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Postsynaptic 5-HT$_1$ Receptors and Offensive Aggression in Rats: A Combined Behavioural and Autoradiographic Study With Eltoprazine

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SIJBESMA, H , J SCHIPPER, E. R DE KLOET, J MOS, H VAN AKEN AND B OLIVIER *Postsynaptic 5-HT$_1$ receptors and offensive aggression in rats A combined behavioural and autoradiographic study with eltoprazine* PHARMACOL BIOCHEM BEHAV **38**(2) 447–458, 1991 —The present study was designed to assess whether the antiaggressive effects of eltoprazine are mediated via presynaptic and/or postsynaptic 5-HT$_1$ receptors We describe the effects of central 5-HT depletion 1) on the behaviour of resident TMD-S3 rats in a territorial situation, 2) on the efficacy of eltoprazine to inhibit offensive aggression, and 3) on the 5-HT$_{1A}$ 5-HT $_{1B}$ and 5-HT$_{1C}$ receptor binding in brains of rats previously used in behavioural studies Male resident rats were given combined 5,7-dihydroxytryptamine (5,7-DHT) injections into the dorsal and median raphe nuclei Two to four weeks after the lesions, rats were confronted with an intruder Wistar rat in their home cage for a 10-min period The 5,7-DHT treatment resulted in a modest reduction of offensive behaviour, while having no effects on other social and nonsocial behaviours Oral administration of eltoprazine (1 mg/kg) specifically reduced offensive aggression in both sham- and 5,7-DHT-lesioned animals, leaving social interest and exploration intact or even increasing it A low dose (0 3 mg/kg) of eltoprazine did not affect the behavioural repertoire of sham-operated rats, whereas this dose significantly reduced offense behaviours in the 5,7-DHT-lesioned residents Quantitative autoradiographic studies 5 weeks after 5,7-DHT treatment revealed a significant increase in radioligand binding to 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ sites in many brain regions studied, except for the raphe nuclei where [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ sites was markedly reduced The concentrations of 5-HT and 5-HIAA in frontal cortex were reduced to approximately 10% of controls The results indicate that serotonin has a stimulatory rather than an inhibitory influence on offensive aggressive behaviour Central 5-HT depletion does not prevent the antiaggressive effects of eltoprazine, indicating a role for postsynaptic 5-HT$_1$ receptors in the modulation of offensive aggression The 5,7-DHT-induced overall upregulation of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites suggests that these three receptor subtypes receive a tonic serotonergic influence It is conceivable that this postsynaptic 5-HT$_1$ receptor supersensitivity is reflected by the increased efficacy of eltoprazine to inhibit offensive aggression

Offensive aggression 5-HT$_1$ receptors Eltoprazine 5,7-Dihydroxytryptamine Resident-intruder
Serotonergic supersensitivity Quantitative autoradiography Rat

AGGRESSION in rats has been categorized into offensive, defensive and predatory behaviours (1, 3, 21, 43). The involvement of the central serotonergic system in the modulation of aggressive behaviour is most explicit for predation. Depletion of brain serotonin (5-HT) consistently facilitates mouse-killing behaviour by rats and, conversely, enhancement of serotonergic neurotransmission attenuates this behaviour [for reviews, see (36) and (52)]

The role of 5-HT in the regulation of offensive aggression seems to be more complex. Isolation-induced aggression in male mice, which is predominantly characterized by offensive behaviour (35,43), has frequently been associated with a reduction in 5-HT turnover (64,65). However, manipulations that either increase or decrease 5-HT neurotransmission have both been shown to inhibit the attack behaviour in this paradigm [reviewed by Miczek and Donat (36)]. Another model to study affective aggressive behaviour is the territorial situation (resident-intruder paradigm) in which resident animals mainly exhibit offensive behaviours, whereas intruders predominantly display defensive postures (8,34).

Studies using the resident-intruder paradigm have further supported the importance of 5-HT in the modulation of offensive aggression Vergnes and colleagues (67) showed that a single injection of PCPA (a 5-HT synthesis blocker) does not affect defensive behaviour when given to intruders, but enhances offensive aggression when administered to resident rats. The latter observation, however, seems to be in contrast with findings of File and Deakin (15) who demonstrated that combined 5,7-DHT lesions into the dorsal and median raphe nuclei do not affect agonistic behaviour of the resident rats. The fact that in these studies the behavioural testing has been preceded by isolation of the residents, which in itself may cause behavioural and neurochemical

changes (63), makes the interpretation of the results rather complex.

