The Journal of Neuroscience, November 1995, 15(11): 7132–7140

Dynamics of Central Nervous 5-HT_{1A}-Receptors Under Psychosocial Stress

Gabriele Flügge

German Primate Center, 37077 Göttingen, Germany

It is well established that stress leads to changes in the serotonergic system. In order to gain a better understanding of the effects of recurrent stressful experiences on the serotonergic system, changes in the 5-HT_{1A}-receptor system resulting from different periods of psychosocial stress (PSS) were analyzed in the present study. Male tree shrews (*Tupaia belangeri*) were submitted to subordination stress for 2, 10, 21, and 28 d. 5-HT_{1A}-receptor binding was quantified by *in vitro* receptor autoradiography using the agonist ^{3}H-8-OH-DPAT (^{3}H-8-hydroxy-2-(di-n-propylamino)tetralin. PSS caused a downregulation of ^{3}H-8-OH-DPAT binding sites in cortical areas and in the hippocampus. After 10, 21, and 28 d of PSS, the number of binding sites was reduced in layers V and VI of the posterior cingulate cortex (by 34%). After 28 d of PSS, the number of binding sites was reduced in the parietal cortex (by 18%), in the prefrontal cortex (by 16%), in the regio retrobulbaris (by 8%), and in region CA1 of the hippocampus (by 11%). In the raphe nuclei, no PSS-induced downregulation of 5-HT_{1A}-receptors occured. A transient increase in ^{3}H-8-OH-DPAT binding was observed in the claustrum after 2 d of PSS (by 15%). There were also transient decreases in affinities for the radioligand probably representing receptor desensitization, for example, in the dorsal raphe nucleus after 28 d of PSS.

In conclusion, the dynamic 5-HT_{1A}-receptor changes occuring during PSS include downregulation and transient desensitization of receptors. They reflect regulatory mechanisms which probably lead to destabilization of the serotonergic system during prolonged PSS.

[Key words: DPAT, 5-HT, 5-HT_{1A}-receptor, stress, downregulation, desensitization, tree shrew]

The effects of stress on central nervous transmitter systems are of biomedical interest because prolonged stress can lead to anxiety disorders and depression (Akil and Morano, 1995). Both stress and anxiety have been related to an activation of the central nervous 5-HT system (Chaouloff, 1993), and it has been reported that the "stress hormone" corticosterone increases the activity of trytophan hydroxylase, the rate limiting enzyme of 5-HT synthesis (Azmitia and McEwen, 1974). The role of the 5-HT system in the regulation of emotional behavior and stress has largely been investigated using specific ligands for 5-HT-receptor subtypes (Zifa and Fillion, 1992). Among the subtypes, 5-HT_{1A}-receptors play a special role because pharmacological studies showed that the selective agonist 8-OH-DPAT can trigger the release of 5-HT and causes a decrease in anxiety (Blanchard et al., 1994). 5-HT_{1A}-receptors have been localized by autoradiography in the human and the rat brain (Hoyer et al., 1986; Vergé et al., 1986). On the cellular level, activation of these G-protein coupled receptors inhibits adenylate cyclase, opens potassium channels, and closes calcium channels to hyperpolarize target cells (Sanders-Bush and Canton, 1995). The somatodendritic 5-HT_{1A}-autoreceptors in the raphe nuclei regulate 5-HT release whereas in the hippocampus 5-HT_{1A}-receptors regulate the activity of pyramidal neurons (Hamon et al., 1990; Beck et al., 1992; Boadle-Biber, 1993). Stimulation of postsynaptic 5-HT_{1A}-receptors in the association cortex of the rat reduced the membrane excitability of pyramidal neurons (Araneda and Andrade, 1991). Experiments in rats demonstrated that the number of 5-HT_{1A}-receptors in the hippocampus, the brain region involved in the regulation of emotional behavior and autonomic functions is reduced by glucocorticoids as well as social stress (Chalmers et al., 1993; McKittrick et al., 1995). In these studies, the experimental factor regulating receptor expression was either a certain concentration of external glucocorticoids or a certain stress period. However, it is not known whether and how receptor expression changes over the course of prolonged stress periods accompanied by repetitive stressful events.

An experimental stress paradigm for studying the neurochemical consequences of prolonged stress should reflect the human situation where recurrent negative psychosocial experiences may lead to depressive symptoms. With regard to this aspect, male tree shrews (*Tupaia belangeri*) provide a useful model to investigate the central nervous consequences of psychosocial stress (PSS; Flügge et al., 1992; Fuchs and Flügge, 1995). In the tree shrew stress paradigm, the coexistence of two males in one cage leads to a stable dominant/subordinate relationship resulting in profoundly disturbed patterns of feeding, circadian rhythm, and sleep, and in clear behavioral depression of the subordinate animal (Aue, 1989). In the subordinate, the activities of the hypothalamic-hypophyseal-adrenal axis and of the sympathoadrenal system are constantly elevated (Fuchs et al., 1993). The distinct stress-induced behavioral and physiological alterations which occur in the subordinate tree shrew are based exclusively on the cognitive interpretation of the continuous presence of the dominant conspecific and can therefore be regarded as psychological stress (Raab and Storz, 1976). In the brains of subordinates, turnover rates of monoaminergic transmitters and their

Received Apr. 13, 1995; revised June 14, 1995; accepted June 20, 1995.

This work was in part supported by the German Science Foundation (SFB 406). Preliminary results of the study have been published as abstract on the Annual Meeting of the Society for Neuroscience (Miami). I thank R. Rudolph, S. Lüert, and A. Heutz for their excellent technical assistance, M. Hampe for making the photos, and E. Fuchs for critical comments on the manuscript.

Correspondence should be addressed to Dr. G. Flügge, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany.

corresponding receptors are affected in areas involved in the regulation of autonomic functions (Raab, 1971; Raab and Storz, 1976; Flügge et al., 1992). To evaluate time dependent effects of prolonged PSS on 5-HT_{1A}-receptors, binding of the agonist ^{3}H-8-OH-DPAT was characterized by autoradiography in 10 different brain areas of subordinate male tree shrews and controls after 2, 10, 21, and 28 d of PSS determining B_{max} and K_d-values in saturation experiments.

Materials and Methods

Experimental procedure and determination of urinary cortisol. Adult male tree shrews (*Tupaia belangeri*; 9–24 months old) from the breeding colony at the German Primate Center were adapted for the study. The animals were accustomed to frequent handling since their birth. They were housed singly on a regular day/night cycle (lights on from 08:00 to 20:00 hr) at 26°C, 55% relative humidity with tree shrew diet (Altromin, Lage, Germany). During the control period of 10 d, each animal lived alone in its cage. During the periods of PSS, the opaque partition between the neighboring cages of two animals unknown to one another was removed. This resulted in an active competition for control over the enlarged territory. After establishment of a stable dominant/subordinate relationship (usually after 1–2 hr), the two males were separated by a transparent wire mesh so that the animals could still see each other. Under these conditions, the subordinate animal reduced its sphere of action in the cage. During these periods of social encounters which lasted for 2, 10, 21, and 28 d, the wire mesh was removed every day for 1–2 hr. Control males lived singly in separate cages elsewhere in the animal facilities. All control males had never been confronted with either a dominant or a subordinate during their adulthood. During control as well as PSS periods, animals were weighed and morning urine samples were collected daily. Urinary cortisol was measured by a scintillation proximity radioimmunoassay (Udenfriend et al., 1985) using anti-rabbit antibodies (Paesel and Lorei, Frankfurt, Germany) bound to fluomicrospheres according to the manufacturers instructions (Type I reagent) and ^{3}H-cortisol as tracer (Amersham). To correct for physiological dilutions, the resulting concentrations were related to creatinine concentrations which were determined with Beckman Creatinine Analyzer 2.

