Ample experimental evidence suggests that β-amyloid (Aβ), when injected into the rat magnocellular nucleus basalis (MBN), impels excitotoxic injury of cholinergic projection neurons. Whereas learning and memory dysfunction is a hallmark of Aβ-induced cholinergic deficits, anxiety, or hypoactivity under novel conditions cannot be attributed to the loss of cholinergic MBN neurons. As mood-related behavioral parameters are primarily influenced by the central serotonergic system, in the present study we investigated whether Aβ(1-42) toxicity in the rat MBN leads to an altered serotonergic innervation pattern in the rat basal forebrain and cerebral cortex 7 days postsurgery. Aβ infusion into the MBN elicited significant anxiety in the elevated plus maze. Aβ toxicity on cholinergic MBN neurons, expressed as the loss of acetylcholinesterase-positive cortical projections, was accompanied by sprouting of serotonergic projection fibers in the MBN. In contrast, the loss of serotonin-negative fiber projections, decreased concentrations of both serotonin and 5-hydroxyindoleacetic acid, and decline of cortical 5-HT1A receptor binding sites indicated reduced serotonergic activity in the somatosensory cortex. In conclusion, the Aβ-induced primary cholinergic deficit in the MBN and subsequent cortical cholinergic denervation bidirectionally modulate serotonergic parameters in the rat basal forebrain and cerebral cortex. We assume that enhanced serotonin immunoreactivity in the damaged MBN indicates intrinsic processes facilitating neuronal recovery and cellular repair mechanisms, while diminished cortical serotonergic activity correlates with the loss of the subcortical cholinergic input, thereby maintaining the balance of neurotransmitter concentrations in the cerebral cortex.

Key Words: β-Amyloid; anxiety; magnocellular nucleus basalis; serotonin; sprouting.

Accumulation of β-amyloid peptides (Aβ) in the form of senile plaques in brain areas associated with learning and memory formation is one of the major neuropathological characteristics of Alzheimer’s disease (AD; Iraizoz et al., 1999; Selkoe, 1999; Sisodia & Price, 1995). Whereas a multitude of recent in vitro and in vivo investigations (Bruce-Keller et al., 1998; Giovannelli et al., 1995, 1998; Harkany et al., 1995, 1999a,b; Laskay et al., 1997; Mattson et al., 1992; Yankner et al., 1990) suggests potent neurotoxin-like properties for
full-length Aβ, consisting of 42 amino acid residues (Aβ1-42), specific molecular pathways by which Aβ compromise nerve cells still remain largely to be elucidated. Nevertheless, ample in vitro evidence points to an excitotoxin-like nature of Aβ (Cowburn et al., 1994, 1997; Joseph & Han, 1992; Laskay et al., 1997; Mattson et al., 1992). In vivo experimental data in our laboratory substantiate Aβ excitotoxicity on cholinergic projection neurons of the rat magnocellular nucleus basalis (MBN), where Aβ toxicity was accompanied by an overt extracellular accumulation of excitatory amino acid neurotransmitters and by a pathologically increased intracellular Ca\(^{2+}\) accumulation (Harkany et al., 1999a,b, 2000a). Taking previous findings together (Abe et al., 1994; Giovannelli et al., 1995, 1998; Harkany et al., 1995, 2000a), it appears that cholinergic basal forebrain neurons are particularly vulnerable to Aβ toxicity.

Loss of cholinergic projection neurons in basal forebrain nuclei is a prominent neuropathological hallmark of AD (Bartus et al., 1982; Mesulam, 1998; Whitehouse et al., 1981), and closely correlates with the progressive loss of memory performance and with the degree of dementia (Iraizoz et al., 1999). However, such a functional breakdown is not restricted to the cholinergic system, as the concurrent reduction of monoaminergic (e.g., serotonergic (5-HTergic) and noradrenergic (NEergic)) function was also associated with the pathogenesis of AD (Curcio & Kemper, 1984; Yamamoto & Hirano, 1985). Since 5-HTergic projection fibers originating in raphe nuclei are reported modulators of the function of cholinergic basal forebrain neurons (Khateb et al., 1993; Smiley et al., 1999), it seems likely that degeneration of postsynaptic cholinergic target cells may directly interact with the activity of the 5-HTergic projection system. Additionally, a cholinergic-5-HTergic interaction has also been established in the neocortex, where the two neurotransmitter systems share identical postsynaptic innervation targets, and coregulate a number of processes that are substantially involved in the regulation of learning and mood (Cassel & Jeltsch, 1995; Nilsson et al., 1988; Riekkinen et al., 1990). Accordingly, ample pharmacological evidence indicates that altered central 5-HTergic activity is critically involved in the development of fear, anxiety, and several mood-related psychiatric disorders (Chaoouloff, 2000; File & Gonzalez, 1996, 1996).

