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Axonal and terminal degeneration in the mediobasal forebrain after lesions of brainstem pathways

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SUMMARY

Lesion studies in animals are widely used for evaluating physiological parameters. However, changes in anatomical structures of the neuropil have often been neglected in the interpretation of the physiological results. Our study shows that anatomical data should also be considered. Here, the Fink-Heimer-method was used to demonstrate degeneration patterns after lesions in the rat brain. In detail, the ascending noradrenergic fibres were incised at the level of the dorsal tegmental area of the mesencephalon. The resulting degeneration pattern corresponds largely with the noradrenergic projection. The extent of degeneration is less than the physiological results in the literature predict. The interruption of the medial forebrain bundle at the posterolateral hypothalamic level led to a degeneration of the supraoptic decussation, where, according to physiological data, locus coeruleus fibres had been assumed to cross to the contralateral hemisphere. Beside axotomy, the lesions caused edema, hemorrhage and necrosis. They were not restricted to the width of the blade and did not only affect the catecholaminergic system but led to degeneration in mediobasal forebrain sites where gonadotropin-releasing hormone-neurons are present, in fibre systems which carry afferents and efferents of these neurons and in hypothalamic and extrahypothalamic structures that influence the release of the gonadotropin-releasing hormone. Thus, both anatomical and physiological aspects are important for a correct evaluation of lesion studies in brain/behavior research.

Key words: Degeneration – Stereotaxic lesion – Fink-Heimer-method – Brainstem projections – GnRH

INTRODUCTION

The brainstem and basal forebrain as well as the hypothalamus are strongly connected by the fibre system of the medial forebrain bundle. The hypothalamus acts as an integration centre for endocrine, autonomic, and behavioral parameters. A complex neurotransmission and neuromodulation system plays an important role in the regulation of its functions. Lesion studies contributed to the discovery of the anatomy and physiology of this system.

Clifton and Sawyer (1979) interrupted the ascending noradrenergic fibres at a mesencephalic level between brainstem and the mediobasal forebrain including the hypothalamus. Using a physiological method they obtained results that indicated that norepinephrine played a modulatory role in the regulation of ovulation.

The lesions used by Gitler and Barraclough (1988) affected the medial forebrain bundle, the anterior commissure and the anterior commis-

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sure together with the supraoptic decussation. Those authors combined surgical interruption of fibre systems with electrical stimulation of the locus coeruleus and electrochemical stimulation of the medial preoptic nucleus. Their physiological results provided evidence of the crossing of locus coeruleus fibres in the supraoptic decussation, which are involved in the excitatory effect on the release of luteinizing hormone.
The interpretations in those studies were based on physiological results after experimental lesions. However, the alterations in the neuropil as a result of the lesions were not examined. Thus, it was an open question as to how specific a lesion could be, and as to whether only one nucleus, several nuclei or complete nerve cell systems were affected. In the first approach it seemed unlikely that a restriction on a single system was possible.
The procedures in both studies influenced the hypothalamo-hypophyseal circuit. Gonadotropin-releasing hormone (GnRH)-neurons regulate the release of luteinizing hormone in the anterior pituitary gland. Approximately 1200 GnRH-neurons are distributed as a loose continuum along the nervus terminalis in a caudal direction up to the retrochiasmatic region (Hoffman and Berghorn, 1997). Those neurons are concentrated in the medial septum-diagonal band complex, the preoptic area, the nucleus of the stria terminalis, and the anterior hypothalamic area (Silverman et al., 1987; Witkin et al., 1982).
In this study, the experimental deafferentations of Clifton and Sawyer (1979) and Gitler and Barraclough (1988) were reproduced to demonstrate the degeneration pattern between the lesion and the mediobasal forebrain, using the silver impregnation method of Fink-Heimer II (Fink and Heimer, 1967). Lesion 1 corresponded largely to the lesion in the study of Clifton and Sawyer (1979) by using a smaller blade. Lesions 2.1 up to 2.3 reproduced the transections in the study of Gitler and Barraclough (1988). The extent of the degeneration of the anatomical structures in the mediobasal forebrain where GnRH-neurons are localized together with the median eminence, which receives afferents from about 50% of the GnRH-neurons (Silverman et al., 1987), was quantified.

Materials and methods

Thirty-two female Sprague-Dawley-rats with a weight between 250 and 350 g were used in this study. This number includes animals on which operations were performed to clarify methodological questions and animals on which stereotaxic lesions were set to demonstrate degeneration patterns. Animals were housed in a room with lights on from 08.00 to 18.00 h. Food and water were provided ad libitum.

Stereotaxic surgery

Experimental surgical methods and maintenance of the animals were approved by the local Animal Care Committee according to German law regulating the experimental use of animals.
The rats were anaesthetized with intraperitoneal (i.p.) chloral hydrate at a dose of 350 mg/100 g body weight. The animals received i.p. atropine at a dose of 0.1 mg/100 g body weight to prevent excessive airway secretions. Dexamethasone was injected subcutaneously (s.c) at a dose of 0.7 mg/100 g body weight to protect against stress. The operation field was anaesthetized locally using 0.5 % bupivacaine solution. A craniotomy was performed using a dentist's drill. Build up of excessive heat was avoided. The stereotaxic surgery was performed with a Stoelting Stellar apparatus with reference to the coordinates of Pellegrino et al. (1979).
Brain transections at the following coordinates were performed:

1. A 1 mm wide blade transected the structures of the left mesencephalon 5.6 mm posterior to the bregma. The blade was lowered 7.5 mm below the dura in the frontal plane. The medial edge of the blade was positioned 0.9 mm lateral to the midline (lesion 1, Fig. 1A).
2. A 2 mm wide blade was lowered in a frontal plane in the mammillary region of the hypothalamus to the base of the sphenoid bone 2 mm posterior to the bregma. The medial edge of the blade was positioned 1.5 mm lateral to the midline in the left hemisphere (lesion 2.1, Fig. 1B).
3. The anterior commissure was transected by a 2 mm wide blade which was lowered in a sagittal plane in the midline 7.5 mm below the dura. The caudal edge of the blade was placed 1.5 mm anterior to the bregma (lesion 2.2, Fig. 1C).
4. The coordinates were the same as in 3. The blade was lowered to the sphenoid bone (lesion 2.3, Fig. 1D).

Histology of degeneration

Five days after surgery the rats were sacrificed by vascular perfusion. The animals were anaesthetized with chloral hydrate (i.p.). Perfusion was started with a balanced physiological rinsing solution (Gonzales-Aguilar and De Robertis, 1963). This step was followed by perfusion with fixative (4% formaldehyde, 1% glutaraldehyde in phosphate buffer) and then of a fixative solution containing 1% saccharose for cryoprotection.
After brain removal, tissue blocks were made and embedded in egg yolk to allow for left/right detection (Ebbesson, 1970).
Forty µm-thick frontal sections were cut with a freezing microtome (Sartorius, Germany). In a systematic sample, up to 70 sections per brain