Tissue preparation and autoradiography. At the end of each confrontation experiment, four subordinate and four control animals were sacrificed between 08:00 and 09:00 hr. One subordinate and one control animal were euthanasized simultaneously and care was taken to exclude nonspecific stress effects. The brains were quickly removed and frozen in liquid nitrogen. Cryostat sections (10 μm) were cut at −18°C, thaw-mounted onto gelatine-coated glass slides and placed under vacuum at 4°C overnight. Corresponding brain sections from one subordinate and one control animal were cut on the same day and processed in the same receptor binding experiment. Saturation experiments were performed with 10 different concentrations of ^{3}H-8-OH-DPAT (^{3}H-8-hydroxy-2-[di-n-propylamino]tetralin; specific activity 143.8 Ci/mmol; New England Nuclear) according to Vergé et al. (1986) with minor modifications. Nonspecific binding was determined in the presence a 1000-fold excess of 5-HT (Sigma). Briefly, consecutive sections (at least three sections per brain area, radioligand concentration, and animal) were preincubated for 30 min at room temperature in buffer (170 mM Tris/HCl, pH 7.6, 4 mM $CaCl_2$, 0.01% ascorbic acid, 10 μM pargyline; 10 μM fluoxetine), incubated with ^{3}H-8-OH-DPAT for 120 min, washed in buffer (twice for 5 min on ice), in distilled water (30 sec on ice), and dried under a stream of cold air.

Analysis of autoradiographic data. Quantification of ^{3}H-8-OH-DPAT binding sites was performed by autoradiography. Brain sections were exposed together with ^{3}H-microscale standards on tritium sensitive Hyperfilm-^{3}H (Amersham) for 5 weeks. The anatomical localization of radioligand binding was performed with the aid of Nissl stained sections adjacent to the sections which had been processed for autoradiography and with a tree shrew brain atlas (Tigges and Shanta, 1969). The films were densitometrically analysed with a computerized image analysis system (MCID, Imaging Inc., St. Catherines, Canada). Gray values of the standards were used to determine the amount of radioactivity bound to tissue sections which was expressed in femtomoles bound per milligram tissue equivalent (Davenport and Hall, 1988). The maximal number of binding sites (B_{max}) and the equilibrium constants (K_d) were derived from saturation experiments. Data were generated with curve fitting programs (SIGMA PLOT, Corte Madeira, CA). For statistical evaluation, group differences were assessed by the Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney U test. A probability level of $p \leq 0.05$ was used to determine statistical significance.

Table 1. Effects of PSS on body weight

Duration of PSS (days)	Controls	Dominants	Subordinates
2	98.8 ± 0.7	99.1 ± 0.9	95.2 ± 1.7*
10	98.6 ± 0.2	100.1 ± 0.7	95.3 ± 0.9*
21	98.7 ± 0.2	100.8 ± 0.6	96.1 ± 0.6*
28	98.5 ± 0.4	100.8 ± 0.4	95.8 ± 0.7*

Body weight is expressed as percent of values from the control period (mean ± SEM). Absolute values during the control period were 228.5 ± 5.6 gm in controls, 227.0 ± 1.3 gm in dominants, and 220.1 ± 2.4 gm in subordinates.
* Significant difference to control period ($p < 0.05$).

Results

Effects of psychosocial stress on body weight and urinary cortisol

When two male tree shrews were kept together in one cage a stable hierarchy was established within a short time. After an initial phase of social encounters, when one male frequently attacked the other until it had achieved the dominant status, the mere visual presence of the dominant was sufficient to induce subordinate behavior in the defeated animal. Within a few days, the subordinates developed clear behavioral depression as evidenced by decreased motor activity and decreased autogrooming. It has been demonstrated previously, that subordinates show a characteristic decrease in body weight (Fuchs et al., 1993). In the present experiments, the body weight of subordinates was reduced by approximately 5% as compared to the control period (Table 1). Subordinates also showed a marked activation of the hypothalamic-hypophyseal-adrenal (HPA) axis as revealed by the increase in urinary cortisol excretion (Table 2). In controls and dominants, the activity of the HPA-axis and body weight remained unchanged.

^{3}H-8-OH-DPAT labeling in the tree shrew brain

The 5-HT_{1A}-receptor agonist distinctly labels several nuclei and areas in the tree shrew brain. In the midbrain, the whole dorsal raphe nucleus is strongly labeled by ^{3}H-8-OH-DPAT (B_{max} 116.3 ± 5.2 fmol/mg; Figs. 1, 2). In the region of the median raphe nucleus, binding sites are clustered in the vicinity of the midline (B_{max} 35.4 ± 2.3 fmol/mg). The central gray is moderately labeled (41. ± 1.6 fmol/mg), and in the superior colliculus, a homogeneous labeling extends from the dorsal layers through the zonal layer to the stratum opticum (B_{max} 30.3 ± 1.5 fmol/mg).

The neocortex exhibits a low to moderate number of ^{3}H-8-OH-DPAT binding sites (Fig. 2). In the parietal cortex, layer I and II are relatively strongly labeled while layers III through V are only moderately labeled (mean B_{max} for layers I to V 29.1 ± 3.1 fmol/mg). Layer IV occasionally appears as distinctly labeled in the parietal cortex (Fig. 2). Many ^{3}H-8-OH-DPAT binding sites are found in the prefrontal cortex where again, labeling in layers I and II predominates (B_{max} for layers I to III 55.0 ± 5.5 fmol/mg). Within the posterior cingulate cortex, a broad band of ^{3}H-8-OH-DPAT binding sites covers layer VI reaching into layer V (B_{max} 50.2 ± 4.9 fmol/mg). Also in the occipital lobe of the neocortex, layers VI–V are distinctly labeled.

Table 2. Effects of PSS on urinary cortisol

Control period[a]			PSS		
Controls[a]	Dominants	Subordinates	Duration of PSS (days)	Dominants	Subordinates
0.115 ± 0.007	0.108 ± 0.015	0.131 ± 0.039	2	0.112 ± 0.012	0.321 ± 0.080*
0.116 ± 0.008	n.d.	0.092 ± 0.003	10	0.118 ± 0.011	0.374 ± 0.090*
0.118 ± 0.011	0.122 ± 0.014	0.091 ± 0.004	21	0.127 ± 0.008	0.299 ± 0.012*
0.134 ± 0.012	n.d.	0.125 ± 0.017	28	0.116 ± 0.010	0.245 ± 0.022*

Cortisol is expressed as ng/μmol creatinine (mean ± SEM). n.d., not determined.

[a] Control periods were 10 d for all animals.

* Significant difference to control period ($p < 0.05$).

A high number of 5-HT$_{1A}$-receptors is found in the region of the anterior olfactory nucleus which corresponds to the regio retrobulbaris as defined by Zilles (1990). Binding sites are diffusely distributed in this region and labeling extends through the tenia tecta into the medial and lateral orbital cortex (B_{max} 130.5 ± 6.7 fmol/mg). On sagittal sections, labeling shows a ray like pattern corresponding to diffuse layers visible on frontal sections (Figs. 1, 2). The olfactory bulb itself remains nearly unlabeled (Fig. 1).

In the thalamus, several areas contain high numbers of 5-HT$_{1A}$-receptors (Fig. 2). Comparison of autoradiograms to adjacent Nissl stained sections revealed that the ^{3}H-8-OH-DPAT binding sites accompany the transtegmental pathway and the mammillothalamic tract giving rise to moderate labeling in the ventromedial thalamic nucleus (B_{max} 64.0 ± 15.7 fmol/mg). Faint labeling was observed close to the internal medullary lamina (Fig. 2). However, the intensity of this labeling varies considerably between individual animals. The area of the paraventricular hypothalamic nucleus is faintly labeled and also other parts of the hypothalamus and the preoptic area exhibit only low numbers of ^{3}H-8-OH-DPAT binding sites. In the hippocampal formation, CA1 region and dentate gyrus are strongly labeled with the labeling extending into the subiculum (B_{max} for CA1: 160.0 ± 3.6 fmol/mg; Fig. 2). The hippocampal region CA3 appears to contain no ^{3}H-DPAT binding sites.