Previous in vivo studies demonstrated that infusion of Aβ fragments or the glutamate analogue N-methyl-D-aspartate (NMDA) into the rat MBN significantly affects locomotor activity (Harkany et al., 1998), anxiety (Harkany et al., 1999b, 2000b), and novelty-induced arousal (Harkany et al., 2000b). Since changes of these behavioral parameters are presumably indirectly related to the primary cholinergic deficit following MBN lesions, in the present study we investigated whether Aβ(1-42) infusion into the MBN alters 5-HTergic responses in both the rat basal forebrain and cerebral cortex. Aβ was unilaterally injected into the MBN, and the degree of anxiety was assessed in the elevated plus maze test 7 days postsurgery. Aβ-induced damage to cholinergic MBN neurons was determined by means of quantitative acetylcholinesterase (AChE; EC 3.1.1.7) histochemistry in the somatosensory cortex, which receives the densest innervation from the damaged MBN subdivision (Luiten et al., 1995). Alterations in the density of 5-HTergic fiber projections and of 5-HT\(_{1A}\) receptors were demonstrated both in the MBN and in the cerebral cortex. Additionally, Aβ-induced changes in serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were measured in the somatosensory cortex.

**MATERIALS AND METHODS**

**Peptide Synthesis**

Aβ\(1-42\) was synthesized on an ABI 430 A automated peptide synthesizer (Biolytic Lab. Performance Inc., Fremont, CA) with amide at the C-terminal by a solid-phase technique involving Boc chemistry as previously described in detail (Harkany et al., 1995, 2000a). Briefly, peptide chains were elongated on 4-methylbenzhydrylamine (MBHA) resin (0.6–0.8 mmol/g). Couplings were performed with dicyclohexylcarbodiimine with the exception of asparagine, which was incorporated in OHBt-ester form. The Boc group was removed by treatment with 50% trifluoroacetic acid in CH\(_2\)Cl\(_2\). After completion of the synthesis, the peptides were cleaved from the resin with liquid hydrogen fluoride (HF). Free peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC) on an 8ST-SI-100S C\(_{18}\) column. Their purity was checked by RP-HPLC on a W-Porex 5C\(_{18}\) column. Amino acid analysis demonstrated the expected amino acid composition and electrospray mass spectrometry confirmed the expected molecular ion.

**Animals, Surgical Procedure, and Tissue Processing**

Young adult male Wistar rats (Charles River, SPF, 250–300 g, \(n = 70\) [\(n = 4–7\) per group]) were caged...
individually at least 3 days prior to the start of the experiments and kept on a normal laboratory diet and tap water ad libitum in an air-conditioned room (21 ± 2°C) with a 12-h light/dark cycle (lights on at 08.00). All efforts were made to minimize animal suffering throughout the experiments. Their care and treatment were in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Local Ethical Committee of the University of Groningen (DEC #2286/1999).

The animals were anesthetized with halothane (1.5%, 1.8 L/min airflow; Zeneca, Ridderkerk, The Netherlands), and their heads were mounted in a stereotaxic frame (Narishige, Japan). Subsequently, for behavioral and quantitative histochemical studies, and for the determination of 5-HT1A receptor density by means of autoradiography 1 μl of 200 μM Aβ(1-42) was injected slowly (0.1 μl/min) into the right MBN with a Hamilton microsyringe (antero-posterior (AP) co-ordinate = −1.5 mm, L = 3.2 mm, DV = 6.5 mm; Paxinos & Watson, 1986), while for biochemical assays (5-HT and 5-HIAA) 2 μl of 200 μM Aβ(1-42), was delivered unilaterally. Previous studies in our laboratory demonstrated that infusion of 2 μl of Aβ(1-42) in the rat MBN produces characteristic biochemical changes in rat neocortex (Harkany et al., 1995; O’Mahony et al., 1998). Aliquots of lyophilized Aβ(1-42) were dissolved in ultrapure water and immediately infused into the MBN (O’Mahony et al., 1998). All solutions were prepared freshly. Sham-operated animals received vehicle injections only. Based on previous evidence a 7-day survival period was used (Harkany et al., 2000a; O’Mahony et al., 1998).