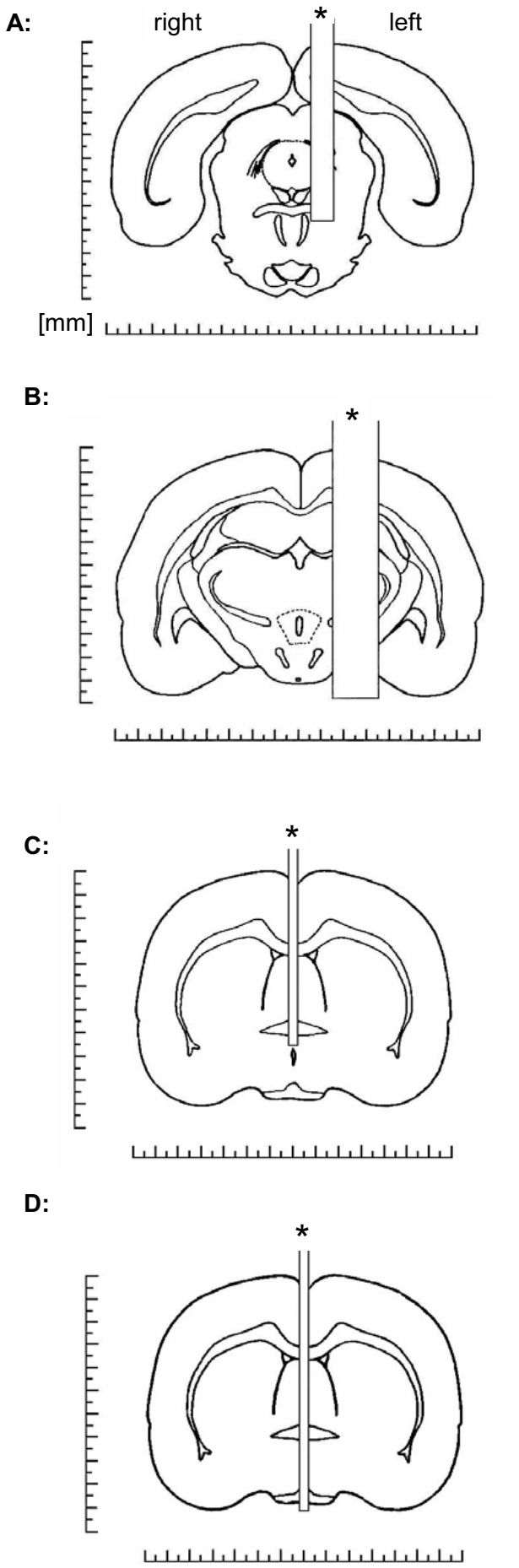

Fig. 1.- Schematic drawings of the localization of the lesions according to Pellegrino et al. (1979). **A:** lesion 1. **B:** lesion 2.1. **C:** lesion 2.2. **D:** lesion 2.3. ***:** marks the blade.

were stained by the Fink-Heimer-II-method to demonstrate axonal and terminal degeneration (Fink and Heimer, 1967; Switzer, 2000): Pretreatment with potassium permanganate, oxalic acid-hydroquinone solution and uranyl-nitrate was followed by impregnation with a weak silver pyridine solution and an ammoniacal silver nitrate solution. Slightly alcoholic dilute formaldehyde acidified with citric acid was used for reduction. In this procedure the silver bound to degenerated axonal and terminal structures and appeared in stained sections as small dark particles with a diameter smaller than 0.5 µm.

The degree and the pattern of degeneration that had developed between the experimental lesion and the mediobasal forebrain was documented by drawings of characteristic sections. These drawings were made using a light microscope equipped with a drawing tube (Wild M20, Switzerland).

Comparable sections of the medial septal nucleus, the nucleus of the diagonal band, the periventricular preoptic nucleus, the median and medial preoptic nucleus, the anterior part of the periventricular hypothalamic nucleus, the bed nucleus of the stria terminalis, the anterior hypothalamic area and the median eminence of the brains were chosen. The anatomic atlases of Pellegrino et al. (1979) and Paxinos and Watson (1998) were used as references. For the evaluation of frontal sections, computer-based counting was performed using the VIDS-picture-analysing system (AI-Tektron, Germany) and an Olympus Vanox-AH2 light microscope. This procedure involved counting the number of silver particles in 10 separate fields. The average size of each field was 2800 µm^2. Silver particles were counted in each nucleus or area at a magnification of 600. Control values were determined by counting the silver particles in 10 separate fields in areas where no degeneration was detectable with a low magnification of 200 to take the background staining into account. The quantitative analysis included a right and a left hemisphere count. The mean and the standard error of the mean were calculated to characterize the distribution of the numerals. Diagrams show the results of the computer-based count by plotting the rounded mean and the rounded standard error of the mean. In comparison with the control values, rounded means greater than 20 were judged as major degeneration; means between 11 and 19 were judged as minor degeneration.

RESULTS

The survival time after an operation has a crucial importance for the demonstration of degenerative changes of nerve cells using the Fink-Heimer-II-method. Boutons have a short-

er degeneration period than fibres (Blackstad et al., 1981). In preceding experiments the degree of degeneration of the medial preoptic nucleus had been examined for survival times ranging from 3 to 9 days. For this study, a post operative survival time of 5 days was chosen to demonstrate both axonal and terminal degeneration. (The abbreviations and the panel used to describe a structure are given in brackets).

Lesion 1

Extent of the lesion

Lesion 1 transected the noradrenergic fibres at mesencephalic level. It also affected the cortex, the corpus callosum, the superior colliculus and its decussation, and the periaqueductal grey. In the area surrounding the lesion, tissue defects (d: Fig. 2B), hemorrhage and edema were present. The abbreviations and panels in this paragraph refer to Fig. 2.

Degeneration pattern

Degeneration could be seen in the ipsilateral cortex (C: Fig. 2A, B), on both sides of the corpus callosum, ipsilateral in the superior colliculus (SC: Fig. 2A, B), on both sides in its decussation (csc: Fig. 2A) and on both sides in the dorsal raphe nucleus (DR: Fig. 2A). Dorsolateral and lateral degeneration of both sides of the periaqueductal grey (PAG: Fig. 2A, B) and ventral to the aqueduct was observed. Degeneration also affected ipsilateral regions traversed by the medial and dorsal longitudinal fascicles. There was ipsilateral degeneration of the dorsal tegmental bundle (dtg: Fig. 2A, B). Within the bundle, the silver particles were arranged in clusters (Fig. 10A). Degeneration in the reticular formation was more pronounced on the contralateral side (RF: Fig. 2B).

Ascending degenerated fibres could be followed through the peduncle of the mammillary body (mp: Fig. 2B) and the medial lemniscus (ml: Fig. 2B). Others could be followed to the interpe-

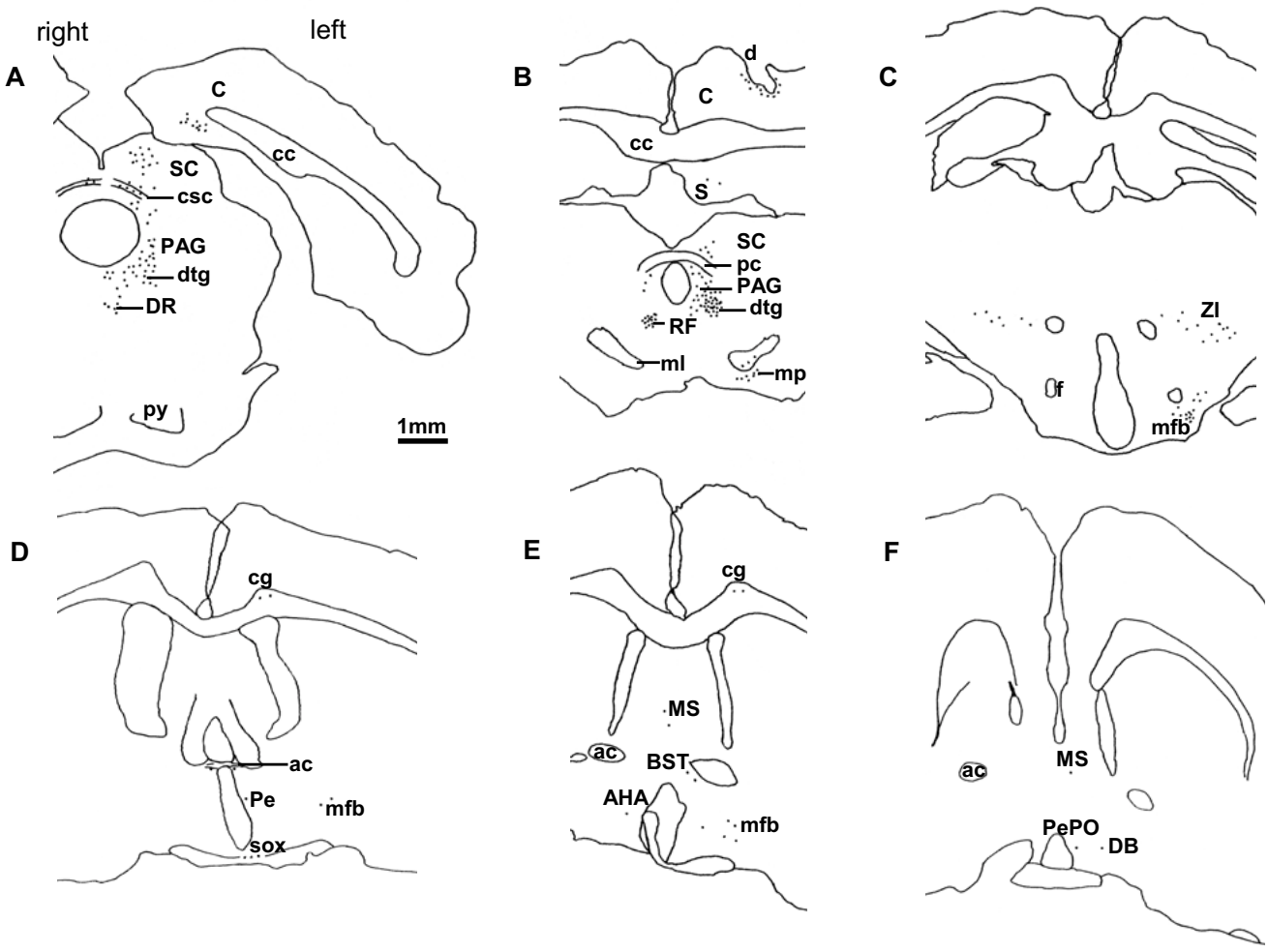

Fig. 2.- Degeneration pattern between lesion 1 and the forebrain. Black dots represent silver particles at degenerated structures.