Territorial aggression in male mice, which were housed together with a female until social encounter with a conspecific intruder, is diminished by both serotonergic agonists and antagonists, though not seldom in a nonspecific manner (30,74). In a similar model with rats, Olivier and co-workers (43–46) investigated the specificity of the antiaggressive activity of various serotonergic compounds It was found that drugs like eltoprazine and fluprazine effectively decrease offensive aggression of the resident rats without causing sedation and without affecting defensive behaviours. These drugs belong to a recently developed series of phenylpiperazines, the so-called serenics, which specifically inhibit offensive aggressive behaviour in several animal models (43,45) In isolation-induced aggression in male mice, eltoprazine reduces offensive behaviours concomitant with an increase of nonaggressive social interactions (44). Radioligand binding and in vitro autoradiographic studies have demonstrated that eltoprazine selectively binds to the 5-HT_1 receptor type (45,58)

The 5-HT_1 receptor class has been subdivided into the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} sites [for review see (49)]. At least three of these subtypes, 5-$HT_{1A, 1B}$ and $_{1C}$, are found in the rat brain (50). The 5-HT_{1D} sites have been characterized in the brains of many species that lack the 5-HT_{1B} receptor subtype [e.g., bovine, pig, guinea pig and human (23, 27, 69, 70)], although one report recently suggested that 5-HT_{1D} sites are also present in rat brain (22) Eltoprazine displays the highest affinity for 5-HT_{1B} and 5-HT_{1A} binding sites and has a 5- to 10-fold lower afffinity for 5-HT_{1C} and 5-HT_{1D} sites, respectively (55). In addition to their postsynaptic locations, both 5-HT_{1A} and 5-HT_{1B} sites are assumed to be also presynaptically present in rat brain, the 5-HT_{1A} sites as somatodendritic autoreceptors on 5-HT neurons in the raphe nuclei (13) and the 5-HT_{1B} sites as terminal autoreceptors in widespread serotonergic projection areas (14).

At present, not much is known about the location or the subtype(s) of the 5-HT_1 receptors that are involved in offensive aggressive behaviour, or more specifically, in the mediation of the antiaggressive effects of eltoprazine. Hence, the first aim of the present study was to assess whether the antiaggressive activity of eltoprazine is mediated via pre- and/or postsynaptic 5-HT_1 receptors To this end, we selectively lesioned central serotonergic fibres of male resident rats by injecting 5,7-DHT into the midbrain raphe nuclei and we studied the behavioural effects of eltoprazine in the resident-intruder paradigm both before and several weeks after the lesions. The resident-intruder paradigm was chosen because it provides an excellent means to study the effects of eltoprazine on the complete behavioural repertoire of rats in a territorial situation The model has the advantage that the elicitation of aggression does not require shock or isolation, but relies on more naturally occurring motivational stimuli (7, 31, 61)

Secondly, it was of interest to determine whether the chronic depletion of 5-HT resulted in adaptive changes of the different 5-HT_1 receptor subtypes, which are possibly related to behavioural alterations For this purpose we have used brains of resident rats in a quantitative autoradiographic study with [^{3}H]5-HT, [^{3}H]8-OH-DPAT and [^{3}H]eltoprazine.

METHOD

Experimental Animals

Adult male rats (400–500 g) of the Tyron Maze Dull strain (TMD-S3), bred and weaned in the Central Institute for the Breeding of Laboratory Animals (CPB-TNO, Zeist, The Netherlands), were individually housed, together with an adult TMD-S3 female rat, in a large wooden home cage (85 × 60 × 50 cm). The front of this cage consisted of Plexiglas and the floor was covered with wood shavings Food pellets and water were available ad lib. The animals were kept in a controlled environment (20–22°C, 60–70% humidity) under a reversed day-night cycle (dark period: 7:00 a.m.–7 00 p.m.) and testing was done between 10 00 a.m and 3.00 p.m in the same room under red light conditions (75 W). Male rats (200–300 g) of the Wistar strain (CBP-TNO) were used as intruders and were used only once.

