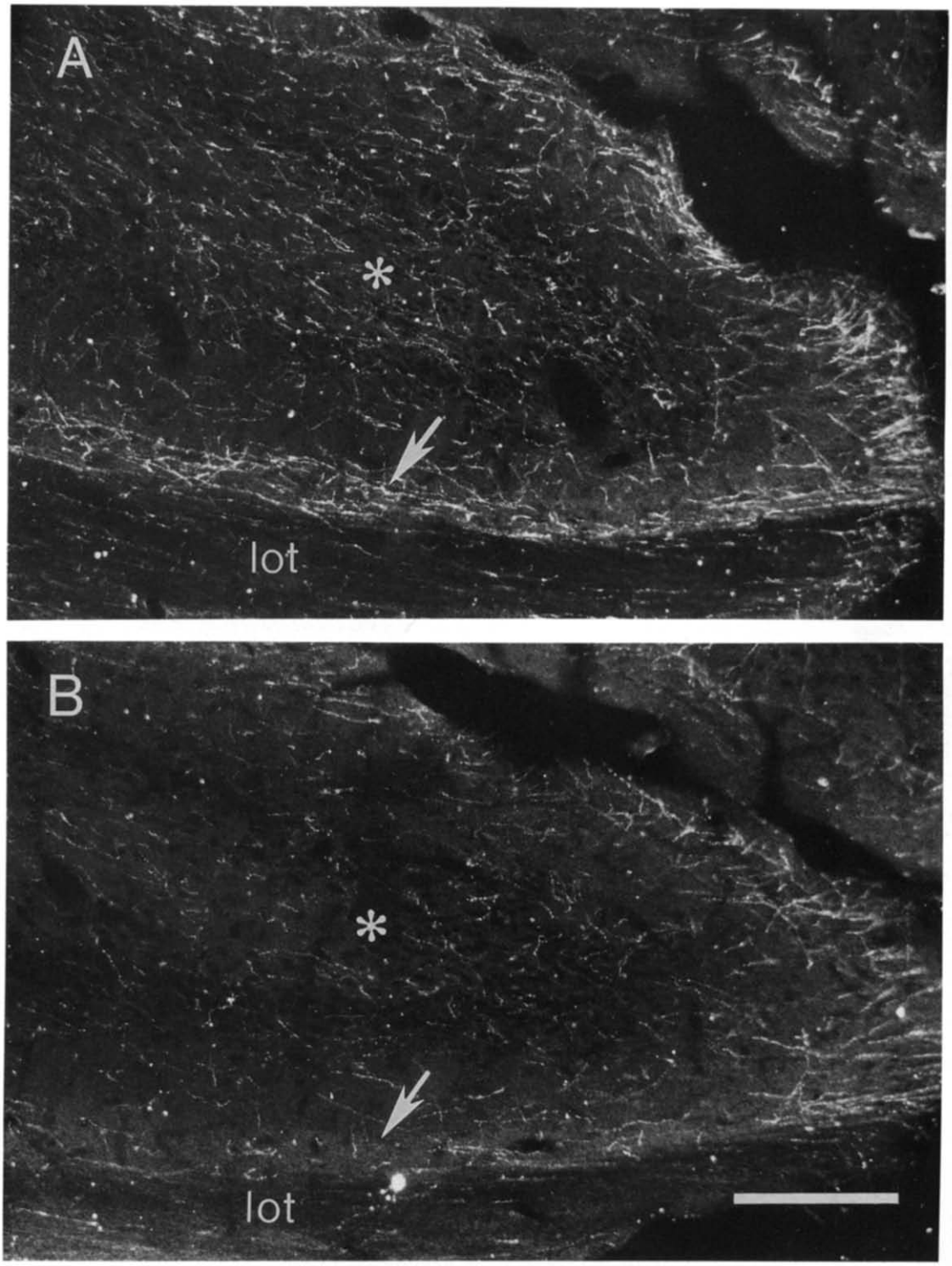

Fig. 6. Darkfield photomicrographs showing DBH-I processes in parasagittal sections through the piriform cortex of an SD rat (A) and GEPR-9 (B). In the SD rat (A), a dense network of fibers was observed within layer 3 (asterisk), layer 2, and layer 1. The latter layer displayed many fibers that were oriented parallel to the lateral olfactory tract (lot) (arrow) and pial surface. In comparison, fewer DBH-I fibers were observed in the GEPR-9 piriform cortex (B); most notably within layer 3 (asterisk) and layer 1 (arrow). Calibration bar = 200 μm.