duncular nucleus. Ipsilateral degeneration developed in the subiculum (S: Fig. 2B). Degenerated fibres ascended bilaterally through the zona incerta (ZI: Fig. 2C) and ipsilaterally through the medial forebrain bundle (mfb: Fig. 2C - E). In the area of the hypothalamus, ipsilateral degeneration of the lateral hypothalamic area and the lateral preoptic area developed. These are both structures through which the medial forebrain bundle ascends. There was degeneration of the supraoptic decussation (sox: Fig. 2D), ventral to the anterior commissure (ac: Fig. 2D) and of the cingulum (cg: Fig. 2D, E).

Quantitative analysis

The results are plotted graphically in Fig. 3. Major ipsilateral degeneration of the bed nucleus of the stria terminalis (BST: Fig. 2E) and of the periventricular hypothalamic nucleus (Pe: Fig. 2D) developed after lesion 1. Minor ipsilateral dege-neration was seen of the anterior hypothalamic area (AHA: Fig. 2E), on both sides of the median preoptic nucleus, ipsilateral of the periventricular preoptic nucleus (PePO: Fig. 2F), of the nucleus of the diagonal band (DB: Fig. 2F) and of the medial septal nucleus (MS: Fig. 2F).

Lesion 2.1

Extent of the lesion

Lesion 2.1 interrupted the medial forebrain bundle at the level of the caudal hypothalamus. The transection also affected structures of the cortex, the corpus callosum, the hippocampus, the thalamus, the medial lemniscus, the zona incerta, the internal capsule, and the cerebral peduncle. Surrounding edema led to additional damage of adjacent structures. There were contralateral edematous changes affecting the hypothalamus and thalamus. The abbreviations and panels in this paragraph refer to Fig. 4.

Degeneration pattern

Rostral to the lesion, ipsilateral degeneration involved the cortex (C: Fig. 4A - D) and the corpus callosum (cc: Fig. 4A). There was bilateral degeneration in the limbic system of the dentate gyrus and of CA1, CA2, and CA3 of the hippocampus. Only the contralateral hippocampus is shown in the panels (Hi: Fig. 4A). Degeneration developed in the ventral hippocampal commissure, the fimbria of the hippocampus (fi: Fig. 4A), the fornix (f: Fig. 4B, C) and the cingulum (cg: Fig. 4A).

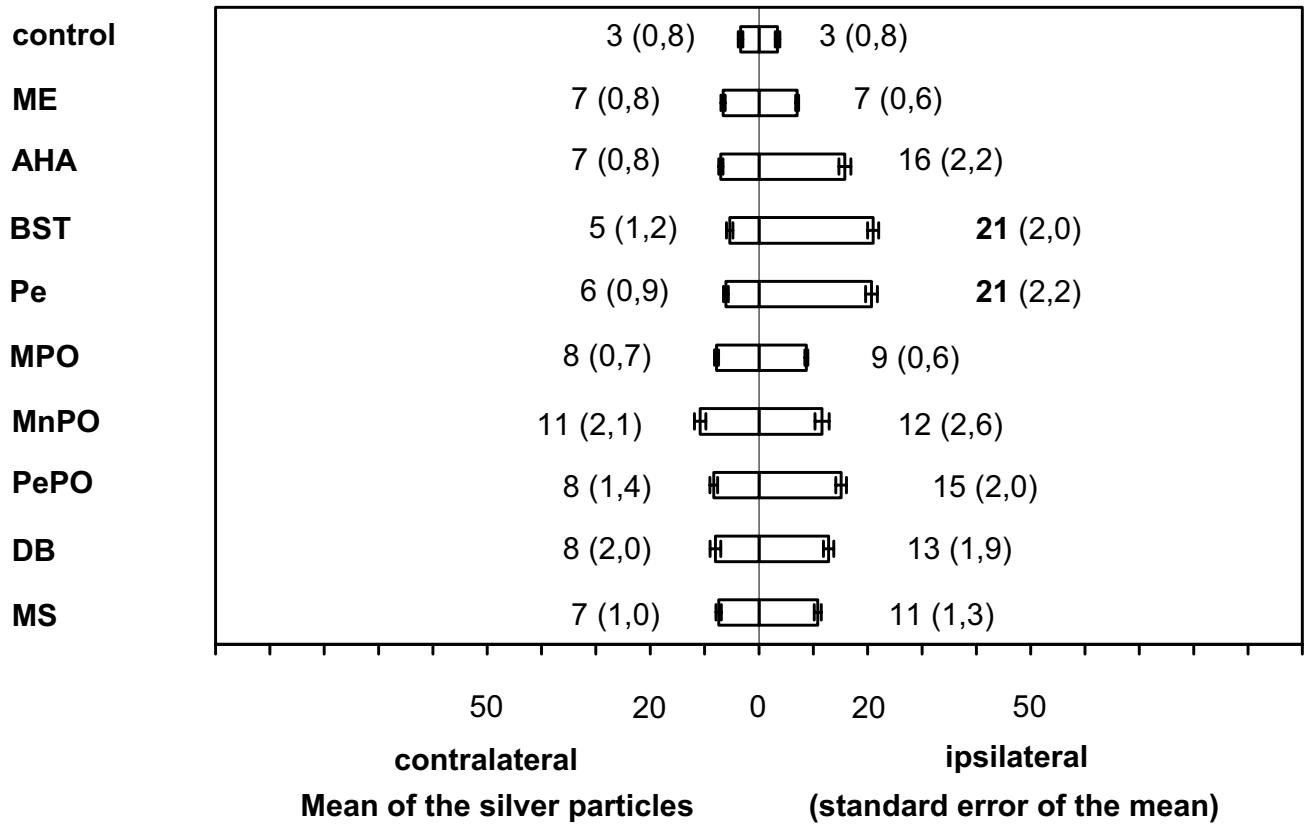

Fig. 3.- Quantitative degeneration pattern of the mediobasal forebrain after lesion 1.