For histochemistry, fixation of the brains was carried out under deep sodium pentobarbital anesthesia by transcardial perfusion with 350 ml fixative composed of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.4), which was preceded by a short prerinse (50 ml) with physiological saline. Brains were then postfixed for 2 h in the same fixative, and cryoprotected by overnight storage in 30% sucrose in 0.1 M PB at 4°C. Thereafter, coronal frozen sections were cut on a cryostat microtome at 20- and 50-μm thickness for AChE histochemistry and 5-HT immunocytochemistry, respectively. For biochemical determination of cortical 5-HT and 5-HIAA concentrations and for the localization of 5-HT1A receptors rats were deeply anaesthetized in ether and decapitated. Whole brains were quickly removed from the skull and stored at −80°C until further processing.

Elevated Plus Maze

To assess the effect of Aβ(1-42) infusion in the MBN on novel stimulation-evoked fear/anxiety as well as on exploratory drive the animals were tested in the elevated plus maze on day 7 (d7) postinjection according to a standard protocol (File & Gonzales, 1996; Harkany et al., 1999b). Briefly, the plus maze had two open arms (50 × 10 cm) surrounded by 0.5-cm-high ledges and two closed arms of the same size with walls 50 cm high, elevated 50 cm above the ground. Each rat was placed in the central square of the apparatus (10 × 10 cm), facing a closed arm, and allowed to explore the maze for 5 min. The times spent on the open arms and the number of both open and closed arm entries were recorded. Additionally, the percentage time spent on the open arms and the percentage of open arm entries (time spent on open arms/total time × 100) [%t] and (open arm entries/total number of entries × 100) [%no]) were calculated. An increase in the percentage of the time spent on the open arms was interpreted as an anxiolytic response. All tests started at 11.00 a.m., and took place under quiet conditions in an environment lit by red light to force the animals to explore the plus maze apparatus.

Quantitative Histochemistry

For AChE histochemistry, free-floating sections were post-fixed by immersion in a 2.5% glutaraldehyde solution in phosphate-buffered saline (PBS; 0.01 M, pH 7.4) overnight at 4°C. Subsequently, cholinergic fibers were visualized by staining for AChE according to Hedreen et al. (1985) using an AgNO3 intensification procedure.

Tissue sections processed for 5-HT immunocytochemistry were rinsed several times in 0.01 M PBS, immersed in 0.3% H2O2 in phosphate-buffered saline (0.01 M, pH 7.4; PBS) for 15 min, rinsed again, exposed to 0.5% Triton X-100 for 30 min, and incubated for 1 h at room temperature (RT) in 5% normal goat serum (NGS; Zymed, San Francisco, CA) diluted in 0.1% Triton X-100 and 0.1% sodium-azide-containing PBS. Subsequently, free-floating sections were incubated with rabbit anti-5-HT IgG, as primary antibody (diluted 1:10000; Freund et al., 1990; Görcs et al., 1985) in 1% NGS and 0.1% Triton X-100 at 4°C for 72 h under gentle movement of the incubation medium. After incubation, sections were thoroughly rinsed in PBS, preincubated for 1 h with 5% NGS at RT followed by the second antibody incubation with biotinylated goat anti-rabbit IgG (1:300; overnight at 4°C; Vector Labo-
AChE fiber density was measured in layer V of the posterior somatosensory cortex according to a standard protocol by using a Quantimet Q-600HR computerized image analysis system (Leica, Rijswijk, The Netherlands; Harkany et al., 1999a, 2000b). Surface area density of cortical AChE-positive fibers was measured in three somatosensory cortical sections (at coordinates −1.3 mm, −1.5 mm, and −1.7 mm; Faxinos & Watson, 1986). After background subtraction and gray-scale threshold determination, the surface area of skeletonized AChE-positive fibers ([the area covered by AChE-positive cholinergic fibers]/[the total sampling area], given as percentages) was computed in each parietal cortical section by using a 599-nm emission filter. The relative value of fiber reduction was calculated in preestablished quadrants as the percentage difference between the surface area density at lesioned and contralateral sides of the brain.