Experimental Procedures

Male TMD-S3 rats were tested once a week in the resident-intruder (RI) paradigm during a four-week training period Based on their aggression levels in the last two training sessions, animals were evenly divided into two groups, which received 5,7-DHT or sham lesions in the midbrain raphe nuclei. Two days after surgery, the animals were returned to their females and allowed a 2-week recovery period. In the following two weeks, the rats were submitted to the RI-aggression test twice a week (three days interval). Sixty min prior to testing, rats were orally injected with either placebo (tragacanth 1%) or eltoprazine (0 3, 1.0 and 5.0 mg/kg, suspended in tragacanth 1%) Each single dose of eltoprazine was tested against placebo in a one-week cross-over design, in which treatments were balanced over the day At the end of the behavioural test period (five weeks after the lesions), the animals were decapitated and brains were used for determination of monoamine levels as well as for autoradiographic analyses of 5-HT_1 receptor binding. The whole experiment was performed twice. In the first experiment (n = 13) eltoprazine was administered in doses of 1 and 5 mg/kg in the RI-tests In the second experiment (n = 14) rats were treated with 0 3 and 1 mg/kg eltoprazine, respectively

Resident-Intruder (RI) Aggression in Rats

Resident male TMD-S3 rats were tested in their home cages for aggression against a male conspecific intruder The residential female was removed from the cage 30 min prior to the start of the test period. After placing an intruder rat in the territorial cage, the behaviour of the resident male was observed and recorded for 10 min according to the method described by Olivier (42) In brief, a total of 49 different behavioural elements were scored and grouped, based on sequential and cluster analysis (42), into the following 7 behavioural categories (the most characteristic acts and postures are listed between brackets): [1] *Offense* (fighting, biting, lateral threat, upright posture, jump attack, boxing and chasing), [2] *Exploration* (locomotion, rearing, digging, sniffing and attention), [3] *Social interest* (move to, nosing, sniff intruder, crawl over and partner grooming), [4] *Inactivity* (sitting, lying and immobility), [5] *Avoidance* (move away, retire and keeping off postures), [6] *Body care* (grooming, washing, feeding and shaking), and [7] *Defense* (defensive upright, flight, lying on back and keeping off postures while lying). For the present purpose the mean durations (amount of time spent on each behavioural category) and frequencies of occurrence per session were used As no differences were found within each category between the pattern of scores of the different behavioural elements, we only present the grouped data of the 7 behavioural categories.

5,7-Dihydroxytryptamine (5,7-DHT) Lesions

Male TMD-S3 rats were pretreated with desmethylimipramine (DMI, 20 mg/kg IP) 20 min before anesthesia with Hypnorm® (1 ml/kg). Animals were then placed into a stereotaxic apparatus with the incisor bar set to 3 3 mm below the interaural line Thirty min after the DMI injection, 5,7-DHT (10 μg, dissolved in 2 μl

0.9% saline containing 0 01% ascorbic acid) was injected into both the dorsal raphe (DR) and median raphe (MR) nucleus, at coordinates· A 1 2 mm, H 3.5 (DR) and 1 5 (MR), L 0.0 at an angle of 10° in order to avoid the superior sagittal sinus. Coordinates were adapted from the stereotaxic rat brain atlas of Paxinos and Watson (47). Injections were made at 1 μl/min using a 10 μl Hamilton syringe and the needle remained in position for 2 min after each injection. Sham-operated animals (controls) received DMI followed by raphe-injections of a corresponding volume of vehicle After surgery, rats were treated with naloxone (1 mg/kg IP) to shorten the narcosis