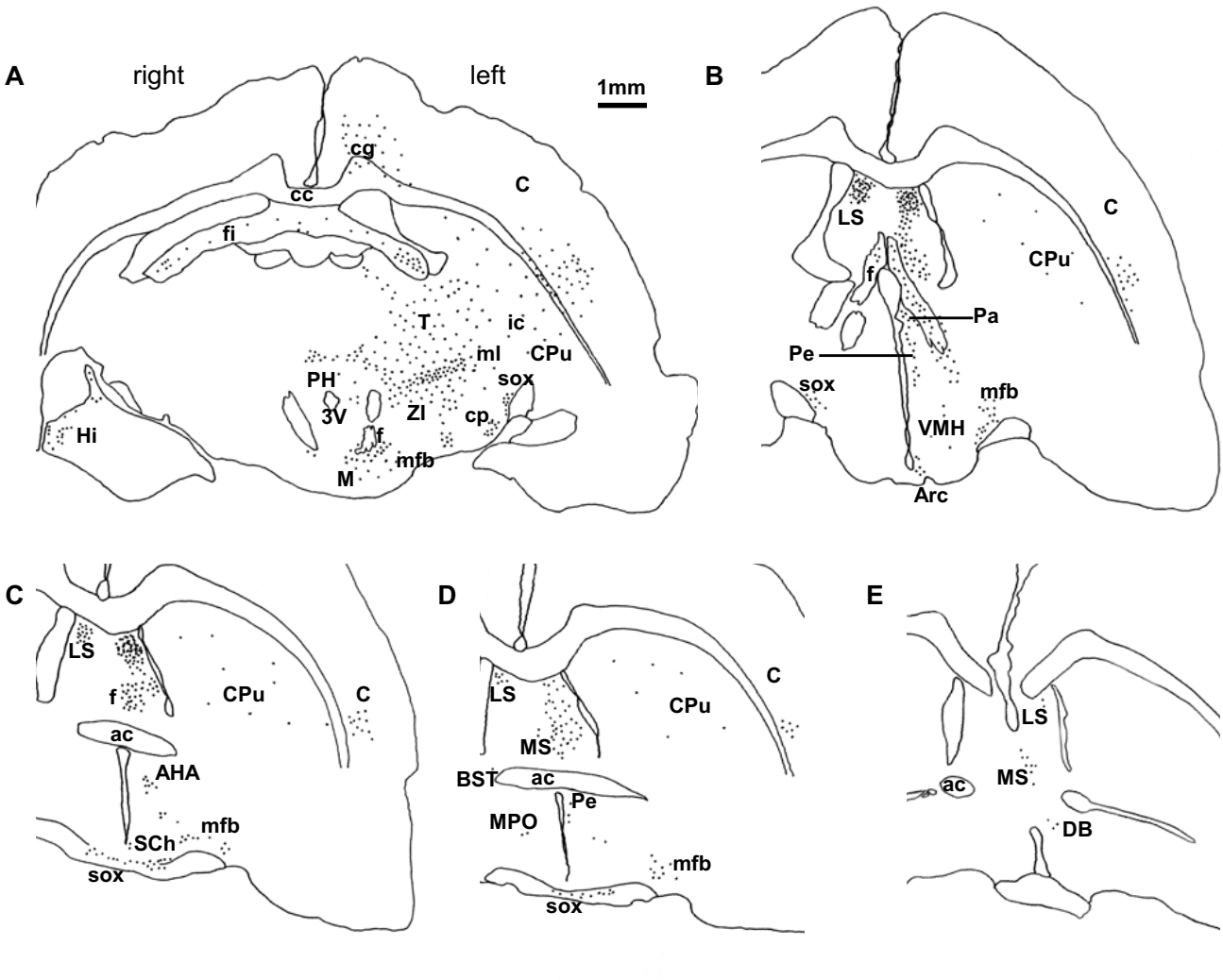

Fig. 4.- Degeneration pattern between lesion 2.1 and the forebrain.

The lesion also affected the ipsilateral medial lemniscus (ml: Fig. 4A). Structures on both sides of the thalamus (T: Fig. 4A) showed signs of degeneration. Ipsilateral degeneration developed in the zona incerta (ZI: Fig. 4A), along the internal capsule (ic: Fig. 4A) to the striatum (CPu: Fig. 4A - D), in a rostral direction along the medial forebrain bundle (mfb: Fig. 4A - D) and to the contralateral side along the supraoptic decussation (sox: Fig. 4A - D and Fig. 10B).
In the hypothalamus there was bilateral degeneration of the mammillary nuclei (M: Fig. 4A) and ipsilateral degeneration of the posterior hypothalamic area (PH: Fig. 4A), the paraventricular (Pa: Fig. 4B), arcuate (Arc: Fig. 4B), suprachiasmatic (SCh: Fig. 4C), ventromedial (VMH: Fig. 4B) and periventricular nuclei (Pe: Fig. 4B, D).
Numerous small silver particles are bilaterally visible in the neuropil of the lateral septal nucleus (LS: Fig. 4B - E and Fig. 10C).

Quantitative analysis

The results are summarized graphically in Fig. 5. Major ipsilateral degeneration of the medial septal nucleus was present. Minor ipsilateral degeneration developed in the median eminence and the anterior hypothalamic area (AHA: Fig. 4C), contralaterally in the bed nucleus of the stria terminalis (BST: Fig. 4D), ipsilaterally in the periventricular hypothalamic nucleus (Pe: Fig. 4D), bilaterally in the medial preoptic nuclei (MPO: Fig. 4D), ipsilaterally in the nucleus of the diagonal band (DB: Fig. 4E) and contralaterally in the medial septal nucleus (MS: Fig. 4D).

Lesion 2.2

Extent of the lesion

This lesion interrupted the anterior commissure and adjacent structures as well as structures of the cortex, the corpus callosum, the septum, and the preoptic area. Tissue defects (d: Fig. 6C - F), hemorrhage (h: Fig. 6C, E, F) and edematous changes developed. The demonstrated degeneration affected ascending and descending fibre systems. The abbreviations and panels in this paragraph refer to Fig. 6.

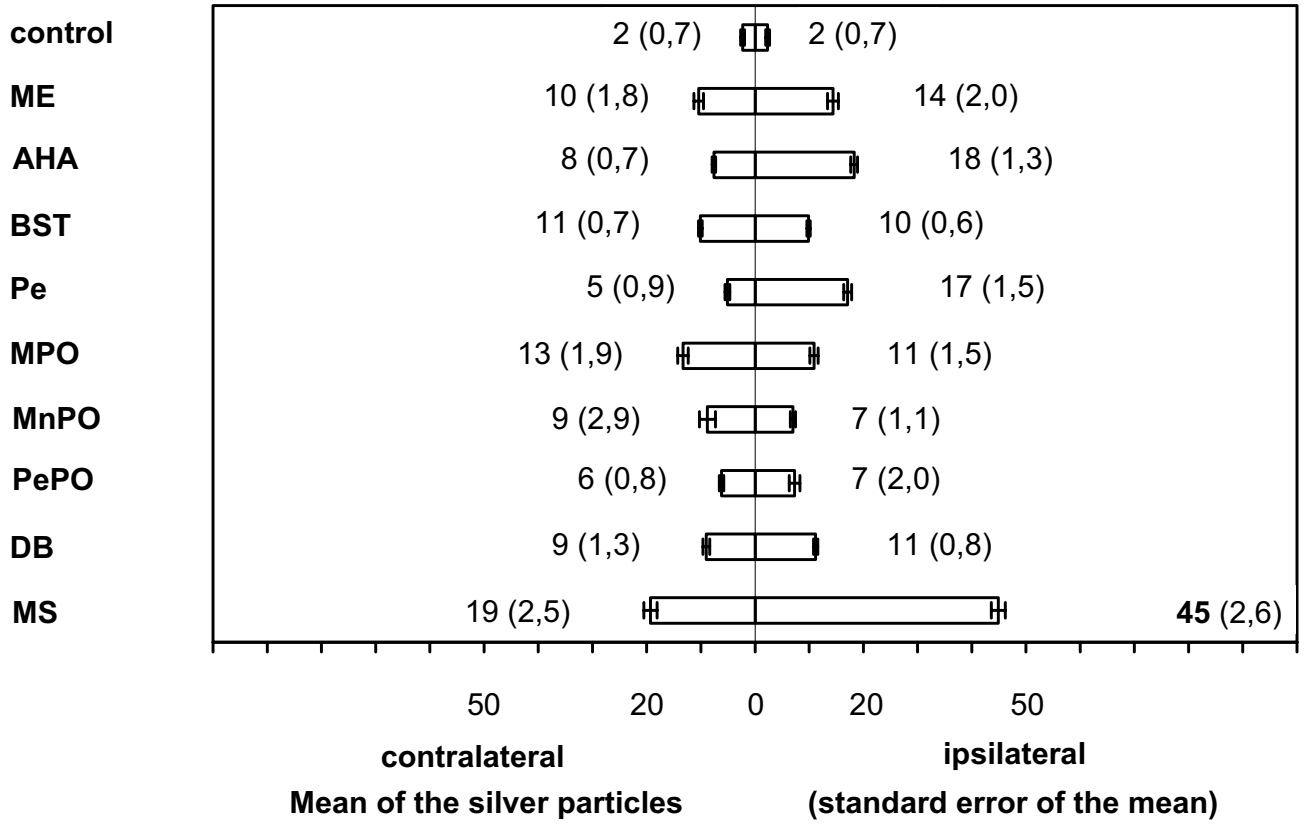

Fig. 5.- Quantitative degeneration pattern of the mediobasal forebrain after lesion 2.1.