The density of 5-HT immunoreactive (5-HT-ir) projections in the MBN and in the somatosensory cortex was determined by an unbiased, random sampling-based method utilizing computer-assisted (Quantimet Q-600HR, Leica) optical densitometry (Harkany et al., 2000b). Briefly, at least three coronal sections were selected from each brain sample at identical AP coordinates (starting at −1.4 mm from bregma; Faxinos & Watson, 1986) with a standard distance of 100 μm. Following manual delineation, the extent of the intermediate MBN (mm²) was determined. As inconsistent surface area determination may be a major pitfall of such a quantitative approach, special attention was given to the standardization of this particular step. Such data were therefore subjected to statistical analysis with nonsignificant differences between the different experimental groups as an obligate prerequisite of further data processing (contralateral hemisphere: 0.573 ± 0.026 mm² [sham-operated], 0.581 ± 0.027 mm² [Aβ(1-42)] vs ipsilateral hemisphere: 0.596 ± 0.026 mm² [sham-operated], 0.581 ± 0.039 mm² [Aβ(1-42)]). Thereafter, a frame grid consisting of 80 primary quantification frames of 110 × 110-μm individual frame size (as 8 frames/lane in 10 consecutive lanes) was superimposed. Each primary quantification frame was generated adjacent to the previous one (without any overlap) to ensure complete coverage of the region of our interest, but with the preservation of the independence of optical density (OD) measurements. Following subsequent threshold determination OD values were independently measured in all primary quantification frames that were in superposition with the delineated MBN structure. In general terms, such a quantification setup yielded 45–55 independent OD values covering the delineated MBN surface depending on the AP coordinates of the sections analyzed. OD values ranged from 0.000–2.500 based on standardized gray-scale values after logarithmic correction. Densitometrically obtained data were subsequently analyzed to determine (1) the mean ± SEM OD value of 5-HT-ir projections in each MBN, and (2) the distribution of OD values for the primary quantification frames at both sides of the brain.

Mean OD. Absolute OD values were averaged for each MBN and subsequently, the average of such values was calculated in the four sections analyzed. To express the effects of Aβ(1-42) infusion on 5-HT-ir structures these values were further processed to test statistically significant differences.

Distribution of independent OD values of primary quantification frames. Gray-scale-based OD determination employs distinct values between 0.000–2.500. Therefore, this range was divided into 25 identical subsets (e.g., 0.0–0.099, 0.1–0.199, etc.) and, accordingly, the frequency of independent measurements in the MBN was grouped and plotted. Such an approach reveals even mild shifts in ODs irrespective of their direction (increasing ODs: rightward, decreasing ODs: leftward) with high sensitivity.

5-HT-ir in cerebral cortex. 5-HT densitometry was only performed in layer V of the somatosensory cortex in sections adjacent to those stained for AChE. As 5-HTergic innervation of layer V of the cerebral cortex exhibits homogeneous staining intensities mean ± SEM OD values were expressed, whereas an OD distribution curve was not plotted.

5-HT<sub>1A</sub> Receptor Autoradiography and Optical Densitometry

Localization of 5-HT<sub>1A</sub> receptors in the MBN and somatosensory cortex was performed as essentially described by Nyakas et al. (1997) using [3H]8-OH-DPAT (2(N,N-di[2,3(9)]H)propylamino)-8-hydroxytetratin, sp act: 221 Ci/mmol; Amersham Life Sci-
ences, Little Chalfont, Buckinghamshire, UK). Briefly, 20-μm-thick coronal brain sections were cut on a cryostat microtome and mounted onto electrically charged SuperFrost slides (Fisher Scientific, Tustin, CA). Subsequently, the sections were incubated in Tris–HCl buffer (0.17 M, pH 7.6) supplemented with 4 mM CaCl₂, 0.01% ascorbate and 10 μM pargyline for 60 min at RT. All chemicals were of analytical purity and purchased from Sigma (St. Louis, MO). Following incubation, the sections were washed 3 × 15 min at 4°C and air-dried. Nonspecific binding was determined in the presence of 1 μM 5-HT. Thereafter, sections were exposed to 3H-sensitive Amersham Hyperfilm (Amersham) for 8 weeks at RT.

Autoradiograms were quantified by using an automated computer-assisted image analysis system (Q-500HC, Leica). After gray-scale threshold determination and background subtraction, OD of [3H]8-OH-DPAT binding was determined as tissue equivalents (nCi/mg tissue) based on standard [3H]Micro-scales (Amersham) and expressed as fmol/mg tissue. Specific binding was defined by subtracting tissue equivalent nonspecific binding separately in each cortical layer. In each brain, three sections were measured at AP coordinates corresponding to those selected for quantitative AChE histochemistry without preliminary knowledge of the case condition, and the average specific binding value of the three measurements was further processed statistically.