Measurement of Monoamine Concentrations

At the end of the behavioural tests (5 weeks after the 5,7-DHT lesions), the male TMD-S3 rats were decapitated and frontal cortices were rapidly dissected and frozen on dry ice. The posterior part of the brain was either further dissected or directly frozen on dry ice and stored at −80°C for autoradiographic experiments. Dissected cortices were weighed and homogenised in 10 volumes (w/v) of ice-cold 0 4 M $HClO_4$ containing 0.1% cysteine as an antioxidant and N-methyl-serotonin as an internal standard. Subsequently, samples were centrifuged at 12,000 × g for 1.5 min in a microfuge (Beckman) and aliquots of the clear supernatant were analysed using an HPLC system (Hewlett Packard 1084) with a reversed phase column (Zorbax-C8, 15 × 0.46 cm, particle size 7 5 μm). The mobile phase consisted of 0.05 M ammonium phosphate, 1 5% n-propanol, 2.25 mM sodium octylsulphonate, 0.1 M $NaClO_4$, 0 1 mM EDTA and 0.5 mM triethylamine (pH was adjusted to 3.0 with H_3PO_4). The flow was set at 2 ml/min and the column temperature was maintained at 18 ± 0 5°C Noradrenaline (NA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were detected with a EG&G model 400 electrochemical detector (Princeton Applied Research). The glassy carbon electrode was set at a potential of 750 mV versus an Ag/AgCl reference electrode The range was set at 50 nA full scale and the output was recorded on a Hewlett Packard 3396 integrator. Peak height values were measured and related to internal and external standards. Concentrations are expressed in ng/g brain tissue.

In Vitro Autoradiography

Coronal brain sections (20 μm) from control TMD-S3 rats (n = 5) and 5,7-DHT-treated animals (n = 3) were cut with a cryostat at −20°C. Sections from 5,7-DHT-treated rats were mounted together with sections from control rats on gelatin-coated slides and stored at −80°C until use. The incubation procedure was carried out as previously described (58). Briefly, frozen rat brain sections were thawed and preincubated for 30 min at room temperature in 0 17 M Tris-HCl (pH 7 6) and 4 mM $CaCl_2$. After drying, sections were incubated for 60 min at room temperature with several [^{3}H]ligands in 400 μl 0.17 M Tris-HCl (pH 7.6), 4 mM $CaCl_2$ and 0.01% ascorbic acid. For 5-HT$_1$ receptor labelling, sections were incubated with 2 nM [^{3}H]5-HT (New England Nuclear, 28 Ci/mmol) or 14 nM [^{3}H]eltoprazine (Amersham, 31 Ci/mmol). For labelling of the 5-HT$_{1A}$ and 5-HT$_{1B}$ subsites we respectively incubated with 0.5 nM [^{3}H]8-OH-DPAT (New England Nuclear, 157 Ci/mmol) and 2 nM [^{3}H]5-HT in the presence of 200 nM 8-OH-DPAT (to block 5-HT$_{1A}$ sites) and 300 nM DOI (to block 5-HT$_{1C}$ sites). In case of [^{3}H]5-HT binding, the incubation buffer was supplemented with 10 μM pargyline and 1 μM zimelidine Nonspecific binding was always determined in the presence of 1 μM 5-HT. The incubation was finished by washing the sections twice for 3 min at 4°C in fresh preincubation buffer, followed by a short dip in ice-cold distilled water to remove buffer salts. Slides were subsequently dried at 60°C and autoradiograms were generated by apposing the labelled tissue sections together with [^{3}H]Standard microscales (Amersham RPA.506 and RPA.507) to [^{3}H]Ultrofilm (Amersham, England). After an exposure time of 7 weeks, films were developed in Kodak D19 developer (5 min, 20°C), fixed and analysed.

TABLE 1

MONOAMINE CONCENTRATIONS IN FRONTAL CORTEX OF RESIDENT TMD-S3 RATS

	n	NA	5-HT	5-HIAA
Controls	15	416 ± 6	461 ± 20	256 ± 10
5,7-DHT	12	401 ± 6	53 ± 14*	26 ± 8*

Animals were sacrificed 5 weeks after injections of vehicle or 5,7-DHT into the midbrain raphe nuclei Concentrations (ng/g of brain) of noradrenaline (NA), 5-HT and 5-HIAA were determined by HPLC analysis and are expressed as the mean ± S E M *p<0 001

Data Analysis

Statistical analyses of the behavioural data were performed with the Mann-Whitney U-test for between-group analysis and by the Wilcoxon matched-pairs test for within-group comparisons between doses. For between-group comparisons of the HPLC measurements we used the two-tailed Student's t-test. Autoradiograms were analysed using a Vidas image analysis system (Kontron, Munich, FRG) The optical density measurements were converted to fmoles of ligand bound/mg tissue equivalent using the [^{3}H]microscales from the same [^{3}H]sensitive film The stereotaxic brain atlas of Paxinos and Watson (47) was used to identify the neuroanatomical regions. Specific binding values were obtained by subtracting nonspecific binding from total binding The binding data of control and 5,7-DHT-treated animals were analysed with ANOVA, and subsequently group means were compared for significant differences using the Student's t-test