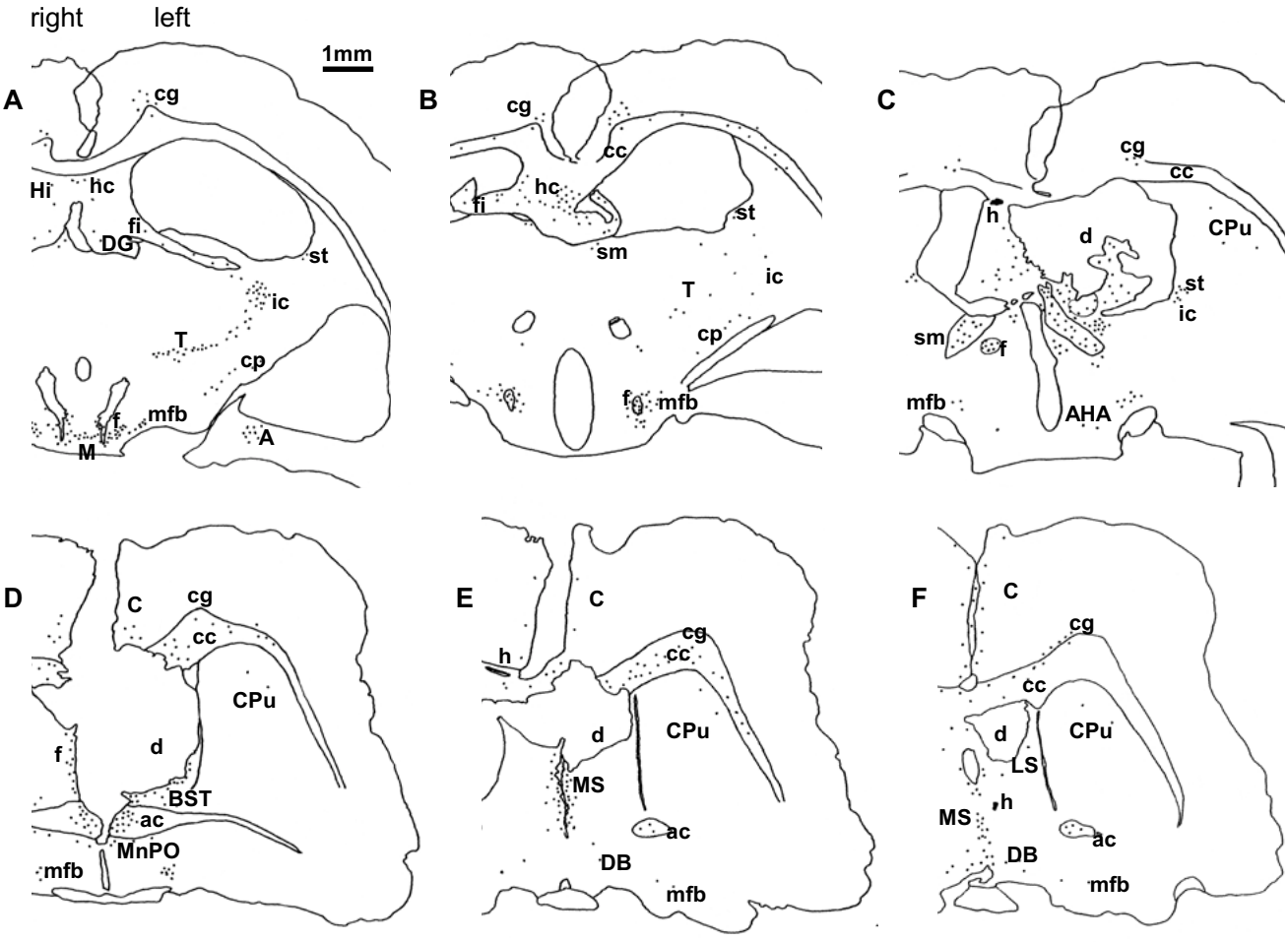

Fig. 6.- Degeneration pattern between lesion 2.2 and the forebrain.

Degeneration pattern

Degeneration developed symmetrically on both sides. The cortex (C: Fig. 6D - F) and the corpus callosum (cc: Fig. 6B - F) were affected. There was bilateral degeneration in the limbic system of the cingulate gyrus, the cingulum (cg: Fig. 6A - F), the fornix (f: Fig. 6A - D), the fimbria of the hippocampus (fi: Fig. 6A, B), the hippocampal commissure (hc: Fig. 6A, B), CA3 of the hippocampus (Hi: Fig. 6A), the dentate gyrus (DG: Fig. 6A) and the amygdala (A: Fig. 6A).
Bilateral degeneration was present of the anterior commissure and the surrounding structures (ac: Fig. 6D - F and Fig. 10D).
Degeneration continued in both caudal and rostral directions along the medial forebrain bundle (mfb: Fig. 6A - F), the stria medullaris (sm: Fig. 6B, C) and the stria terminalis (st: Fig. 6A - C).
In the area of the hypothalamus, the mammillary nuclei were affected (M: Fig. 6A). There was degeneration of the cerebral peduncle and the internal capsule (ic: Fig. 6A-C), the striatum (CPu: Fig. 6C-F) and the thalamus (T: Fig. 6A, B).
Further rostral degeneration of the lateral septum (LS: Fig. 6F) was present.

Quantitative analysis

The quantitative results are summarized in Fig. 7. In the mediobasal forebrain major bilateral degeneration was present in the medial septal nucleus (MS: Fig. 6E, F), in the nucleus of the diagonal band (DB: Fig. 6E, F), in the median preoptic nucleus (MnPO: Fig. 6D) and in the bed nucleus of the stria terminalis (BST: Fig. 6D). The degree of degeneration of the anterior hypothalamic area was major on the left side and minor on the right side (AHA: Fig. 6C), with only a small difference in the mean. Minor degeneration developed in the left medial preoptic nucleus.

Lesion 2.3

Extent of the lesion

Both the anterior commissure and supraoptic decussation, as well as surrounding structures, were transected. This lesion also affected the cerebral cortex, the corpus callosum, the septum, the adjacent preoptic area and the 3rd ventricle. There were tissue defects (d: Fig. 8C - F), hemorrhage (h: Fig. 8A - F) and edematous changes. The abbreviations and panels in this paragraph refer to Fig. 8.

Degeneration pattern

Degeneration developed symmetrically on both sides. The cortex (C: Fig. 8D - F) and corpus callosum (cc: Fig. 8A - F) were affected. In the limbic system, degeneration was present in the cingulate gyrus, the cingulum (cg: Fig. 8A - F), the hippocampal commissure (hc: Fig. 8A, B), the fimbria of the hippocampus (fi: Fig. 8A, B) and the fornix (f: Fig. 8A-D). In other sections, de-

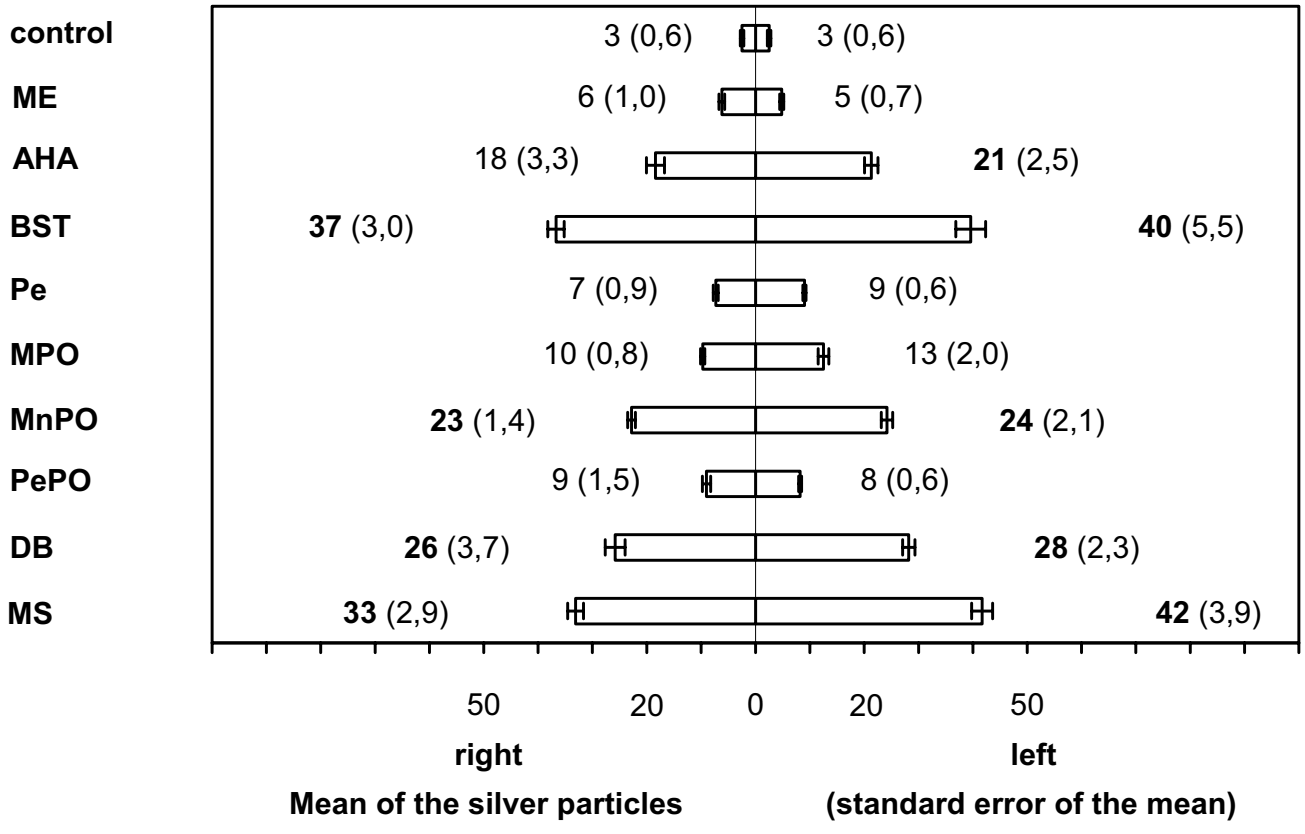

Fig. 7.- Quantitative degeneration pattern of the mediobasal forebrain after lesion 2.2.