**Determination of 5-HT and 5-HIAA Concentrations in the Neocortex**

Biochemical analysis of 5-HT and 5-HIAA concentrations in the neocortex was carried out by means of HPLC with electrochemical detection as initially described by Seyfried et al. (1986). Briefly, neocortical samples were sonicated in 1 ml buffer (pH 2.8, adjusted with 4 N NaOH) containing 0.1 M citric acid, 0.1 M NaH₂PO₄, 1.4 mM octane-1-sulfonic acid, 0.1 mM etylenediaminetetraacetic acid, and 9% ethanol (mobile phase) to which 2 ng/50 μl N-methyl-dopamine had been added as internal standard that allowed correction for processing losses. Subsequently, aliquots were injected onto a LI Chrosorb RP-18 column and neurotransmitter fractions were detected electrochemically at 0.8V, with a mobile phase flow rate of 1 ml/min at a pressure of 200 bar. These data, together with those of the external standards were used to calculate 5-HT and 5-HIAA concentrations of the samples. Data were expressed as ng/g fresh weight of tissue.

**RESULTS**

ALβ(1-42) infusion in the MBN elicited anxiety in the elevated plus maze on d7 postsurgery (Figs. 1A and 1B). Such a behavioral response was characterized by the significantly decreased frequency of open arm entries (22.69% (Aβ(1-42)-lesioned) and by the apparently reduced time spent on the open arms of the apparatus (8.95% (Aβ(1-42)-lesioned), as compared to both naive control and sham-operated animals (%no: 36.65% (sham-operated), 56.25% (naive control) (medians), W = 11.96, P < 0.01; Fig. 1A; %: 25.50% (sham-operated), 34.33% (naive control), W = 15.18, P < 0.01; Fig. 1B).
In accordance with previous data (Harkany et al., 2000a; O’Mahony et al., 1998), AChE histochemistry revealed a significant loss of cholinergic projection fibers invading the somatosensory cortex after Aβ_{1-42} injections (Fig. 2B), relative to the sham-operated control group (Fig. 2A). Quantitative analysis of the density of AChE-positive projections in layer V of the somatosensory cortical area indicated 18.47 ± 1.62% reduction of cholinergic fiber processes as a consequence of Aβ_{1-42} infusion that significantly exceeded the effects of sham-operation (6.85 ± 1.09%, F = 11.72, P = 0.008; Fig. 3A).

Aβ_{1-42}-induced lesions of the MBN elicited a bi-directional change in the density of 5-HTergic innervation of the rat forebrain. Neurotoxic lesions resulted in a strongly increased density of 5-HT fiber innervation in the lesioned cholinergic MBN (Fig. 2D), while in sham-lesioned animals only a lightly stained 5-HTergic projection network was present (Fig. 2C). Whereas the compact core of the Aβ_{1-42} lesion was virtually devoid of 5-HT-ir fibers, two morphologically distinct types of 5-HTergic fibers were visualized in a strictly organized distribution pattern surrounding the Aβ_{1-42}-induced lesion. While 5-HT-positive fibers with swollen spherical or oval varicosities encircled the core of the lesion, a network of smooth 5-HT-ir fibers was predominantly present more distally in a likely “penumbral zone.” As Fig. 3B shows, increased OD of the 5-HTergic innervation of the damaged MBN became apparent following analysis of

FIG. 2. Effect of Aβ_{1-42} infusion on cortical cholinergic projections (A, B), and 5-HTergic innervation in the MBN (C, D). Note the apparent loss of acetylcholinesterase (AChE)-positive projection fibers in layer V of the somatosensory cortex (B), and the accumulation of 5-HT-ir projection neurites surrounding the Aβ_{1-42}-induced lesion (D), as compared to the effects of the sham-operative insult (A, C). Insets in Figs. C and D show representative 5-HT-immunoreactive projections in the MBN. Arrowheads point to sprouting 5-HTergic fibers, whereas arrows denote swollen—presumably degenerating—5-HT-positive varicosities in the core of Aβ_{1-42} lesion. Horizontal bars in Fig. A denote the cortical layer, which was subjected to quantitative determination of surface area density of AChE-positive structural elements. Scale bar: (A) 50 μm; (D) 150 μm; (insets) 20 μm.

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mean OD values (0.78 ± 0.09 (Aβ(1-42)-lesioned) vs 0.51 ± 0.07 (sham-operated), F = 5.46, P < 0.05). Distribution analysis of the OD values of primary quantification frames further substantiated the significant increase of 5-HT labeling in the ipsilateral MBN, as it was indicated by an increase in the mean optical density (OD; B) and by a considerable rightward shift in the OD distribution profile (E), and by decreased OD of cortical 5-HTergic projections (F), as compared to the effects of sham-operation (B, D). Note that unilateral Aβ infusion did not affect 5-HTergic activity in the contralateral MBN (mean OD, C; OD distribution, D, E insets), or in somatosensory cortex (data not shown). *P < 0.05 vs sham-operated (n = 6).