Drugs

Eltoprazine [1-(2,3-dihydro-1,4-benzodioxin-5-yl) piperazine hydrochloride] was synthesized at our chemical laboratories (Duphar B.V., Weesp, The Netherlands). The following drugs were obtained commercially 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino) tetralin] and DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] (Research Biochemical Inc , USA), 5-HT (5-hydroxytryptamine creatinine sulfate, serotonin), N-methyl-serotonin, 5,7-dihydroxytryptamine (5,7-DHT), desmethylimipramine (DMI), naloxone and pargyline HCl (Sigma Chemical Co , St. Louis, MO), zimelidine (Astra, Sweden) Hypnorm® (containing 10 mg fluanison and 0 2 mg fentanyl per ml) (Janssen, Belgium).

RESULTS

Biochemical Assays

In both experiments, the 5,7-DHT injections resulted in a highly significant reduction of 5-HT (ca 89%) and 5-HIAA (ca. 90%) concentrations in the frontal cortex of the resident rats. Noradrenaline levels were only slightly but not significantly decreased (ca. 4%). The mean monoamine contents of control and lesioned animals are presented in Table 1

TABLE 2

BEHAVIOURAL COMPARISON BETWEEN CONTROL AND 5,7-DHT-TREATED ANIMALS

	Mean Total Duration (s) per Session						Mean Frequency (counts) per Session					
	Experiment 1			Experiment 2			Experiment 1			Experiment 2		
Behaviours	Control n=8	5,7-DHT n=5	*p*-Value	Control n=7	5,7-DHT n=7	*p*-Value	Control n=8	5,7-DHT n=5	*p*-Value	Control n=7	5,7-DHT n=7	*p*-Value
Offense	217 ± 16	91 ± 27	0 006	140 ± 27	95 ± 13	0 16	113 ± 8	53 ± 12	0 004	93 ± 13	69 ± 9	0 38
Exploration	172 ± 12	215 ± 21	0 13	244 ± 27	280 ± 17	0 32	99 ± 8	125 ± 13	0 22	132 ± 15	161 ± 10	0 26
Social interest	152 ± 15	148 ± 23	0 94	135 ± 9	149 ± 26	1 00	76 ± 6	74 ± 12	1 00	79 ± 3	82 ± 11	0 90
Inactivity	27 ± 7	109 ± 63	0 13	37 ± 6	46 ± 12	0 80	7 ± 1	14 ± 3	0 05	11 ± 2	12 ± 2	0 71
Avoidance	18 ± 2	25 ± 7	0 62	15 ± 2	19 ± 2	0 16	20 ± 3	25 ± 5	0 43	15 ± 3	20 ± 2	0 10
Body care	13 ± 5	12 ± 5	0 94	27 ± 17	10 ± 2	0 80	4 ± 1	5 ± 1	0 76	5 ± 2	4 ± 1	0 62
Defense	0 ± 0	0 4 ± 0 3	0 17	1 7 ± 1 7	0 ± 0	0 71	0 1 ± 0 1	0 4 ± 0 2	0 17	0 3 ± 0 3	0 ± 0	0 71

Each resident TMD-S3 rat was confronted for a 10-min period with an intruder Wistar rat The behavioural scores are expressed as the mean ± S E M Statistical analyses were performed with the Mann-Whitney U-test, two-tailed

Resident-Intruder Behaviour

During the first few weeks of the four-week training period, there was a gradual increase in aggression levels of the residents. Statistical comparisons revealed no differences in attack behaviour between the third and fourth week. Two weeks after sham or 5,7-DHT lesions, the animals were submitted to 4 RI-aggression tests over a two-week period Each animal received placebo once a week and a dose of eltoprazine once a week. No time-related changes occurred between the two placebo treatments in any of the observed behaviours The scores of the placebo-treated rats are therefore presented as the mean values from two behavioural test sessions in one experiment (Table 2).