In the amygdala complex, it is the magnocellular basal nucleus which exhibits the highest numbers of ^{3}H-8-OH-DPAT binding sites (B_{max} 163.1 ± 3.6 fmol/mg). Other subnuclei of the amygdala complex contain only low 5-HT$_{1A}$-receptors numbers (see also Flügge et al., 1994). A strong labeling is found in the claustrum (B_{max} 82.8 ± 3.8 fmol/mg). Saturation experiments showed that ^{3}H-8-OH-DPAT bound with high affinity to 5-HT$_{1A}$-receptors in the different brain areas (Fig. 3). The highest affinities were detected in region CA1 of the hippocampal formation (K_d 0.16 ± 0.02 nM) while, in contrast, relatively low affinities were detected in the superior colliculus (K_d 1.17 ± 0.10 nM).

Effects of psychosocial stress on ^{3}H-8-OH-DPAT binding sites

Psychosocial stress led to time dependent alterations in the number of ^{3}H-8-OH-DPAT binding sites in some brain areas. In layers V–VI of the posterior cingulate cortex, the number of binding sites was reduced by 23% after only 10 d of PSS and this effect persisted until day 28 of PSS (see Fig. 4). In the parietal and the prefrontal cortex (layers I–V), a significant downregulation of ^{3}H-8-OH-DPAT binding sites occured after 28 d of PSS. In region CA1 of the hippocampus and in the area of the anterior olfactory nucleus, ^{3}H-8-OH-DPAT binding was also reduced after 28 d of PSS (Figs. 3, 4). The only PSS-induced increase in 5-HT$_{1A}$-receptor number was detected in the claustrum after 2 d of PSS. In the prefrontal cortex, there was a tendency towards an upregulation seen after 2 and 10 d, but values did not significantly differ from controls. No PSS-induced changes in receptor numbers were observed in the dorsal raphe nucleus (Figs. 3, 4) and in the median raphe nucleus, where the mean B_{max} for all PSS periods was 35.4 ± 2.3 fmol/mg in controls versus 35.9 ± 1.5 fmol/mg in subordinates. As stated above, ^{3}H-8-OH-DPAT labeling in the thalamic nuclei showed considerable inter-individual differences. The mean B_{max} for the ventromedial thalamic nucleus in subordinates from all groups

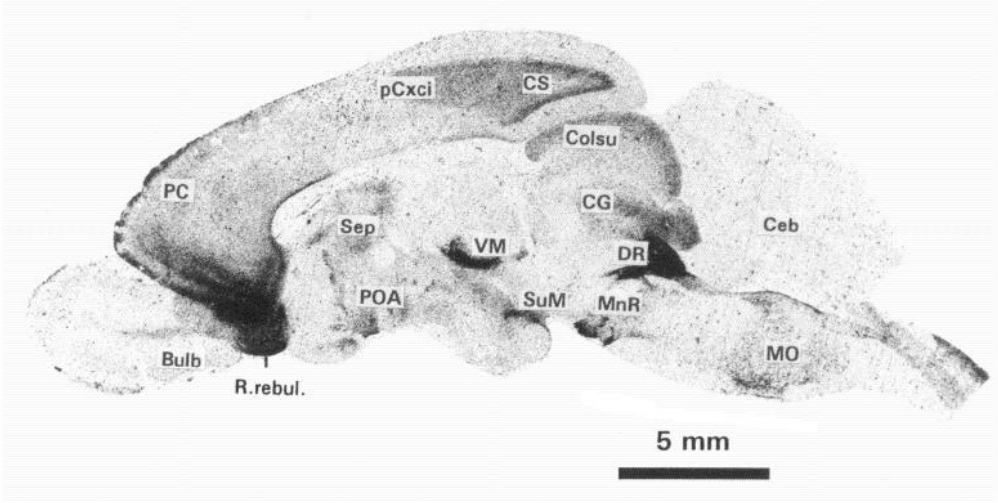

Figure 1. Distribution of 5-HT$_{1A}$-receptors in the brain. Autoradiogram shows ^{3}H-8-OH-DPAT binding to a sagittal section of the tree shrew brain approximately 0.2 mm lateral from the midline. Section was incubated with 4 nM ^{3}H-8-OH-DPAT. For abbreviations see list. Scale bar, 5 mm.

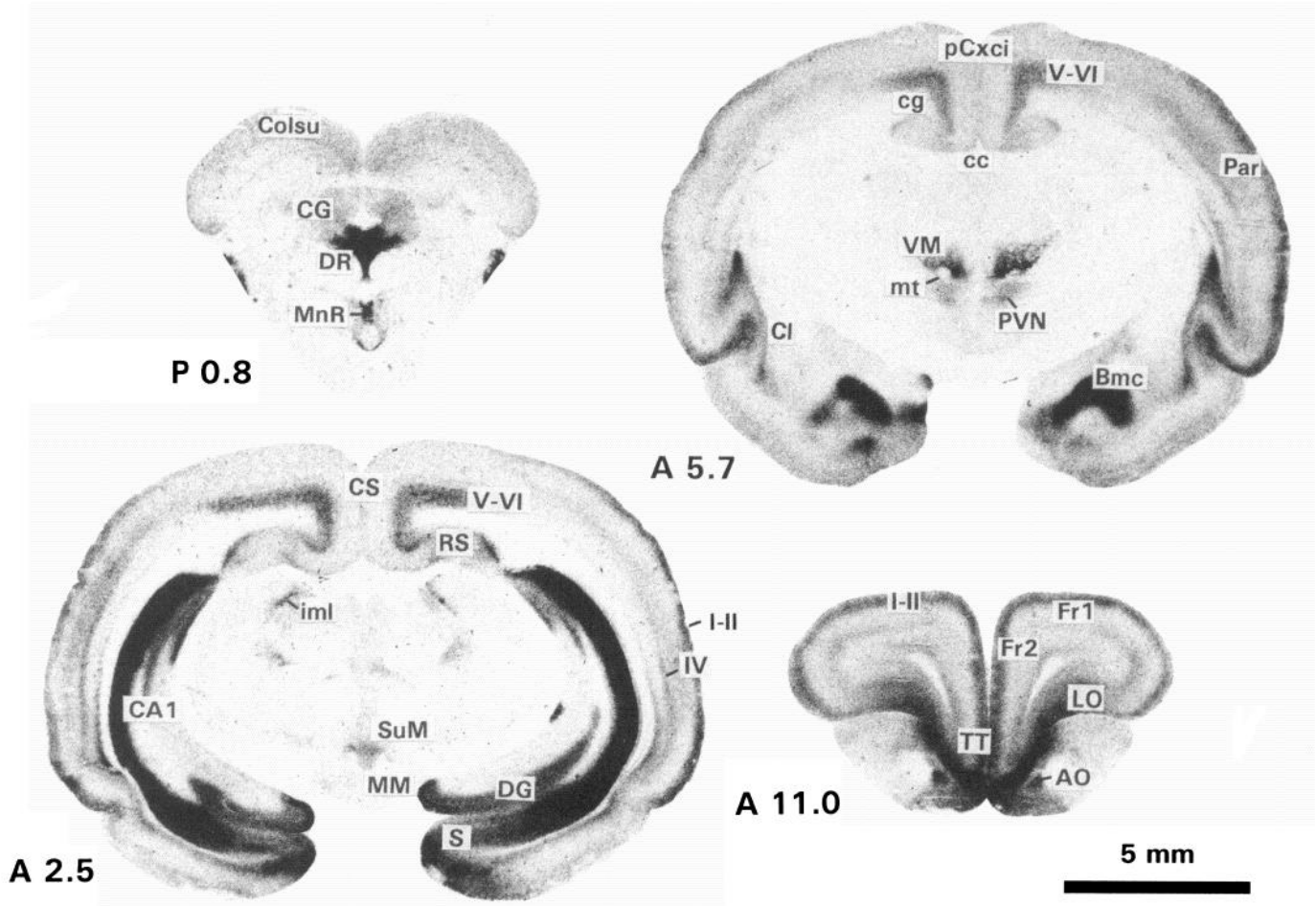

Figure 2. Distribution of 5-HT_{1A}-receptors in the brain. Autoradiograms show ^{3}H-8-OH-DPAT binding to frontal sections of the tree shrews brain. Sections were incubated with 4 nM ^{3}H-8-OH-DPAT. Numbers on the lower left side of each section denote the anatomical level according to Tigges and Shanta (1969). The area around the anterior olfactory nucleus (*AO*) corresponds to the regio retrobulbaris indicated in Figure 1. Scale bar, 1 mm.