In contrast, a significantly decreased 5-HT signal was detected in layer V of the somatosensory cortex 7 days after Aβ(1-42) infusion in the MBN, as compared to the effects of sham operation (0.15 ± 0.01 (Aβ(1-42)) vs 0.21 ± 0.02 (sham-operated), F = 5.41, P < 0.05; Fig. 3F), whereas 5-HT immunoreactivity in the contralateral neocortex was not altered (0.18 ± 0.03 (Aβ(1-42)) vs 0.17 ± 0.04 (sham-operated).

Localization of 5-HT₁A receptors in the MBN by means of [³H]8-OH-DPAT autoradiography revealed a slight, nonsignificant decrease in the density of

FIG. 3. Changes of cortical cholinergic innervation (A) and the density of 5-HTergic projections in the MBN (B–E) and in the neocortex (F) as a result of Aβ(1-42) infusion into the intermediate MBN subdivision, as detected by means of quantitative AChE histochemistry and 5-HT immunocytochemistry. Aβ(1-42) elicited a significant loss of cortical cholinergic innervation (A). Such a cholinotoxic injury was accompanied by sprouting of 5-HT-ir projection fibers in the ipsilateral MBN, as it was indicated by an increase in the mean optical density (OD; B) and by a considerable rightward shift in the OD distribution profile (E), and by decreased OD of cortical 5-HTergic projections (F), as compared to the effects of sham-operation (B, D). Note that unilateral Aβ infusion did not affect 5-HTergic activity in the contralateral MBN (mean OD, C; OD distribution, D, E insets), or in somatosensory cortex (data not shown). *P < 0.05 vs sham-operated (n = 6).
**FIG. 4.** Distribution of [3H]8-OH-DPAT binding in the parietal cortex after vehicle (A) or Aβ(1-42) (B) infusion into the MBN (~1.7 mm from bregma, Paxinos & Watson, 1986). Quantitative densitometric analysis was carried out in the cortical area bordered by dashed lines. Distinct neocortical layers were separated based on their different [3H]8-OH-DPAT binding capacities, as shown in B. A significant decrease of [3H]8-OH-DPAT binding was determined as a consequence of Aβ(1-42) infusion into the MBN (C). *P < 0.05 (one-way analysis of variance), data on [3H]8-OH-DPAT binding were expressed as fmol/mg tissue based on standard [3H]Micro-scales. Scale bar, 1 mm.

<table>
<thead>
<tr>
<th></th>
<th>Layer I - II</th>
<th>Layer III - IV</th>
<th>Layer V</th>
<th>Layer VI</th>
<th>Entire Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>11.40 ± 0.91</td>
<td>11.72 ± 0.54</td>
<td>26.92 ± 1.22</td>
<td>17.61 ± 0.95</td>
<td>16.21 ± 0.36</td>
</tr>
<tr>
<td>Aβ(1-42) (n = 4)</td>
<td>9.41 ± 0.95</td>
<td>10.36 ± 1.13</td>
<td>22.31 ± 1.13*</td>
<td>13.48 ± 1.09*</td>
<td>13.85 ± 0.41*</td>
</tr>
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[^H]8-OH-DPAT binding sites following Aβ(1-42) infusion (1.86 ± 0.30 fmol/mg tissue), as compared to the effect of sham-operation (2.15 ± 0.36 fmol/mg tissue). It is noteworthy, however, that the low density of 5-HT1A receptors on diffusely localized cholinergic neurons of the rat MBN is close to the sensitivity threshold of the detection procedure, as gray-scale values of the samples usually do not exceed that of the lowest standard point (1.4 nCi/mg tissue) of the [^H]Microscales applied to plot a logarithmic calibration curve. As it is shown in Figs. 4A and 4B, the cortical binding pattern of [^H]8-OH-DPAT closely resembled the distribution profile of AChE-positive cholinergic innervation fibers (Fig. 2B). Quantitative analysis of the distinct cortical layers, in particular of layer I-II, III-IV, V, and VI revealed a consistent decrement of [^H]8-OH-DPAT binding in each neocortical layer investigated, with significant differences in layer V (17.14 ± 4.20% loss of the sham-operated value, \( F = 7.10, P < 0.05 \)), layer VI (23.39 ± 6.16% loss of the sham-operated value, \( F = 7.67, P < 0.05 \)), and in the [^H]8-OH-DPAT binding of the entire neocortex (14.53 ± 2.51% of the sham value, \( F = 16.58, P < 0.05 \); Fig. 4C).