The amount of time spent by the sham-lesioned residents on offensive aggression was quite high, but different in the two experiments (relative duration of offense 36% and 23% in Experiment 1 and Experiment 2, respectively). Also the frequency of offensive elements was rather eminent (relative frequency of offense. 35% and 28%) In both experiments, 5,7-DHT treatment caused a decrease in duration and frequency of offensive behaviours, such as biting, fighting and lateral threat. This decrease reached levels of significance ($p<0.01$) in Experiment 1, but not in the second experiment ($p=0.16$ for duration). The reduction in offense was counterparted by increases in exploration, inactivity and to a lesser extent avoidance behaviour, which, however, all failed to reach statistical significance. Social interest and self-care were unaltered. Defensive postures were almost absent in both control and lesioned residents (Table 2). Comparisons, either in the sham- or in the 5,7-DHT-lesioned group, of individual levels and/or changes in aggressive behaviour with the individual 5-HT and 5-HIAA levels, did not reveal any significant correlations.

Administration of 1 0 or 5.0 mg/kg eltoprazine to sham-lesioned residents drastically reduced the mean duration and frequency of offensive aggression concomitant with an increase in exploration and social interest (Fig 1). Both doses of eltoprazine also significantly inhibited offensive behaviours in the 5,7-DHT-lesioned residents (Fig. 1) In these animals 1 mg/kg eltoprazine had less effect on the duration and frequency of other behaviours, although a consistent, but not significant, increase in exploration was observed. The high dose of eltoprazine (5 mg/kg) decreased not only offensive aggression, but also social interest and exploration concurrent with a dramatic increase of inactivity in 5,7-DHT-lesioned rats.

Administration of 0.3 mg/kg eltoprazine had different effects on offensive behaviour of sham- and 5,7-DHT-lesioned animals This dose did not significantly alter duration and frequency of any of the behaviours in control residents, whereas the same dose given to 5,7-DHT residents significantly inhibited offensive aggression and concurrently enhanced exploration The different behavioural effects of eltoprazine in sham- and 5,7-DHT-lesioned rats are illustrated in Fig 2, in which the relative effects of eltoprazine on the three main behavioural categories (offense, exploration and social interest) are presented combined for the two experiments.

Quantitative Autoradiography

The effects of 5,7-DHT treatment on 5-HT_1 recognition sites labelled by [^{3}H]5-HT or [^{3}H]eltoprazine are presented in Table 3 The 5,7-DHT treatment resulted in a marked increase in both [^{3}H]5-HT and [^{3}H]eltoprazine binding in many brain regions, including lateral septum, basal ganglia, hypothalamic region, amygdala, dorsal subiculum, hippocampus (CA_1–CA_2 region), dentate gyrus, thalamus, cingulate cortex, piriform cortex and several midbrain structures. The increase was least evident in the CA_3 region of the hippocampus and in the occipital and parietal cortex Although the height and significance of the increases varied somewhat between the two labels, all brain regions studied (except for the dorsal raphe nucleus where ca. 60% reduction was found) displayed higher binding levels in 5,7-DHT-lesioned rats as compared to controls.

In brains of 5,7-DHT-lesioned rats, the binding of [^{3}H]8-OH-DPAT to 5-HT_{1A} sites was also clearly increased in several areas such as lateral septum, dentate gyrus, entorhinal cortex, central grey and colliculi, as compared to the sham-operated animals (Fig. 3). In contrast, [^{3}H]8-OH-DPAT binding was strongly decreased in dorsal (82%) and median (63%) raphe nuclei (Fig 3) The decrease in binding in the median raphe nucleus did, however, not reach significance ($p=0.08$) due to a relatively high variation in the binding values This variation is probably a consequence of the fact that it is rather difficult to clearly delineate the median raphe nucleus in which the serotonergic cell bodies are diffusely distributed 5,7-DHT treatment did not cause a decrease in [^{3}H]8-OH-DPAT binding in any of the other brain areas examined.