(59.6 ± 6.6 fmol/mg) did not differ significantly from the value in controls (65.2 ± 5.7 fmol/mg), although a tendency towards downregulation was apparent.

In some brain areas, PSS did not only affect the number of ^{3}H-8-OH-DPAT binding sites but also their affinity in a time dependent manner (Table 3). In the superior colliculus, the claustrum, and the parietal cortex, there was a significant decrease in affinity (increase in K_d) after only 2 d of PSS. In the claustrum, the low affinity persisted throughout the whole PSS period although values did not significantly differ from controls on all experimental days. In the parietal cortex, a tendency towards persistently low affinity of 5-HT_{1A}-receptors was also observed. On day 21 of PSS, a significant decrease in affinity was observed in the superior colliculus and in region CA1 of the hippocampus preceeding the downregulation in receptor number in the later structure. The only significant increase in affinity (decrease in K_d) was detected in the central gray after 21 d of PSS.

Discussion

Psychosocial stress in tree shrews

As shown previously, chronic psychosocial stress (PSS) in subordinate tree shrews induces an activation of the neurosympathetic tone and of the hypothalamic-hypophyseal-adrenal (HPA) axis (Fuchs et al., 1993). The accompanying elevation of circulating catecholamines and glucocorticoids may have detrimental effects on behavior and various bodily functions by affecting the endocrine, the cardiovascular and the nervous systems (Bohus et al., 1987). In the present experiments, the urinary excretion of cortisol was constantly increased during PSS periods indicating hyperactivity of the HPA-axis.

Anatomy of the 5-HT_{1A}-receptor system

It is known from many studies that the ascending serotonergic system innervates nearly all midbrain and forebrain structures (Moore et al., 1978; Parent et al., 1981; Steinbusch, 1981) but 5-HT_{1A}-receptors exist only in selected raphe projection fields. The distribution of ^{3}H-8-OH-DPAT binding sites in the tree shrew brain largely resembles that in rats (Palacios et al., 1990) although the strong binding in the region of the anterior olfactory nucleus which extends into the tenia tecta and the orbital cortex has not been described previously. This region represents a cortical structure called regio retrobulbaris which receives projections from the raphe nuclei (Azmitia and Segal, 1978; Zilles, 1990).

^{3}H-8-OH-DPAT binding sites are abundant in the cerebral cortex which is innervated by two 5-HT projection systems (Mulligan and Törk, 1988; Törk, 1990). In the prefrontal cortex, the overall distribution of binding sites matches that of ^{3}H-5-HT binding sites in rhesus monkeys (Goldman-Rakic et al., 1990). In the posterior cingulate cortex, a prominent ^{3}H-8-OH-DPAT

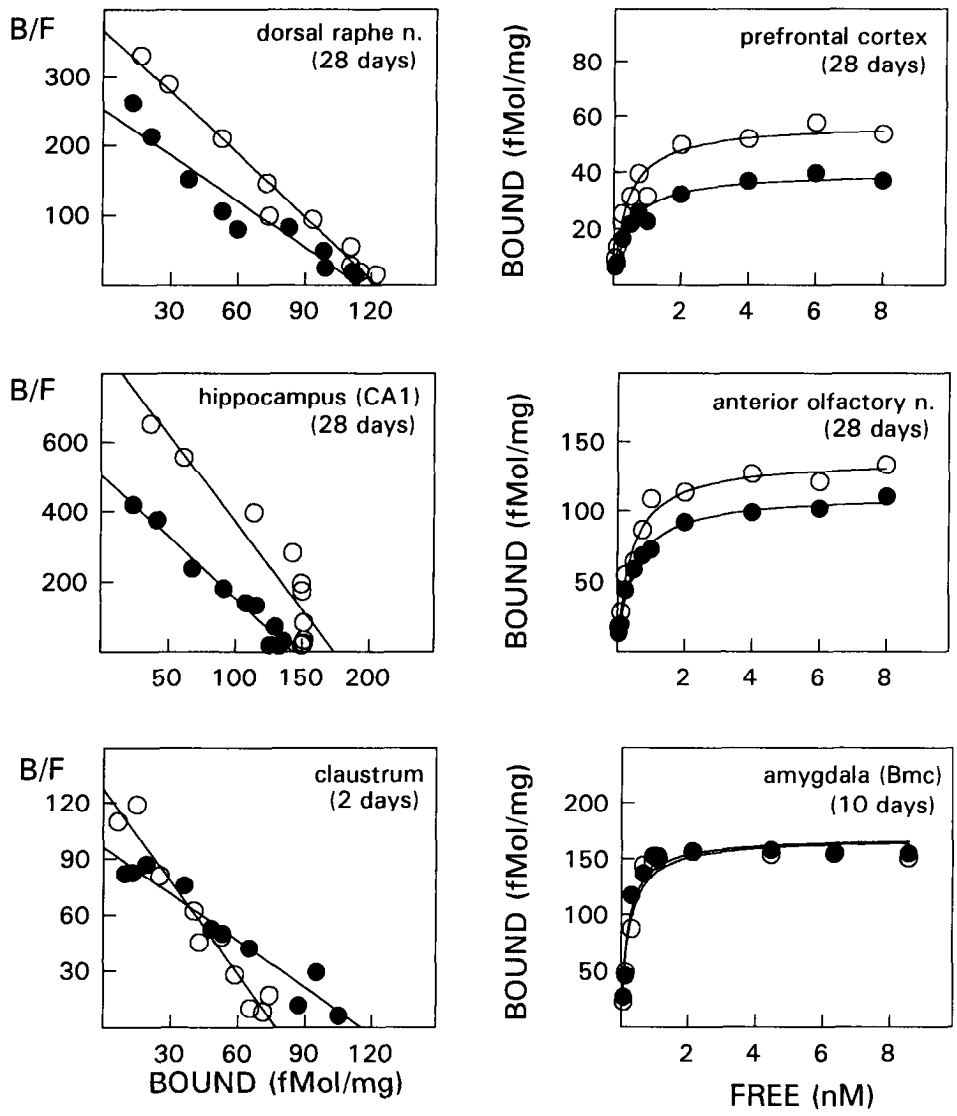

Figure 3. Quantification of ^{3}H-8-OH-DPAT binding in different brain areas. Examples of Rosenthal plots (*left*) and saturation curves (*right*) for binding of the radioligand in brains of control (*open circles*) and subordinate tree shrews (*solid circles*). Text in parentheses denotes durations of the stress periods.

labeling is observed in layers V and VI, which contain relatively low numbers of serotonergic fibers (Morrison et al., 1982). The labeling extends into the retrosplenial cortex thus covering isocortical and allocortical areas as defined by Zilles et al. (1986). Also in the striate and the whole occipital cortex, layers V and VI are labeled.