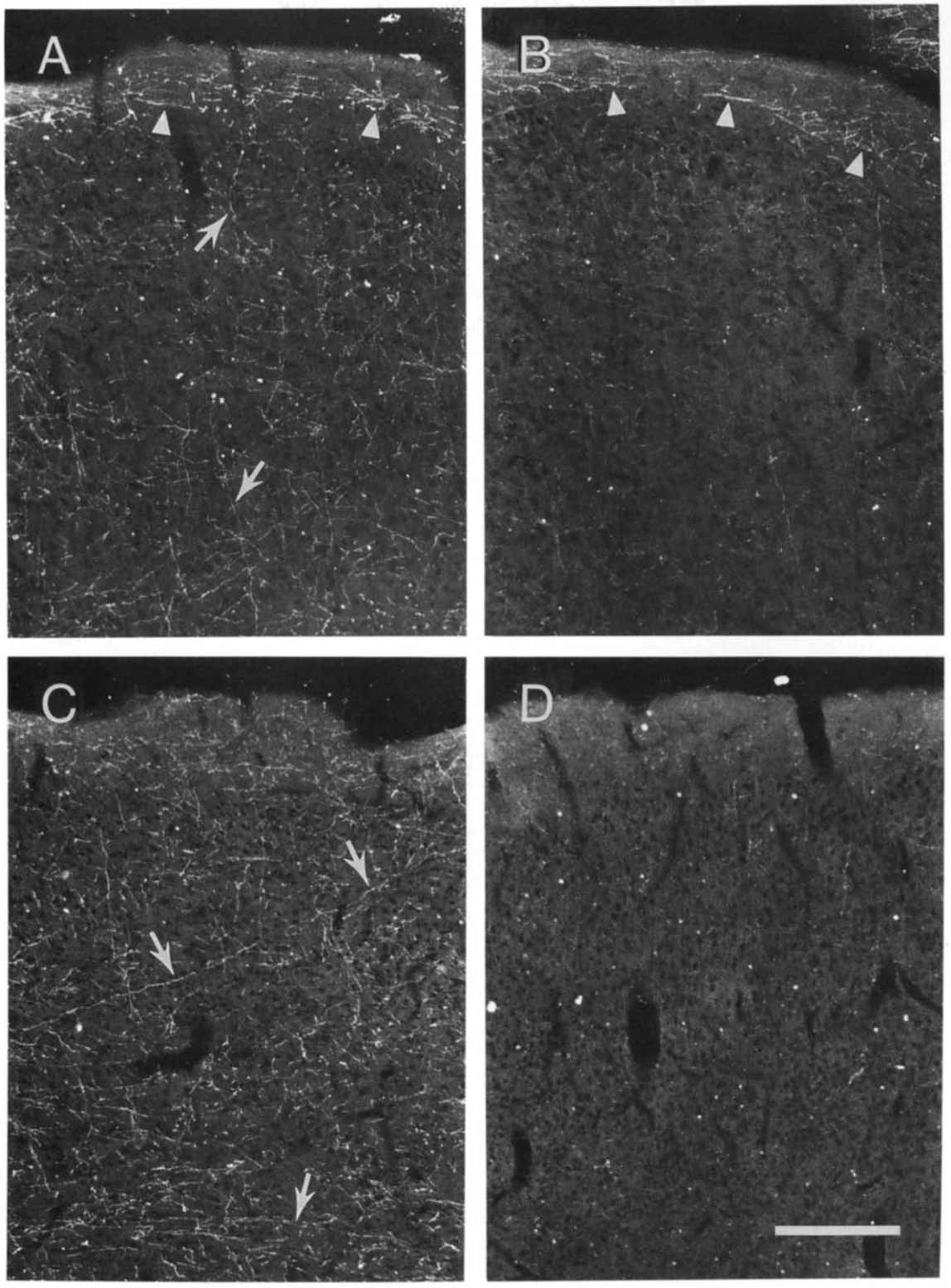

Fig. 7. Darkfield photomicrographs showing 2 areas of cortex, orbital (A and B) and somatosensory (C and D), that displayed significant differences in DBH immunoreactivity between SD rats (A and C) and GEPR-9s (B and D). The lateral orbital cortex of GEPR-9s (B) contained reduced numbers of DBH-I fibers in comparison to SD rats (A) in all layers except layer 1 (arrowheads). In addition, there was a reduction in the number and staining intensity of DBH-I fibers within somatosensory cortex of GEPR-9s (D) as compared to SD rats (C). Arrows (A and C) show individual, well-stained DBH-I processes observed in SD cortices that were not observed in these cortical areas of the GEPR-9s. Calibration bar = 150 μm.