Labelling of 5-HT_{1B} sites by [^{3}H]5-HT in the presence of 8-OH-DPAT and DOI (to block 5-HT_{1A} and 5-HT_{1C} sites, respectively) demonstrated an increase in binding densities in 5,7-DHT-lesioned rats very similar to the increases in 5-HT_1 sites labelled with [^{3}H]5-HT or [^{3}H]eltoprazine (Table 3) A 30 to 40% increase was, for instance, measured in basal ganglia, dorsal subiculum, hypothalamus and the superficial grey layer of the superior colliculus, all regions containing relatively high levels of 5-HT_{1B}

TABLE 3

[^{3}H]5-HT AND [^{3}H]ELTOPRAZINE BINDING IN CORONAL RAT BRAIN SECTIONS OF CONTROL AND 5,7-DHT-TREATED RATS AS DETERMINED BY QUANTITATIVE AUTORADIOGRAPHY

	Specific [^{3}H]5-HT Binding (fmol/mg tissue equivalent)			Specific [^{3}H]Eltoprazine Binding (fmol/mg tissue equivalent)			Specific [^{3}H]5-HT + DPAT + DOI Binding (fmol/mg tissue equivalent)		
	5-HT$_1$ Sites			5-HT$_1$ Sites			5-HT$_{1B}$ Sites		
Brain Area	Control	5,7-DHT	Δ%	Control	5,7-DHT	Δ%	Control	5,7-DHT	Δ%
Cortex									
Cingulate	48 1 ± 5 3	56 6 ± 2 2	18*	42 4 ± 3 2	49 3 ± 1 1	16†	22 4 ± 0 9	25 7 ± 1 0	15‡
Piriform	51 5 ± 5 5	69 8 ± 0 8	36†	52 8 ± 3 6	63 1 ± 3 8	20*	—	—	—
Parietal									
lamina I–III	31 5 ± 2 3	35 7 ± 0 5	13†	32 9 ± 2 4	34 7 ± 1 5	5	23 2 ± 2 2	26 0 ± 1.8	12
lamina IV–VI	43 7 ± 3 1	48 6 ± 2 4	11	32 5 ± 2 0	35 0 ± 1 0	8	23 6 ± 2 0	26 5 ± 1 8	12*
Occipital									
lamina I–III	34 9 ± 2 7	38 5 ± 2 7	11	36 0 ± 2 9	39 5 ± 1 6	10	25 2 ± 1 7	26 1 ± 1 0	3
lamina IV–VI	49 9 ± 6 3	54 5 ± 1 3	9	38 6 ± 3 4	42 6 ± 1 7	10	28 0 ± 2 3	29 3 ± 1 8	5
Entorhinal	173 3 ± 11 1	196.4 ± 11 3	13	—	—	—	64 9 ± 5 4	74 1 ± 11 0	14
Septal Region									
Lateral septal nucleus dorsal part	127.2 ± 27 8	165.5 ± 4 0	30*	103 8 ± 11 8	157 8 ± 8 3	52†	—	—	—
Lateral septal nucleus intermediate part	114.2 ± 20 3	155 3 ± 6 0	36*	98.4 ± 10 2	144 6 ± 11.1	47†	—	—	—
Lateral septal nucleus ventral part	98 7 ± 19.2	135 1 ± 4 4	37*	88 2 ± 10 2	127 3 ± 9.8	44†	—	—	—
Basal Ganglia									
Caudate putamen	37.8 ± 5 2	46 7 ± 1 2	24*	46.2 ± 3 3	53 1 ± 1 1	15*	33 1 ± 2 5	41 6 ± 1 8	26†
Ventral pallidum	86 0 ± 14 9	119.4 ± 6.6	39*	121 6 ± 15 7	162 8 ± 29 8	34*	73 4 ± 10 2	97 3 ± 15 2	33*
Globus pallidus	79 1 ± 8 6	100 8 ± 5 1	27*	119 2 ± 10 8	149 8 ± 8 5	26†	64 7 ± 6 4	91 8 ± 2 0	42†
Hypothalamic Region									