In the thalamus, ^{3}H-8-OH-DPAT binding sites occur in and close to the medullary lamina which is traversed by serotonergic fibers of the transtegmental pathway (Lavoie and Parent, 1991). Further binding sites are found close to the mammillothalamic tract. In the hypothalamus, binding sites accompany the median raphe forebrain tract (Azmitia and Segal, 1978).

Psychosocial stress-induced downregulation of 5-HT$_{1A}$-receptors

As shown previously, PSS downregulates α_2-adrenoceptors in brain nuclei containing nor/adrenergic cells and in areas innervated by noradrenergic fibers and involved in the control of autonomic functions (Flügge et al., 1992). The present study demonstrates that 5-HT$_{1A}$-receptors are downregulated in cortical 5-HT projection fields and in the hippocampus.

In region CA1 of the hippocampus, 5-HT$_{1A}$-receptors were downregulated after 4 weeks of PSS. Similar results have been obtained after exposing rats to chronic social stress in colonies followed by an acute restraint stress leading to decreased ^{3}H-8-OH-DPAT binding in the hippocampus (McKittrick et al., 1995). Decreased levels in 5-HT$_{1A}$-receptor binding have also been observed in several brain regions after treating rats experimentally with corticosterone (DeKloet et al., 1986; Mendelson and McEwen, 1992a,b). This regulation of 5-HT$_{1A}$-receptor numbers by the corticosteroids obviously occurs on the transcriptional level since adrenalectomy-induced overexpression of receptor mRNA is suppressed by exogenous corticosterone in rats (Chalmers et al., 1993; Meijer and DeKloet, 1994). Stress-induced downregulation might therefore be—at least in part—due to transcriptional regulation by glucocorticoids. Because 5-HT$_{1A}$-receptor activation induces multiple intracellular signaling processes reduced numbers of these membrane bound receptors might have great consequences for neuron physiology (Sanders-Bush and Canton, 1995). Stimulation of postsynaptic 5-HT$_{1A}$-receptors in the hippocampus elicits hyperpolarization and a decrease in membrane resistance of CA1 pyramidal neurons which can be counteracted by glucocorticoids (Joëls and DeKloet, 1994). It is interesting to note that 5-HT is involved in the con-

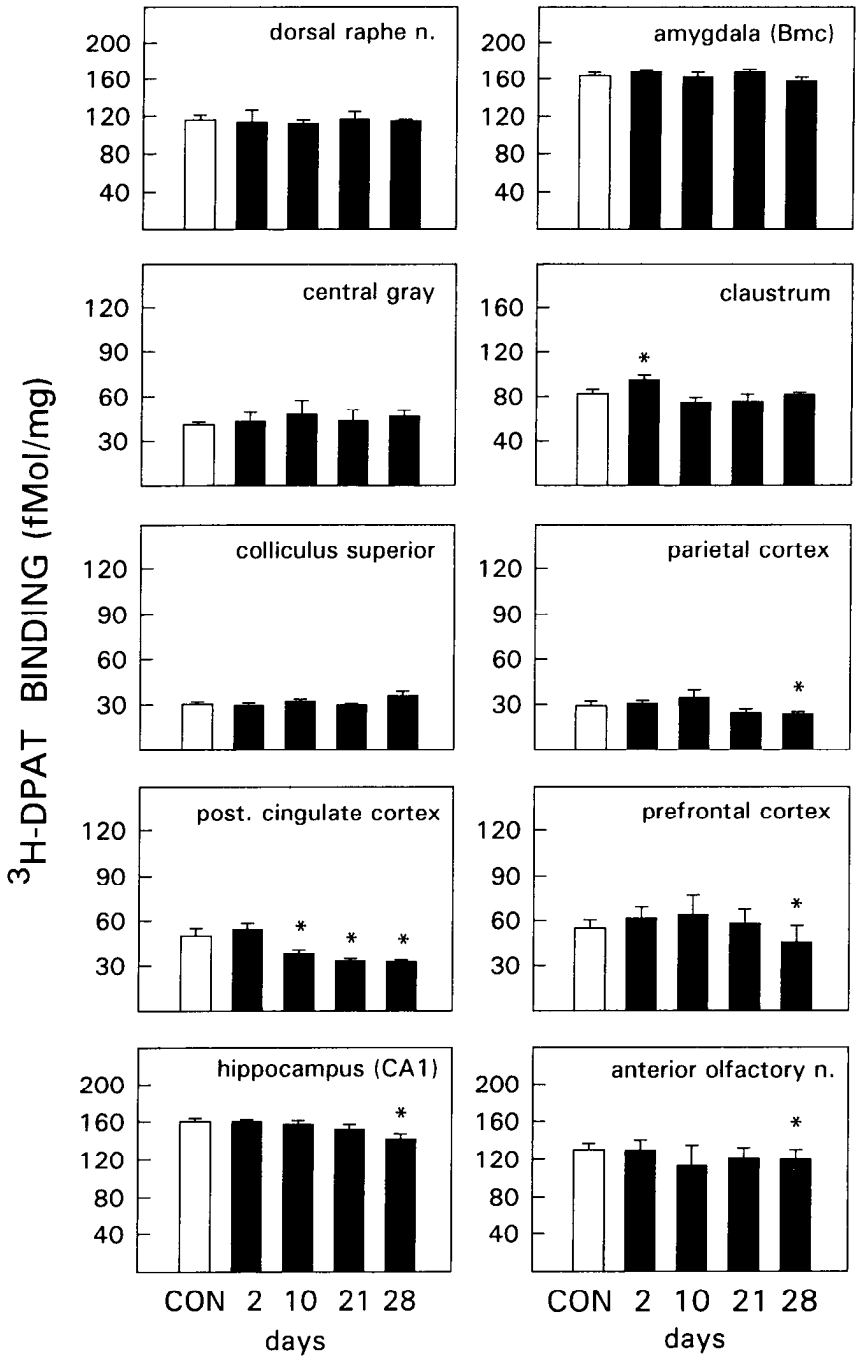

Figure 4. Numbers of ^{3}H-8-OH-DPAT binding sites in the tree shrew brain (B_{max} values derived from saturation experiments; means ± SEM) after different durations of psychosocial stress. Controls, *open bars* (n = 16); subordinates, *solid bars* (n = 4 per group). *Asterisks* denote significant differences as compared to controls ($p < 0.05$). ^{3}H-8-OH-DPAT binding sites were quantified in the following structures: whole dorsal raphe nucleus; dorsal part of the periaqueductal central gray; colliculus superior, zonal layer throughout optical layer; posterior cingulate cortex, layers V and VI; whole hippocampal region CA1; amygdala (Bmc), whole magnocellular basal nucleus; claustrum at the level of the amygdala formation; parietal cortex, layers I to V; prefrontal cortex, layers I to III; area of the anterior olfactory nucleus corresponding to the regio retrobulbaris.

trol of hippocampal theta-rhythm, an electroencephalographic pattern characteristic for emotional stress (Vertes et al., 1994). The cognitive impairment observed in rats after repeated stress experiences might be related to these neurophysiological events in the hippocampus (Luine et al., 1994). The reduction in numbers of 5-HT-receptors might also reflect another aspect of stress effects on neurons. Stress has been reported to induce an atrophy in the dendritic arbor of pyramidal neurons in hippocampal region CA3 (Magariños and McEwen, 1994). In region CA1 which contains the highest numbers of 5-HT$_{1A}$-receptors in the brain, and where neurons are known to be vulnerable to ischemic and other pathological influences, 5-HT$_{1A}$-receptors might counteract neuronal degeneration by mediating regulatory signals of cell growth (Lauder, 1993).