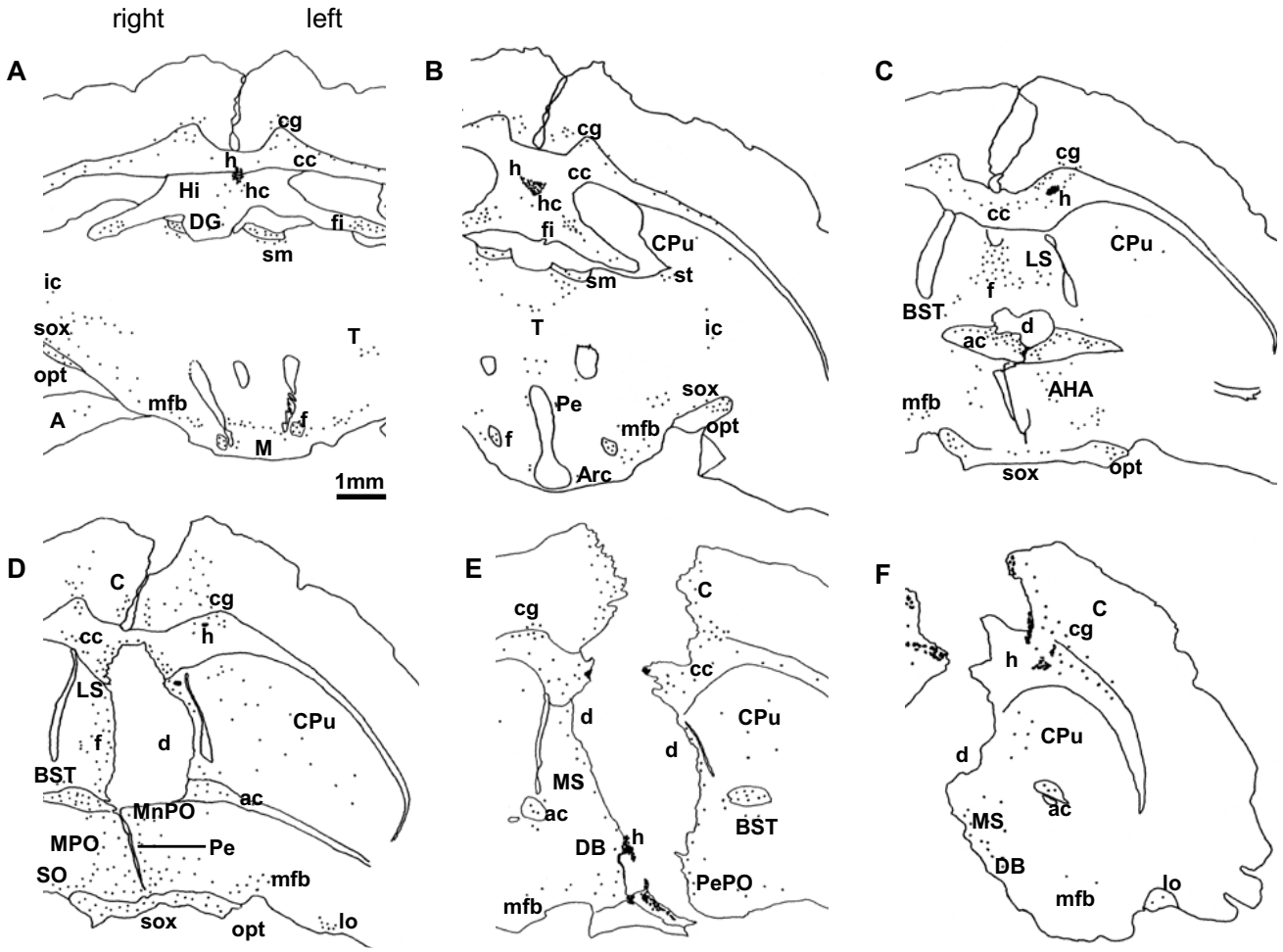

Fig. 8.- Degeneration pattern between lesion 2.3 and the forebrain.

generation developed on both sides (not shown here) of the dentate gyrus (DG: Fig. 8A), in CA3 of the hippocampus (Hi: Fig. 8A) and the amygdala (A: Fig. 8A).

The lesion directly affected the anterior commissure (ac: Fig. 8C, D) and the supraoptic decussation (sox: Fig. 8C). There was degeneration of the optic tract (opt: Fig. 8A - D) and the optic chiasm.

Degeneration continued in caudal and lateral directions through the lateral preoptic area, the lateral hypothalamic area and the medial forebrain bundle (mfb: Fig. 8A - F). There was degeneration of the cerebral peduncle and the internal capsule (ic: Fig. 8A, B) and of the striatum (CPu: Fig. 8B - F). In the area of the hypothalamus, degeneration affected the supraoptic (SO: Fig. 8D), arcuate (Arc: Fig. 8B) and mammillary nuclei (M: Fig. 8A), the stria medullaris (sm: Fig. 8A, B), the adjacent habenula, the thalamus (T: Fig. 8A, B) and the stria terminalis (st: Fig. 8B).

In a rostral direction, there was degeneration of the lateral septum (LS: Fig. 8C) and of the lateral olfactory tract (lo: Fig. 8D, F).

Quantitative analysis

The quantitative results are summarized in Fig. 9. There was a major bilateral degeneration in the mediobasal forebrain of the medial septal nucleus (MS: Fig. 8E, F), of the nucleus of the diagonal band (DB: Fig. 8E, F), of the periventricular preoptic nucleus (PePO: Fig. 8E), of the median preoptic nucleus (MnPO: Fig. 8D), of the medial preoptic nucleus (MPO: Fig. 8D), of the periventricular hypothalamic nucleus (Pe: Fig. 8D), of the bed nucleus of the stria terminalis (BST: Fig. 8C-E) and of the anterior hypothalamic area (AHA: Fig. 8C).

DISCUSSION

The results of this study support the assumption that the lesions made by us affected several pathways at the level of the brain at which the axotomy was performed. The fibres in these pathways varied in their anatomy. There were small myelinated and unmyelinated and coarse myelinated fibres. They belonged to the limbic

system, the sensorimotor system and several other functional systems, and they carried different monoaminergic and other transmitters. Thus, axotomy of a pathway led to a plethora of functional disturbances such as the "lateral hypothalamic syndrome", which comprises a recovery in feeding and drinking via successive stages, and a number of smaller impairments in the regulation of food and water intake, taste-preference abnormalities, and sensorimotor deficits after lateral hypothalamic damage (Nieuwenhuys et al., 1982). Besides the effects of the axotomy itself, the lesions led to edema, hemorrhage and consequently necrosis. Therefore, the lesion always extended beyond the width of the blade. Thus, the degeneration observed could be attributed to several processes in addition to the axotomy itself.

In this study, degeneration occurred in the hypothalamus after unilateral transection of the mesencephalic ascending noradrenergic pathway. Bilateral transections of the ascending noradrenergic pathway led to an 83% depletion of hypothalamic norepinephrine (Clifton and Sawyer, 1979). Compared to the degree of reduction of norepinephrine in the hypothalamus, the degree of degeneration was expected to be higher in the mediobasal forebrain than was actually found after lesion 1 (Fig. 3). However, the smaller blade in this study has to be considered. Gitler and Barraclough (1988) assumed a direct or indirect influence of the locus coeruleus on the contralateral medial preoptic nucleus. Lesion 2.1 led to degeneration in the supraoptic decussation and minor degeneration in the contralateral medial preoptic nucleus (Fig. 5). However the method in use did not differentiate between locus coeruleus and non-locus coeruleus fibres. Crossing fibres in the supraoptic decussation were indeed affected by lesion 2.3 (Fig. 8), as assumed in Gitler and Barraclough (1988).