Concomitant with the loss of [^H]8-OH-DPAT binding sites, significant reduction of 5-HT and 5-HIAA concentrations was measured in the somatosensory cortex as a consequence of Aβ(1-42) infusion in the MBN (Figs. 5A and 5B). Aβ(1-42) injections resulted in a 59.37 ± 4.43% decrease of the neocortical 5-HT concentration (314.1 ± 34.3 (Aβ(1-42)) vs 773 ± 98.6 (sham-operated) ng/g tissue; \( F = 4.41, P < 0.05 \); Fig. 5A), which was paralleled by the levels of the 5-HT metabolite 5-HIAA. In fact, Aβ(1-42) induced a significant 55.34 ± 3.87% decline in cortical 5-HIAA concentration (199.3 ± 17.3 (Aβ(1-42)) vs 446.3 ± 77.2 (sham-operated) and 415.6 ± 48.9 (naive control) ng/g tissue; \( F = 3.44, P < 0.05 \); Fig. 5B). It is noteworthy that sham-operation resulted in increased cortical 5-HT levels (543.5 ± 64.1 ng/g tissue), which were nonsig-
presented as means and 5-HIAA concentrations were expressed as ng/g tissue; data are significantly different from those of the naive control group.

DISCUSSION

The present data indicate that Aβ-induced injury to cholinergic neurons of the basal forebrain elicits anxiety-driven escape behavior in the elevated plus maze. A bi-directional change in 5-HTergic innervation of MBN and the cerebral cortex provided anatomical correlate for the Aβ-induced loss of cholinergic MBN neurons and their cortical projections 7 days post-lesion. Whereas abundant accumulation of 5-HT-ir fibers became evident in the close proximity of the neurotoxic lesion in the MBN, a significant decrease in the density of 5-HTergic projections paralleled the Aβ-induced decrement of cholinergic innervation in the cerebral cortex. Additionally, a significant loss of 5-HT1A receptor-binding sites and concomitant reduction of 5-HT and 5-HIAA concentrations substantiated the decline of 5-HTergic input to the cerebral cortex.

Lesions to basal forebrain cholinergic nuclei were previously shown to impair sensory information processing in several learning paradigms (Harkany et al., 1998, 1999a,b; Riekkinen et al., 1990), whereas relatively little is known about their involvement in the development of anxiety (Harkany et al., 2000b). As connection of monoaminergic, particularly 5-HTergic and NEergic systems to the development of anxiety is a well-established phenomenon (Chaouloff, 2000; Leonard, 1996; Ressler & Nemeroff, 2000), anxiety-related behavioral consequences of MBN lesions may imply changes in the monoaminergic innervation of the basal forebrain and/or its projection areas, such as the somatosensory cortex. Hence, we investigated as to whether changes in escape behavior may be linked to altered 5-HTergic innervation of the MBN following Aβ(1-42) infusion.

Aβ infusion into the MBN results in the accumulation of 5-HT-ir projection fibers in a concentric fashion. Accordingly, 5-HT-ir fibers can be sub-divided into three major morphologically distinct categories: (a) the lesion core devoid of 5-HTergic sprouting, (b) inner zone with large (swollen) varicose 5-HT-ir-positive fibers, and (c) a marginal “penumbra-like” zone of the neurotoxic lesion containing smooth 5-HTergic fibers. The “beaded” appearance of 5-HT-ir neurites in the inner zone of Aβ(1-42) lesions resembles morphological characteristics of degenerating 5-HTergic projection fibers during aging and in AD (Whitehouse et al., 1981). We assume therefore that such 5-HTergic projections in the inner zone of Aβ lesion may undergo neurodegeneration as they lose their postsynaptic cholinergic targets or can directly be damaged by high concentrations of Aβ. Another explanation for the morphological diversity of 5-HT fibers may derive from the capacity of adult 5-HT fibers of heterotypic sprouting in response to damage of their postsynaptic targets, as it was shown after excitotoxic lesions to MBN, hippocampus, and striatum (Harkany et al., 2000b; Zhou et al., 1995). As the degree of 5-HTergic sprouting in the MBN closely correlates with the loss of AChE-positive projections in the somatosensory cortex (5-HT = 0.426 + 0.0128 AChE, corr. coeff. = 0.8486, F = 30.88, P < 0.01), we suggest that the density of 5-HTergic sprouting may correlate with the degree of neuronal damage elicited by Aβ.