The strongest stress-induced effects on 5-HT$_{1A}$-receptor numbers were observed in layers V and VI of the posterior cingulate cortex where downregulation occured after only 10 d of PSS and persisted until 28 d of PSS. This time dependent effect correlated with the downregulation in the hippocampus when calculated for the individual animals (correlation coefficient 0.8; significance: $p < 0.05$). It is therefore possible that the 5-HT$_{1A}$-receptors in the posterior cingulate cortex and the hippocampus are regulated by the same signal, and it is interesting to note that glucocorticoid receptors, which are concentrated in the hippocampus, are also expressed in relatively high number in the cingulate cortex of the tree shrew (Jöhren et al., 1994). In both structures, hippocampus and cingulate cortex, 5-HT$_{1A}$-receptor downregulation may therefore be due to a stress-induced in-

Table 3. Effect of PSS on 5-HT_{1A}-receptor affinity

Area	Duration of PSS				
	Control	2 d	10 d	21 d	28 d
Dorsal raphe n.	0.43 ± 0.08	0.46 ± 0.04	0.50 ± 0.05	0.52 ± 0.01	0.53 ± 0.03*
Median raphe n.	0.75 ± 0.15	0.74 ± 0.02	0.52 ± 0.09	0.72 ± 0.11	0.92 ± 0.27
Central gray	0.88 ± 0.05	1.08 ± 0.01*	0.98 ± 0.09	0.69 ± 0.06■	0.81 ± 0.07
Colliculus sup.	1.17 ± 0.10	1.28 ± 0.17	1.30 ± 0.08	1.34 ± 0.05*	1.48 ± 0.18
Post. cingul. cortex	0.70 ± 0.08	0.60 ± 0.08	0.68 ± 0.04	0.61 ± 0.09	0.72 ± 0.01
Hippocampus (CA1)	0.16 ± 0.02	0.13 ± 0.01	0.19 ± 0.01	0.25 ± 0.01*	0.19 ± 0.02
Amygdala (Bmc)	0.28 ± 0.06	0.22 ± 0.02	0.35 ± 0.02	0.54 ± 0.22	0.29 ± 0.12
Claustrum	0.60 ± 0.03	0.73 ± 0.06*	0.65 ± 0.08	0.69 ± 0.05*	0.72 ± 0.13
Parietal cortex	1.12 ± 0.20	2.00 ± 0.26*	1.63 ± 0.33	1.31 ± 0.25	2.15 ± 0.54*
Prefrontal cortex	0.59 ± 0.12	0.60 ± 0.09	0.52 ± 0.12	0.62 ± 0.17	0.59 ± 0.08
Regio retrobulbaris	0.43 ± 0.07	0.44 ± 0.12	0.3 ± 0.11	0.51 ± 0.10	0.51 ± 0.06

K_d values (nM; mean ± SEM) for binding of ^{3}H-8-OH-DPAT were derived from saturation experiments. Controls, $n = 16$; subordinates, $n = 4$ per group. Asterisks indicate significantly higher K_d and squares significantly lower K_d as compared to controls ($p < 0.05$).

crease in glucocorticoid concentrations. The fact that 5-HT_{1A}-receptor downregulation in the posterior cingulate cortex was the same after 10, 21, and 28 d of PSS may indicate that the reduction in receptor expression is a final phenomenon persisting as long as there are challenging events. It is known from neurosurgical interventions that an impairment of the cingulate cortex leads to severe disturbances in affectivity (Zilles, 1990) so that it is tempting to assume that the cingulate 5-HT_{1A}-receptors play an important role in the regulation of emotionality. Since layers V and VI contain high numbers of intracortical synaptic contacts, receptor down-regulation might have significant consequences for intracortical information transfer. Betz cells and GABAergic neurons have been discussed as targets for serotonergic inputs in these cortical layers (Törk et al., 1990; Spain, 1994). However, layers V and VI receive only sparse 5-HT projections from the raphe nuclei, but moderate to dense noradrenaline projections (Morrison et al., 1982). The 5-HT_{1A}-receptor expression may therefore also underlie nonserotonergic transmitter signals, for example, from the noradrenergic system. Furthermore, because 5-HT_{1A}-receptors could be present on glial elements (Whitaker-Azmitia and Azmitia, 1986; Whitaker-Azmitia et al., 1993) one has to consider more indirect effects on synaptic transmission in this part of the neocortex. However, layers V and VI are not particularly rich in glial elements when compared to the other layers, which is in contrast to the retrobulbar region where glial structures are abundant (own unpublished observations in tree shrews). Further investigations are needed to elucidate the cellular localization of 5-HT_{1A}-receptors in different regions of the brain. PSS also reduced 5-HT_{1A}-receptor number in the prefrontal cortex which is involved in the regulation of agonistic behavior (de Bruin, 1990). Therefore, with exception of the hippocampus, all structures exhibiting PSS-induced 5-HT_{1A}-receptor down-regulation have been defined as cortical regions according to phylogenetic and morphometric criteria (Zilles et al., 1986; Zilles, 1990).

No alterations in the number of ^{3}H-8-OH-DPAT binding sites was observed in the dorsal and median raphe nuclei. Since there is electropysiological evidence for a large receptor reserve with respect to 5-HT_{1A}-receptor mediated inhibition of dorsal raphe neuronal firing (Cox et al., 1993) one must conclude that the presynaptic raphe 5-HT_{1A}-receptors are regulated differently than the receptors in the projection areas. A region specific coupling of 5-HT_{1A}-receptors to G-proteins may underlie these differences in receptor regulation (Iben et al., 1995).

The upregulation of 5-HT_{1A}-receptors in the claustrum cannot be explained at present, although it is interesting to note that the claustrum contains no glucocorticoid receptors (Flügge et al., 1988). It is interconnected with visual and somatosensory areas of the neocortex and stress-induced up-regulation of claustral 5-HT_{1A}-receptors might have implications on these functions.

Psychosocial stress-induced decrease in 5-HT_{1A}-receptor affinities

Besides the downregulation of 5-HT_{1A}-receptors, there were also PSS-induced decreases in the affinities for ^{3}H-8-OH-DPAT in some brain areas, probably representing desensitization of receptors. Agonist binding to G-protein coupled receptors can be modified by the agonist itself and a loss of high-affinity ^{3}H-8-OH-DPAT binding sites accompanied by desensitization of receptor-adenylate-cyclase-coupling was observed after exposure of 5-HT_{1A}-receptor expressing cells to high 8-OH-DPAT concentrations (Harrington et al., 1994). In rats, restraint stress leads to an increase in concentrations of 5-HT and 5-hydroxyindolacetic acid in the hippocampus (Kirby et al., 1994), and in tree shrews, increased concentrations of brain 5-HT and its metabolites were measured during PSS (Raab, 1971). The increase in hippocampal and raphe 5-HT concentrations might bring about agonist-mediated 5-HT_{1A}-receptor desensitization, so that the heterogeneity of hippocampal 5-HT_{1A}-receptors showing different affinities for 8-OH-DPAT (Nenonene et al., 1994) might in fact represent different affinity states of the same receptors (Mongeau et al., 1992). The low-affinity binding sites possibly play an important role for cellular signal transfer since the potency of 5-HT_{1A}-agonists to inhibit adenylate-cyclase-activity appears to be a function of their affinity for the low-affinity sites (Chamberlain et al., 1993). Also in the dorsal raphe nucleus, the decrease in affinity of 5-HT_{1A}-receptors on day 28 of PSS probably represents agonist-mediated receptor-desensitization since an increased 5-HT turnover-rate has been detected in the raphe nuclei of the rat during stress (Clement et al., 1993). The PSS-induced receptor-desensitizations in the tree shrew brain are not directly related to down regulation of receptors, but preceed the later process in some areas. As demonstrated recently, the extent of brain 5-HT release varies with the duration of the stress (Kir-

by et al., 1994) so that the dynamic changes in the receptor affinities described here might be related to changes in transmitter release. Since in male rats, social defeat induces a blunted adrenocortical response to 8-OH-DPAT probably due to a decrease in 5-HT-receptor sensitivity (Korte et al., 1995) the 5-HT-receptor downregulation and desensitization detected in the present study can be regarded as a central nervous adaptational mechanism aiming at a reduction of the adrenocortical hyperactivity during stress. At present, the behavioral consequences of the stress-induced changes in 5-HT-release and 5-HT_{1A}-receptor downregulation and desensitization are unknown. However, the behavior of subordinate tree shrews (e.g., withdrawal from the dominant, reduction in motor activity, reduction in autogrooming; Aue, 1989) indicates an increase in anxiety. Since overstimulation of postsynaptic 5-HT_{1A}-receptors has been hypothesized to increase anxiety (Hamon, 1994) the 5-HT_{1A}-receptor changes in the tree shrew brain may reflect repetitive anxiety-inducing signals which lead to subordination behavior.