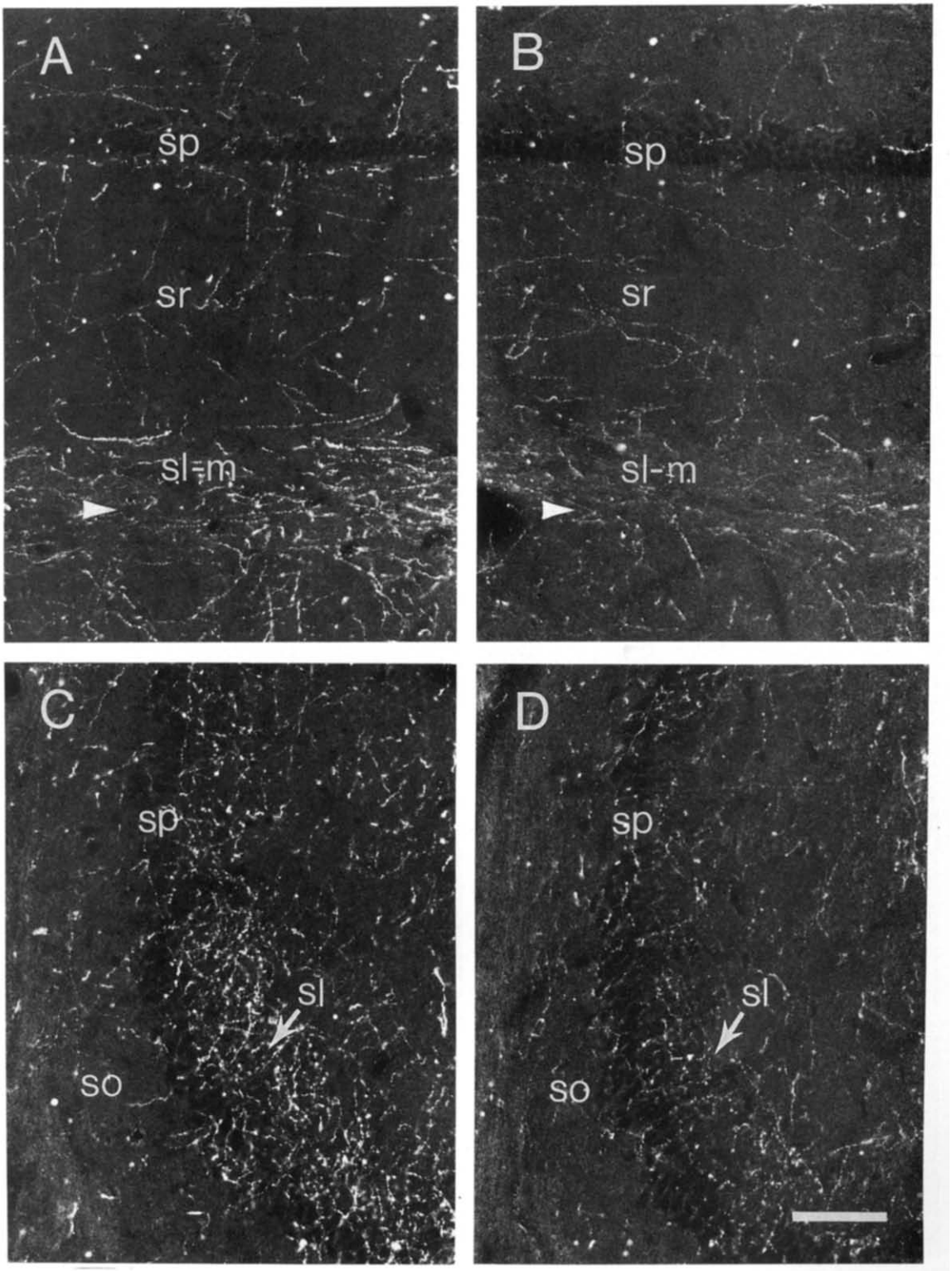

Fig. 8. Darkfield photomicrographs of the hippocampus showing differences in the number and staining intensity of DBH-I fibers between SD rats (A and C) and GEPR-9s (B and D). Fewer DBH-I processes were observed in stratum lacunosum-moleculare (sl-m) of the CA1 region in GEPR-9s (B) as compared to SD rats (A). Arrowhead indicates hippocampal fissure. In the CA3 region, DBH-I fibers were concentrated in stratum lucidum (sl) in the SD rat (C). In contrast, the number of DBH-I fibers in sl was reduced in GEPR-9s (D). Abbreviations: so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. Calibration bar = 150 μm.

In contrast to the findings in the SD rats, the DBH-I fibers in many of the hippocampal subfields appeared to be reduced in number in GEPR-9s. For example, DBH-I processes were nearly absent in stratum lacunosum-moleculare of region CA1 in GEPR-9 with only occasional faintly immunoreactive axons visible (Fig. 8A and B). In region CA3, DBH-I fibers were found distributed throughout stratum lucidum however the density was much less in GEPR-9s as compared to SD rats (Fig. 8C and D). This difference was greater in the septal pole of the hippocampus where the number of DBH-I fibers was less as compared to the temporal pole for both GEPR-9s and SD rats. Finally, in the GEPR-9 dentate gyrus only a few immunoreactive fibers were observed extending from the dentate molecular layer through the granule cell layer as compared to that in the SD rat. In contrast, the hilar region of the dentate gyrus contained a dense network of DBH-I fibers and no apparent differences were observed between GEPR-9s and SD rats.

DISCUSSION

These results support biochemical findings that have shown reduced levels of norepinephrine (NE) in many brain regions of GEPR-9s as compared to SD rats[16]. In particular, reduced numbers and staining intensity of DBH-I fibers (putative NE fibers) were observed in the inferior colliculus, thalamus, and several cortical regions including the orbital, somatosensory and piriform cortices as well as the hippocampus of GEPR-9s. In addition, the findings of this study provide anatomical data that suggest some brain regions of GEPR-9s lack alterations in the NE system. This is particularly evident in the locus coeruleus, cerebellum, striatum and some areas of neocortex where the density of DBH-I neurons and fibers appeared relatively similar between GEPR-9s and SD rats. The findings in the cerebellum and striatum are consistent with previous biochemical data[16]. However, the data for the locus coeruleus, inferior colliculus, hippocampus and cerebral cortex provide new details about the NE system in GEPR-9s.