It is well established that Aβ when injected into the basal forebrain initiates a glutamate-triggered excitotoxic cascade, which impels the degeneration of cholinergic projection neurons (Harkany et al., 2000a). It should be noted that sprouting of monoaminergic projection fibers is not a unique Aβ-related phenomenon (Gasser & David, 1987; Peterson, 1994; Zhou et al., 1995) and, particularly, can be triggered by a variety of excitotoxins, such as ibotenic acid (Zhou et al., 1995) or NMDA (Harkany et al., 2000b). Hence, it cannot be ruled out that changes in the density of 5-HTergic innervation appear as a consequence of altered local glutamate signaling and stimulation of glutamatergic receptors situated on 5-HTergic terminals, and are at
least partly unrelated to the loss of postsynaptic target neurons. From a functional point of view, if such a 5-HTergic sprouting response also leads to concomitant increase of 5-HT concentrations in the damaged MBN, as was shown in rat striatum after ibotenic acid injection (Zhou et al., 1995), then it might be interpreted as a potential intrinsic defense mechanism in response to focal excitotoxic injury. Subsequently, 5-HT may act on 5-HT1A receptors of cholinergic MBN neurons (Nyakas et al., 1997) and thereby counteract excitotoxicity via antagonism of voltage-dependent Ca2+ channel currents (Bayliss et al., 1997; Strosznajder et al., 1996) and postsynaptic membrane hyperpolarization (Davies et al., 1987; Williams et al., 1998).

Another plausible explanation for 5-HTergic sprouting after Aβ(1-42) infusion may rise from the observation that Aβ accumulation in plaques may induce aberrant axonal sprouting and formation of ectopic terminals in amyloid precursor protein (APP) transgenic mice (Phinney et al., 1999). Accordingly, exogenous Aβ(1-42) infusion in the MBN might have initiated the 5-HTergic sprouting response in our present model. An additional factor triggering the observed axonal sprouting may be APP itself, as it is known that APP expression significantly and persistently increases following MBN lesions (Wallace et al., 1991). APP may play a role in synapse formation and neurite extension (Allinquant et al., 1995), and α-secretase-cleaved APP isoforms may be involved in neuroprotective mechanisms following acute brain damage (Mattson, 1997).

Depletion of 5-HTergic activity in the cerebral cortex has long been regarded as an early hallmark of neurodegenerative disorders (Palmer, 1996; Sparks, 1989). Neuropathological findings are supported by experimental data indicating the involvement of cortical 5-HTergic innervation in cognition in a substantial interaction with subcortical cholinergic afferents (Dringenberg & Zalan, 1990; Nilsson et al., 1988; Riekkinen et al., 1990). Our present data substantiate earlier observations on a close relationship between cortical cholinergic and 5-HTergic systems, as Aβ-induced damage to cholinergic MBN neurons and the concomitant loss of their cortical projections resulted in a marked decline of the density of 5-HT-ir projections, the number of 5-HT1A receptors-binding sites and that of 5-HT and 5-HIAA concentrations in the somatosensory cortex. The simultaneous decrease of the 5-HTergic parameters investigated may hint compensatory down-regulation of cortical 5-HTergic activity in correlation with the decline of the subcortical cholinergic input instead of metabolic dys-balance (alteration in the 5-HT/5-HIAA ratio), or profound changes in postsynaptic 5-HT1A receptor sensitivity. In functional terms, the simultaneous decline of cholinergic and 5-HTergic activity in the cerebral cortex may lead to decreased behavioral responsiveness under novel conditions and to increased anxiety/fear-related escape behavior. As development of anxiety correlates with the decrease of brain 5-HT function (Leonard, 1996), the plastic response of 5-HT innervation to Aβ-induced cholinergic hypo-activity in the rat neocortex provides neurochemical substrates to the apparent behavioral dysfunctions.

In conclusion, our present findings show that the loss of cholinergic projection neurons in the MBN as a consequence of Aβ-induced brain damage bidirectionally modulates 5-HTergic activity in the basal forebrain and cerebral cortex. The above-characterized interactions of basal forebrain cholinergic and raphe 5-HTergic systems may provide vistas for the development of neuroprotective drugs and therapeutic strategies that beneficially influence AD-related loss of memory functions.

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REFERENCES


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