The only increase in affinity of ^{3}H-8-OH-DPAT binding sites was observed in the central gray on day 21 of PSS. Dorsal raphe 5-HT neurons exert an inhibitory influence on cells in the periaqueductal gray (Lovick, 1994) and 5-HT_{1A}-receptors are thought to be the mediators of this inhibition (Behbehani et al., 1993). The increase in receptor sensitivity might indicate that in this center of aversive behavior (Graeff, 1994), many 5-HT_{1A}-receptors are already desensitized under control situations and become activated during PSS.

In conclusion, the present findings demonstrate that PSS induces downregulation as well as desensitization of 5-HT_{1A}-receptors in selected brain regions. Some of these receptor changes are transient and might be related to the stress-induced high glucocorticoid concentrations and to increased 5-HT release in certain brain areas. They represent dynamic processes leading to destabilization of the brain 5-HT system in its adaptational/maladaptational attempt to stabilize homeostasis in repeatedly challenging situations.

Appendix

List of abbreviations

AO	anterior olfactory nucleus
Bmc	magnocellular basal nucleus of the amygdala
Bulb	bulbus olfactorius
CA1	region CA1 of the hippocampal formation
cc	corpus callosum
Ceb	cerebellum
CG	central gray
cg	cingulum
Cl	claustrum
Colsu	colliculus superior
CS	cortex striatus
DG	dentate gyrus
DR	dorsal raphe nucleus
Fr1	area 1 of the prefrontal cortex
Fr2	area 2 of the prefrontal cortex
5-HT	5-hydroxytryptamine (serotonin)
iml	internal medullary lamina
LO	lateral orbital cortex
MO	medulla oblongata
MM	medial mammillary nucleus
MnR	median raphe nucleus
mt	mammillothalamic tract
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
Par	parietal cortex
PC	prefrontal cortex
pCxci	posterior cingulate cortex
POA	preoptic area
PSS	psychosocial stress
PVN	paraventricular hypothalamic nucleus
R.rebul.	regio retrobulbaris
RS	retrosplenial cortex
S	subiculum
Sep	septum
SuM	supramammillary nucleus
TT	tenia tecta
VM	ventromedial thalamic nucleus

References

Akil HA, Morano MI (1995) Stress. In: Psychopharmacology: the fourth generation of progress (Bloom FE, Kupfer DJ, eds), pp 773–785. New York: Raven.

Araneda R, Andrade R (1991) 5-hydroxytryptamine$_2$ and 5-hydroxytryptamine$_{1A}$ receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 40:399–412.

Aue D (1989) Konfrontationen zwischen männlichen Spitzhörnchen (*Tupaia belangeri*). Konsequenzen der Sozialkontakte für Verhalten und Physiologie sowie der Einfluss individueller und äußerer Faktoren auf die Dominanzentscheidung. Thesis, Güttingen.

Azmitia EC, McEwen BS (1974) Adrenocortical influence on rat brain tryptophan hydroxylase activity. Brain Res 78:291–302.

Azmitia EC, Segal M (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641–668.

Beck SG, Choi KC, List TJ (1992) Comparison of 5-hydroxytryptamine$_{1A}$-mediated hyperpolarization in CA1 and CA3 hippocampal pyramidal cells. J Pharmacol Exp Ther 263:350–359.

Behbehani MM, Liu HY, Jiang MR, Pun RYK, Shipley MT (1993) Activation of serotonin$_{1A}$ receptors inhibits midbrain periaqueductal gray neurons of the rat. Brain Res 612:56–60.

Blanchard DC, Shepherd JK, Rodgers RJ, Blanchard RJ (1992) Evidence for differential effects of 8-OH-DPAT on male and female rats in the anxiety/defense test battery. Psychoneuroendocrinology 106: 531–539.

Boadle-Biber MC (1993) Regulation of serotonin synthesis. Prog Biophys Mol Biol 60:1–15.

Bohus B, Benus, RF, Fokkema DS, Koolhaas JM, Nyakas C, van Oortmerssen GA, Prins AJA, de Ruiter AJH, Scheurink AJW, Steffens AB (1987) Neuroendocrine states and behavioral and physiological stress responses. Prog Brain Res 72:57–70.

Chamberlain J, Offord SJ, Wolfe BB, Tyau LS, Wang HL, Frazer A (1993) Potency of 5-hydroxytryptamine$_{1A}$ agonists to inhibit adenylyl cyclase activity is a function of affinity for the low-affinity state of ^{3}H-hydroxy-N,N-dipropylaminotetralin (^{3}H-8-OH-DPAT) binding. J Pharmacol Exp Ther 266:618–625.

Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ (1993) Corticosteroids regulate brain hippocampal 5-HT_{1A}-receptor messenger RNA expression. J Neurosci 13:914–923.

Chaouloff F (1993) Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res Rev 18:1–32.

Clement HW, Schäfer F, Ruwe C, Gemsa D, Wesemann W (1993) Stress-induced changes of extracellular 5-hydroxyindoleacetic acid concentrations followed in the nucleus raphe dorsalis and the frontal cortex of the rat. Brain Res 614:117–124.

Cox RF, Meller E, Waszczak BL (1993) Electrophysiological evidence for a large receptor reserve for inhibition of dorsal raphe neuronal firing by 5-HT_{1A} agonists. Synapse 14:297–304.

Davenport AP, Hall MD (1988) Comparison between paste and polymer (^{125}I) standards for quantitative receptor autoradiography. J Neurosci Methods 25:75–82.

DeKloet ER, Sybesma H, Reul HMHM (1986) Selective control by corticosterone of serotonin$_1$-receptor capacity in raphe-hippocampal system. Neuroendocrinology 42:513–521.

Flügge G, Schniewind A, Fuchs E (1988) The corticosterone receptive

system in the brain of *Tupaia belangeri* visualized by *in vivo* autoradiography. Exp Brain Res 72:417–424.

Flügge G, Jöhren O, Fuchs E (1992) ^{3}H-Rauwolscine binding sites in the brains of male tree shrews are related to social status. Brain Res 597:131–137.

Flügge G, Ahrens O, Fuchs E (1994) Monoamine receptors in the amygdaloid complex of the tree shrew (*Tupaia belangeri*). J Comp Neurol 343:597–608.

Fuchs E, Flügge G (1995) Modulation of binding sites for corticotropin-releasing hormone by chronic psychosocial stress. Psychoneuroendocrinology 20:33–51.

Fuchs E, Jöhren O, Flügge G (1993) Psychosocial conflict in the tree shrew: effects on sympathoadrenal activity and blood pressure. Psychoneuroendocrinology 18:557–565.

Goldman-Rakic PS, Lidow MS, Gallager DW (1990) Overlap of dopaminergic, adrenergic, and serotonergic receptors and complementarity of their subtypes in primate prefrontal cortex. J Neurosci 10: 2125–2138.