Methodological considerations

The previous biochemical measurements of NE content in different brain regions of both GEPR-9s and SD rats have shown lower levels of NE in all brain areas except for the cerebellum and striatum[16]. From our anatomical data, it appears that there are some brain areas (e.g., parts of the cerebral cortex) that show no differences in staining even though the biochemical data suggested otherwise. This morphological observation could either be incorrect or could be real in that the regions with no anatomical differences were obscured by areas of cortex that showed large biochemical differences. In any event, there are several technical reasons that involve the immunocytochemical method which could explain this discrepancy between the biochemical and anatomical data.

First, the sensitivity of immunocytochemistry is not comparable to biochemical assays for quantitative determination of the levels of neuroactive substances. In the present study, it was only possible to make a qualitative analysis of the differences in the numbers of DBH-containing fibers because the NE fiber system is quite extensive and difficult to quantitate in brain sections. Since it is unclear what the relationship is between the intensity of DBH-I and the actual quantity of NE within fibers, it is possible that some differences between GEPR-9s and SD rats were not recognized with the immunocytochemical technique.

Secondly, differences in the number of NE fibers might be more reliably observed in moderately innervated areas or highly laminated structures than in areas that contain a dense plexus. For example, the central nucleus of the inferior colliculus and the thalamic reticular nucleus were both moderately innervated structures that showed reduced numbers of NE fibers in GEPR-9s as compared to SD rats. Similarly, it was noted that somatosensory cortex in the SD rat contained a smaller number of DBH-I fibers as compared to the surrounding cortical regions, and it also was an area of cortex that was recognized to contain a reduction of DBH-I fibers in GEPR-9s as compared to SD rats. In addition, the hippocampus which is a highly laminated structure displayed reduced numbers of NE fibers in specific laminae of the GEPR-9s ver-

sus SD rats. Furthermore, this difference was more obvious in the septal pole of the hippocampus where the number of DBH-I fibers was lower than in the temporal pole in both GEPR-9s and SD rats.

Finally, it cannot be overlooked that animal variability may occur. This may account for the fact that some pairs of SD rats and GEPR-9s did not display differences in some of the brain regions. To minimize this variability in our study, comparisons were made using GEPR-9s which exhibited maximal motor seizures and SD rats which exhibited no seizure activity during the same testing parameters.

A selective down-regulation of the NE system in GEPR-9s?

Despite the possible discrepancy between the biochemistry and anatomy of the NE system, it is very interesting that the entire NE system does not seem to be altered in GEPR-9s. It would be expected that since the locus coeruleus gives rise to the massive NE containing projection throughout the neuroaxis that all efferent targets, including for example the cerebellum, would be affected similarly. However, the evidence suggests that there are specific brain regions in the GEPR-9 which display differences in NE.

There are at least two possible explanations for the regional differences in the NE plexus between GEPR-9s and SD rats observed in the present study. First, as mentioned above, it is possible that the level of NE is lower in the entire monoaminergic system but that noticeable differences are only observed in those areas where the density of the fiber plexus is relatively low. Highly innervated areas would tend to obscure the detection of smaller numbers of immunoreactive fibers. Furthermore, it should be pointed out that a decrease in the number of DBH-I fibers is not exclusively indicative of reduced numbers of NE fibers. Rather, it may also reflect a decreased level of NE content within these fibers to the point where it remains undetectable with immunocytochemical methods. This latter notion seems to be supported by the frequent observation that the intensity of the immunoreactive staining was lower in many regions of the GEPR-9 brain. However, the biochemical findings of no differences in NE content in the cerebellum, in addition to the present anatomical findings in the cerebellum that showed no differences in DBH-I fibers between GEPR-9s and SD rats, would suggest that the entire NE fiber system is not similarly affected.

Alternatively, it could be argued that different populations of cells within the locus coeruleus are responsible for the regional variability. Using retrograde tracing methods it has been shown that the LC is comprised of specific subpopulations of neurons that are spatially organized in this brain region with respect to their efferent targets[19,20,33]. For example, projections to the hippocampus originate solely from the dorsal portion of the nucleus. Furthermore, differences in efferent projections of the LC neurons were observed with respect to differences in their morphology in that fusiform cells, located dorsally, project to the hippocampus and cortex, whereas large multipolar cells, found more ventrally, project to the spinal cord and cerebellum. This would suggest that there is the potential for alterations to occur among the individual subpopulations of the LC and may be the basis for the differences observed between the cerebellum and the rest of the brain in GEPR-9. Although alterations within the LC could be in the form of (i) fewer cells or (ii) differential regulation of NE content within the subpopulations of LC neurons, it should be noted that the relative distribution of cells within the LC appeared equivalent between GEPR-9s and SD rats.