Besides the catecholaminergic pathway, degeneration appeared in fibre systems which convey afferents and efferents of GnRH-neurons and in hypothalamic and extrahypothalamic structures which influence GnRH release. Afferents from the rhinencephalon, from limbic structures, and from the brainstem project to specific preoptic and hypothalamic sites where neurosecretory neurons reside. Afferents enter the periventricular hypothalamus by one of four routes: the medial forebrain bundle, the periventricular system, the fornix, and the stria terminalis (Page, 1994). All lesions affected one or more of these fibre systems. The preoptic area-GnRH system projects almost exclusively to the median eminence (Oka, 2002). The GnRH fibers reach the median eminence by more than one route. Axons of GnRH neurons in more caudal and lateral aspects of the preoptic area and hypothalamus travel in or near the medial forebrain bundle. All 4 lesions showed degeneration in the medial forebrain bundle. It is essential that all such pathways to the median eminence be taken into account when paradigms involving surgical interruption of pathways or placement of lesions are used (Silverman et al., 1994).

Major degeneration (Fig. 7) of the medial septum/diagonal band complex, the median preoptic nucleus, the periventricular nucleus of the hypothalamus, the nucleus of the stria terminalis and the anterior hypothalamic area developed after lesion 2.2. No degeneration was seen of structures further ventral. If a lesion was set further ventral up to the supraoptic decussation (lesion 2.3, Fig. 9) major degeneration of all the structures examined, with the exception of the median eminence, developed. The data of the diagrams suggest that lesion 2.3 affected more local connections than lesion 2.2 in the mediobasal forebrain (Swanson, 1987). Input into the medial preoptic area as one of the sites where hypophysiotropic GnRH cells reside in the rat arises from several hypothalamic nuclei in the medial preoptic area-hypophysotropic periventricular complex (Page, 1994).

Limbic structures are known to have a modulating influence on gonadotropin release. Electrochemical stimulation elicited a facilitating and inhibitory effect of different nuclei of the amygdala and an inhibitory hippocampal effect on the release of gonadotropins (Kawakami et al., 1973; Taleisnik and Beltramino, 1975). All lesions in this study led to degeneration in limbic structures, lesion 1, 2.1, 2.2 and 2.3 in the hippocampal formation (Figures 4, 6, 8) and lesion 2.2 and 2.3 in the amygdala (Figures 6, 8).

The interruption of ovulation was overcome by a lesion of the medial cortico-hypothalamic tract (Velasco and Taleisnik, 1969). In the study of Gitler and Barraclough (1988), lesion T2 (here 2.2) together with a locus coeruleus electrical stimulation and a medial preoptic nucleus-electrochemical stimulation led to a significantly prolonged elevated luteinizing hormone (LH) peak. These data suggested that inhibitory inputs from areas outside the hypothalamic regions enter the hypothalamus and suppress luteinizing hormone release. Lesion 2.2 led to interruption and degeneration of the anterior commissure (Fig. 6D) and adjacent structures (Fig. 10D), as assumed in Gitler and Barraclough (1988). Lesion 2.2 and lesion 2.3 caused marked tissue damage in the area of the medial corticohypothalamic tract (Fig. 6D, E; Fig. 8C - E). Therefore, it cannot be excluded that both lesion 2.2 and also lesion 2.3 might influence the inhibitory effect of the hippocampus by damaging a connecting pathway.

Almost all neurotransmitters or neuropeptides have some effect on GnRH release (Jennes et al., 2002). Catecholamines affect LH release by modifying the activity of LHRH neurons (Martins-Afféri et al., 2003). Lesion 1 led to degeneration in

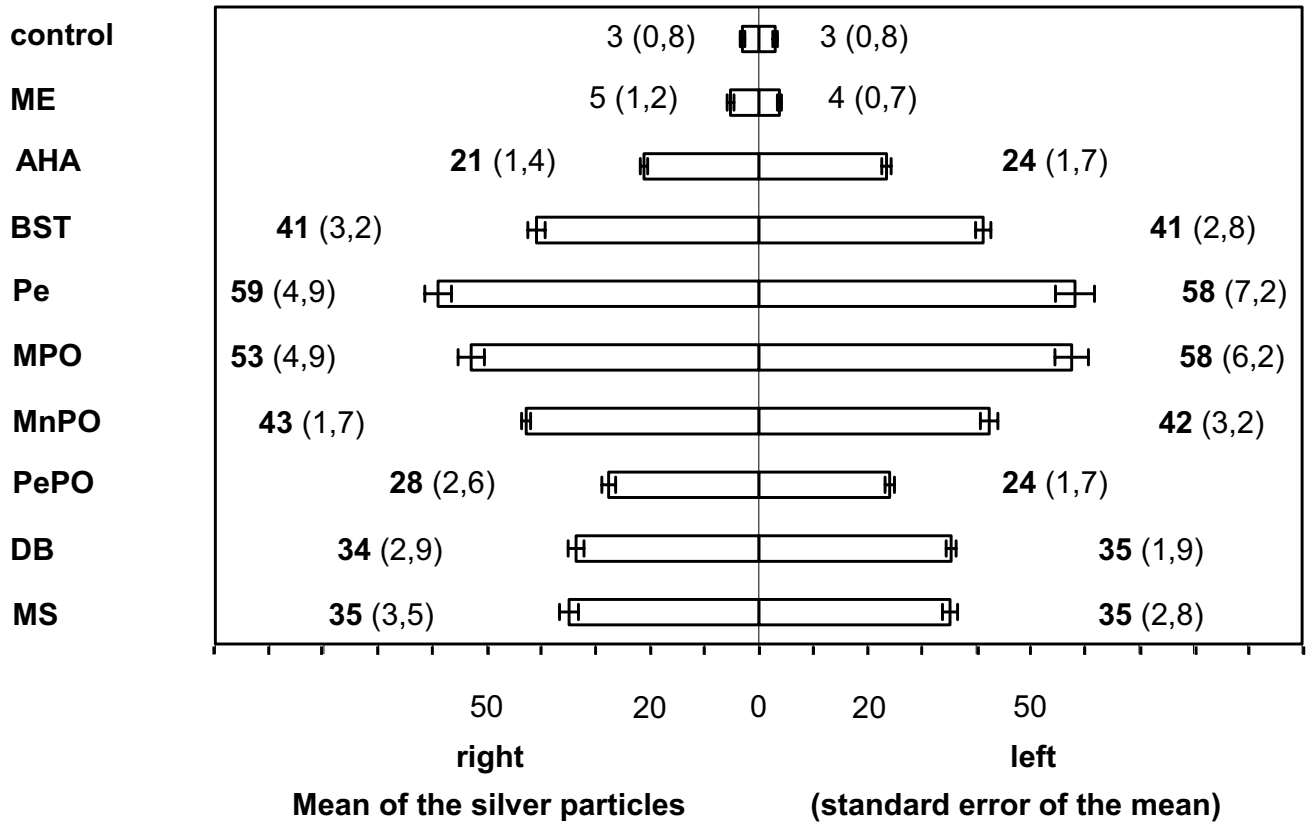

Fig. 9.- Quantitative degeneration pattern of the mediobasal forebrain after lesion 2.3.

di- and telencephalic structures that receive catecholaminergic afferents (Lindvall and Björklund, 1974; Ungerstedt, 1971). However, no degeneration is detectable in the nuclei of the amygdala although afferents have been described in retrograde tracer studies from the catecholaminergic neurons of cell group A1 (Roder and Ciriello, 1994). Swanson and Hartman (1975) concluded that noradrenergic fibres from the locus coeruleus travel with other ascending noradrenergic systems in the principal noradrenergic system, in the central tegmental tract, and enter the hypothalamus from a lateral position in a discrete bundle in the zona incerta. Lesions 1 and 2.1 affected the zona incerta.

The degeneration caused by the lesions was not restricted to the catecholaminergic system. It was found in structures involved in several neurotransmitter and neuropeptide systems. Arginine-vasopressin neurons in the suprachiasmatic nucleus mediate circadian clock information to the GnRH surge generator (Funabashi et al., 2002). There is degeneration of the suprachiasmatic nucleus after lesions 2.1 and 2.3. Both lesions may influence ovulation by interfering with this mechanism.