Graeff FG (1994) Neuroanatomy and transmitter regulation of defensive behaviors and related emotions in mammals. Braz J Med Biol Res 27:811–829.

Hamon M (1994) Neuropharmacology of anxiety: perspectives and prospects. Trends Pharmacol Sci 15:36–39.

Hamon M, Gozlan H, El Mestikawy S, Emerit MB, Bolanos F, Schechter L (1990) The central 5-HT_{1A}-receptors: pharmacological, biochemical, functional and regulatory properties. In: Annals of the New York Academy of Sciences, Vol 600, The neuropharmacology of serotonin (Whitaker-Azmitia PM, Peroutka SJ, eds), pp 114–129. New York: New York Academy of Sciences.

Hoyer D, Pazos A, Probst A, Palacios JM (1986) Serotonin in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. Brain Res 376:85–96.

Harrington MA, Shaw K, Zhong P, Ciaranello RD (1994) Agonist-induced desensitization and loss of high-affinity binding sites of stably expressed human 5-HT_{1A}-receptors. J Pharmacol Exp Ther 268: 1098–1106.

Iben LG, Mahle CD, Yocca FD (1995) Differential sensitivity of ^{3}H-agonist binding to pre- and postsynaptic 5-HT_{1A}-receptors in bovine brain. Br J Pharmacol 113:1400–1406.

Joëls M, DeKloet ER (1994) Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. Prog Neurobiol 43:1–36.

Jöhren O, Flügge G, Fuchs E (1994) Hippocampal glucocorticoid receptor expression in the tree shrew: regulation by psychosocial conflict. Cell Mol Neurobiol 14:281–296.

Kirby LG, Allen AR, Chou J, Lucki I (1994) A regional analysis of the effects of different stressors on extracellular levels of 5-HT. Soc Neurosci Abstr 128.18.

Korte SM, Buwalda B, Mejer O, DeKloet ER, Bohus B (1995) Socially defeated male rats display a blunted adrenocortical response to a low dose of 8-OH-DPAT. Eur J Pharmacol 272:45–50.

Lauder J (1993) Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci 16:233–240.

Lavoie B, Parent A (1991) Serotoninergic innervation of the thalamus in the primate: an immunohistochemical study. J Comp Neurol 312: 1–18.

Luine V, Villegas M, Martinez C, McEwen BS (1994) Repeated stress causes reversible impairments of spatial memory performance. Brain Res 639:167–170.

Magariños AM, McEwen BS (1994) Stress-induced dendritic atrophy of hippocampal neurons involves NMDA receptors and serotonin. Soc Neurosci Abstr 160.11.

McKittrick CR, Blanchard DC, Blanchard RJ (1995) Serotonin receptor binding in a colony model of chronic social stress. Biol Psychiatry 37:383–393.

Meijer OC, DeKloet ER (1994) Corticosterone suppresses the expression of 5-HT_{1A}-receptor mRNA in rat dentate gyrus. Eur J Pharmacol 266:255–261.

Mendelson SD, McEwen BS (1992a) Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT_{1A} and 5-HT_{1B} receptors in the dorsal hippocampus and cortex of the rat. Neuroendocrinology 55:444–450.

Mendelson SD, McEwen BS (1992b) Quantitative autoradiographic analyses of the time course and reversibility of corticosterone-induced decreases in binding of 5-HT_{1A}-receptors in rat forebrain. Neuroendocrinology 56:881–888.

Mongeau R, Welner SA, Quirion R, Suranyicadotte BE (1992) Further evidence for differential affinity states of the serotonin$_{1A}$-receptor in rat hippocampus. Brain Res 590:229–238.

Moore RY, Halaris AE, Jones BE (1978) Serotonin neurons of the median raphe: ascending projections. J Comp Neurol 180:417–438.

Morrison JH, Foote SL, Molliver ME, Bloom FE, Lidov HGW (1982) Noradrenergic and serotonergic fibers innervate complementary layers in monkey primary visual cortex: an immunohistochemical study. Proc Natl Acad Sci USA 79:2401–2405.

Mulligan KA, Törk I (1988) Serotoninergic innervation of the cat cerebral cortex. J Comp Neurol 270:86–110.

Nenonene EK, Radja F, Carli M, Grondin L, Reader TA (1994) Heterogeneity of cortical and hippocampal 5-HT_{1A}-receptors—a reappraisal of homogenate binding with ^{3}H-8-hydroxydipropylaminotetralin. J Neurochem 62:1822–1834.

Palacios JM, Waeber C, Hoyer D, Mengod G (1990) Distribution of serotonin receptors. In: Annals of the New York Academy of Sciences, Vol 600, The neuropharmacology of serotonin (Whitaker-Azmitia PM, Peroutka SJ, eds), pp 36–51.

Parent A, Descarries L, Beaudet A (1981) Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of ^{3}H-5-hydroxytryptamine. Neuroscience 6:115–138.

Raab A (1971) Der Serotoninstoffwechsel in einzelnen Hirnteilen vom Tupaia (*Tupaia belangeri*) bei soziopsychischem Stress. Z Vergl Physiol 72:54–66.

Raab A, Storz H (1976) A long term study on the impact of sociopsychic stress in tree shrews (*Tupaia belangeri*) on central and peripheral tyrosine hydroxylase activity. J Comp Physiol 108:115–131.

Sanders-Bush E, Canton H (1995) Serotonin receptors: signal transduction pathways. In: Psychopharmacology: the fourth generation of progress (Bloom FE, Kupfer DJ, eds), pp 431–441. New York: Raven.

Spain WJ (1994) Serotonin has different effects on two classes of Betz cells from the cat. J Neurophysiol 72:1925–1937.

Steinbusch HWM (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat—cell bodies and terminals. Neuroscience 6:557–618.

Tigges J, Shanta TR (1969) A stereotaxic brain atlas of the tree shrew (*Tupaia glis*). Baltimore: Williams and Wilkins.

Törk I (1990) Anatomy of the serotonergic system. In: Annals of the New York Academy of Sciences, Vol 600, The neuropharmacology of serotonin (Whitaker-Azmitia PM, Peroutka SJ, eds), pp 9–34. New York: New York Academy of Sciences.

Udenfriend S, Diekmann-Gerber L, Brink L, Spector S (1985) Scintillation proximity radioimmunoassay utilizing ^{125}I-labeled ligands. Proc Natl Acad Sci USA 82:8672–8676.

Vergé D, Daval G, Marcinkiewicz M, Patey A, El Mestikawy S, Gozlan H, Hamon M (1986) Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J Neurochem 6:3474–3482.

Vertes RP, Kinney GG, Kocsis B, Fortin WJ (1994) Pharmacological suppression of the median raphe nucleus with serotonin$_{1A}$-agonists, 8-OH-DPAT and buspirone, produces hippocampal theta rhythm in the rat. Neuroscience 60:441–451.

Whitaker-Azmitia PM, Azmitia EC (1986) ^{3}H-5-Hydroxytryptamine binding to brain astroglial cells: differences between intact and homogenized preparations and mature and immature cultures. J Neurochem 46:1186–1189.

Whitaker-Azmitia PM, Clarke C, Azmitia EC (1993) Localization of 5-HT_{1A}-receptors to astroglial cells in adult rats – implications for neuronal–glial interactions and psychoactive drug mechanism of action. Synapse 14:201–205.

Zifa E, Fillion G (1992) 5-Hydroxytryptamine receptors. Pharmacol Rev 44:401–458.

Zilles K (1990) Cortex. In: The human nervous system (Paxinos G, ed), pp 757–802.

Zilles K, Armstrong E, Schlaug G, Schleicher A (1986) Quantitative cytoarchitectonics of the posterior cingulate cortex in primates. J Comp Neurol 253:514–524.