It is unlikely that all of the brain regions that exhibit differences in the NE system contribute to the epileptogenesis of seizures in GEPR-9s because most of the brain regions that display a decrease are rostral to the inferior colliculus. It is significant that this brain region is the most rostral one that both shows a difference in NE[16] and is an essential structure for audiogenic seizures[13,32]. Recently, Browning et al.[1] have attempted to assess the functional loss of NE in the inferior colliculus by injecting micromolar amounts of NE or NE agonists into this structure. They found that audiogenic seizures were not blocked by these injections, even though intracerebroventricular injections of NE or NE agonists reduced the severity of audiogenic seizures in GEPR-9s. These findings,

taken together with the large reductions in DBH-I fibers observed in the present study for thalamic and cortical brain structures including the hippocampus, indicate that reduced NE control of forebrain structures may have a facilitatory effect on midbrain structures that could, in fact, propagate the audiogenic seizures. Such an effect could be mediated by a number of descending pathways that may influence the midbrain and pontine reticular formations which act as the common motor outflow for seizures in GEPR-9s. Within the reticular formation, the reticular pontine oralis nucleus appears to be an important site because lesions of this nucleus suppress tonic components of audiogenic convulsions[2].

The inability of NE to stop seizures when it is injected into the inferior colliculus shifts the emphasis of a neurotransmitter defect to GABA and glutamate which also display abnormal levels in this structure[26]. As discussed in the Introduction, the GABAergic system is significantly altered in the inferior colliculus in that it displays increased numbers of GABAergic neurons[28,29] and steady state levels of GABA[26] as well as a reduced effectiveness of GABA at postsynaptic receptors[6]. When GABA agonists are injected into the inferior colliculus, seizures are blocked in GEPR-9s[1]. Glutamate is also involved with epileptogenesis in this structure because glutamate levels in GEPR-9s are increased over levels found in non-epileptic rats[26] and injections of glutamate antagonists will block seizures in GEPR-9s[7]. Thus, the GEPR-9s display abnormalities in multiple neurotransmitter systems. It remains to be determined which system is the primary cause of the defect that involves epileptogenesis. Furthermore, it could be speculated that each neurotransmitter abnormality is associated with a different chromosomal alteration because the phenotypic expression of seizures in offspring of GEPR-9s and non-epileptic rats is suggestive of a polygenetic inheritance of an autosomal dominant feature[27].

It is apparent that the primary defect in the NE system of GEPR-9s involves the distribution of DBH-I fibers in forebrain and midbrain structures. The regional specificity of the differences observed between GEPR-9s and SD rats may indicate a selective down-regulation of the NE system. The fact that specific portions of the cerebral cortex display reduced numbers of DBH-I fibers indicates that certain target structures may have the ability to modify the axonal plexus of NE terminals. This suggestion is an attractive one and would require a sensitive receptor system on the axonal plexus that could modify the levels of DBH and NE within its fibers in response to an appropriate stimulus. It is important to note that the results of the present study are based on the ability of the immunocytochemical method to localize detectable levels of DBH within NE fibers. If the levels of DBH are evenly distributed throughout the NE fiber system so that most NE fibers stain with a similar intensity, then the present results could be interpreted to indicate that specific alterations occur in the axonal arborization of individual NE fibers that arise from the same neuron in the locus coeruleus in selected brain regions. Future studies will assess this possibility by tracing forebrain axonal arborizations of the NE system following labeling of locus coeruleus neurons with an anterograde neuroanatomical marker that labels the entire axonal distribution.

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