The medial preoptic area is related to ovulation and the feedback relationship of estrogen on GnRH-containing neurons and to sexual behaviour (Page, 1994). Estrogen may have an indirect negative feedback action on LHRH neurons in the preoptic area (Moreno and Franci, 2004). Lesions 2.1, 2.2 and 2.3 led to different amounts of degeneration in this structure (Figures 5, 7, 9).

Substance P-containing neurons from the arcuate nucleus were found to synapse with GnRH-like neurons in the septum and the preoptic area of the rat (Page, 1994). The arcuate nucleus was affected by lesions 2.1 and 2.3 (Figures 4, 8).

Beside the changes at microscopic level, the rats lost up to 23% of their body weight in the 5 days between lesioning and fixation, which was accompanied by different neurohormonal disturbances in the hypothalamo-hypophysial complex and reduced food intake. Ovulatory cycles are inhibited by caloric restriction. Reproductive inhibition in response to undernutrition correlates with alterations in GnRH pulsatility. (Temple and Rissman, 2002). Leptin is secreted by adipocytes and stimulates LHRH release by receptors on arcuate neurons. During fasting, the leptin signal is removed and LH pulsatility and reproductive function decline quite rapidly (McCann et al., 2002).

According to Clifton and Sawyer (1979), in all animals the reproductive cycle restarted 23 days after the bilateral transections of the ascending noradrenergic pathway. The return of ovulation was surprising, considering the 83% decrease in norepinephrine content of the hypothalamus fol-

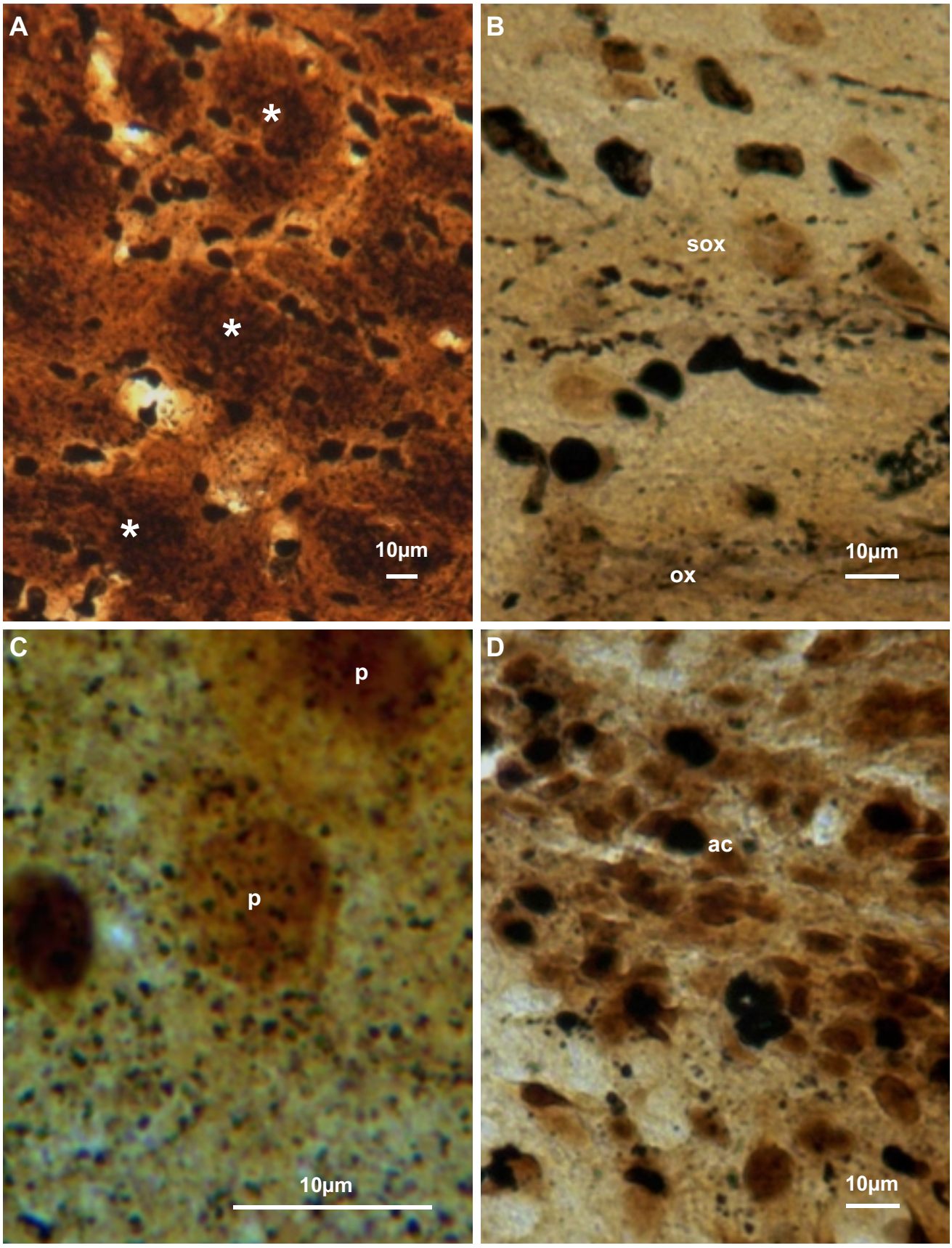

Fig. 10.- A: Dorsal tegmental bundle at the level of Fig. 2B after lesion 1 ipsilateral to the lesion. Fink-Heimer-II-method. The ascending fibres are assembled in clusters (*). **B:** Lesion 2.1. Axotomy of ascending fibres at the level of the lateral posterior hypothalamic region led to degeneration of the supraoptic decussation (sox). Fink-Heimer-II-method. (ox: optic chiasm). **C:** Left lateral septal nucleus after ipsilateral lesion 2.1 and silver impregnation with the Fink-Heimer-II-method. Many small silver particles are seen in the neuropil. (p: perikaryon). **D:** Degeneration has developed ventrally - in the lower part of the picture - to the anterior commissure (ac) after lesion 2.2. Fink-Heimer-II-method.

lowing surgical transection. The development of denervation supersensitivity can only partially explain the return of function post-surgery. This finding supports the existence of multiple control mechanisms for ovulation, thereby allowing other systems to compensate for the loss of noradrenergic regulation. Neurosecretory cells without intact afferent inputs may not express receptors normally seen under physiological conditions (Kordon et al., 1994). It is interesting that the entire apparatus for maintaining the GnRH pulse may be capable of extreme plasticity and reorganization (Silverman et al., 1994).
In conclusion, surgical lesions affect several pathways and are not restricted to a single neuronal system. In each case it needs to be shown which structure is affected by the lesion and what can be interpreted from the physiological results in the highly dynamic brainstem-hypothalamo-hypophyseal-system.

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ABBREVIATIONS

A	amygdala
ac	anterior commissure
AHA	anterior hypothalamic area
Arc	arcuate hypothalamic nucleus
BST	bed nucleus of the stria terminalis
C	cortex
cc	corpus callosum
cg	cingulum
cp	cerebral peduncle
CPu	caudate putamen (striatum)
csc	commissure of the superior colliculus
d	tissue defect
DB	nucleus of the diagonal band
DG	dentate gyrus
DR	dorsal raphe nucleus
dtg	dorsal tegmental bundle
f	fornix
fi	fimbria of the hippocampus
GnRH	gonadotropin-releasing hormone
h	hemorrhage
hc	hippocampal commissure
Hi	hippocampus
ic	internal capsule
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
lo	lateral olfactory tract
LS	lateral septal nucleus
M	mammillary nucleus
mfb	medial forebrain bundle
ml	medial lemniscus
MnPO	median preoptic nucleus
mp	mammillary peduncle
MPO	medial preoptic nucleus
MS	medial septal nucleus
opt	optic tract
ox	optic chiasm
p	perikaryon
Pa	paraventricular hypothalamic nucleus
PAG	periaqueductal gray
pc	posterior commissure
Pe	periventricular hypothalamic nucleus
PePO	periventricular preoptic nucleus
PH	posterior hypothalamic area
py	pyramidal tract
RF	reticular formation
S	subiculum
SC	superior colliculus
SCh	suprachiasmatic nucleus
sm	stria medullaris
SO	supraoptic nucleus
sox	supraoptic decussation
st	stria terminalis
T	thalamus
VMH	ventromedial hypothalamic nucleus
ZI	zona incerta