Anterior hypothalamic area	56 4 ± 8 2	77 8 ± 7 5	38†	75 1 ± 1 8	81 3 ± 5 9	8	37 1 ± 5 7	50.9 ± 4 4	37*
Ventromedial hypothalamic nucleus	46 2 ± 4 0	60 4 ± 6 8	31†	61 5 ± 9 2	68.2 ± 4 9	11	—	—	—
Lateral hypothalamic area	34 0 ± 1 9	44 5 ± 5 2	31†	42 4 ± 1 7	50 2 ± 0 6	18†	—	—	—
Medial preoptic area	63 3 ± 10 5	83 0 ± 6 6	31†	85 3 ± 11 8	93 8 ± 21 5	10	39 6 ± 5 8	54 6 ± 0 8	38†
Amygdaloid Nuclei									
Posteromedial cortical amygdaloid nucleus	54 5 ± 5 7	72 9 ± 3 0	34*	57 8 ± 3 9	69 2 ± 2 7	20*	—	—	—
Posterolateral cortical amygdaloid nucleus	55 1 ± 6 2	72 2 ± 2.6	31†	60 9 ± 6 0	74 1 ± 3 5	22*	33 8 ± 4 2	43 8 ± 2 9	30*
Hippocampus									
CA1 field	118 3 ± 3 7	150 3 ± 10.3	27†	96 8 ± 2 7	114 6 ± 3 2	18†	22 2 ± 1.1	24 1 ± 1 7	8*
CA2 field	119 1 ± 10 5	149 9 ± 9 2	26†	97 2 ± 4 8	115 6 ± 4 5	19‡	21 5 ± 0 5	24 5 ± 1 4	14*
CA3 field	34 9 ± 7 3	37 4 ± 3 8	7	37 0 ± 5 0	39 1 ± 4 7	6	—	—	—
Dentate gyrus									
Inner blade	115 1 ± 9 5	140.7 ± 9 8	22‡	103 6 ± 7.6	121 4 ± 11 3	17‡	—	—	—
Outer blade	118.8 ± 8 7	150 3 ± 7 7	26‡	108 2 ± 4 6	125 8 ± 10 7	16†	—	—	—
Polymorph layer	70 6 ± 7 1	79 6 ± 3 8	13*	58 0 ± 4 7	61 2 ± 5 7	6	—	—	—
Dorsal subiculum	155 7 ± 16 5	213 9 ± 12.7	37†	235.1 ± 16 3	294 2 ± 10 9	25†	118 6 ± 7 4	159 9 ± 1 7	35‡
Thalamus									
Anterior pretectal area	29 0 ± 1 7	36.5 ± 2 6	26†	38 1 ± 3 5	44 4 ± 4 7	16	25 2 ± 0 9	28 2 ± 4 0	12
Ventroposterior thalamic nucleus	21 6 ± 3 1	25 9 ± 2.9	20†	26.3 ± 3 6	26 8 ± 2 7	2	—	—	—
Medial geniculate nucleus	27 7 ± 2 3	31 4 ± 1 7	14*	36 1 ± 1 5	37 9 ± 3 3	5*	20.8 ± 2 1	21 9 ± 3 9	5
Lateral geniculate nucleus	31 3 ± 3 9	44 3 ± 0 5	42†	46 4 ± 3 2	54 6 ± 3 4	18*	27 5 ± 4 1	34 9 ± 1 7	27*

TABLE 3 (continued)

Brain Area	Specific [^{3}H]5-HT Binding (fmol/mg tissue equivalent) 5-HT$_1$ Sites Control	5,7-DHT	Δ%	Specific [^{3}H]Eltoprazine Binding (fmol/mg tissue equivalent) 5-HT$_1$ Sites Control	5,7-DHT	Δ%	Specific [^{3}H]5-HT + DPAT + DOI Binding (fmol/mg tissue equivalent) 5-HT$_{1B}$ Sites Control	5,7-DHT	Δ%
Midbrain									
Substantia nigra	124 4 ± 3 8	164 1 ± 22 4	32†	177 9 ± 18 7	198 6 ± 16 4	12	101 6 ± 10 0	119 8 ± 7 3	18
Central grey	55 7 ± 3 0	74 1 ± 0 9	33‡	79 8 ± 5 0	91 3 ± 3 7	14*	41 3 ± 2 6	51 0 ± 2 1	23*
Interpeduncular nucleus	135 0 ± 11 1	142 8 ± 17 3	6	115 7 ± 13 2	127 6 ± 2 2	10	—	—	—
Ventral tegmental area	36 7 ± 2 6	40 7 ± 7 4	11	43 1 ± 4 5	52 0 ± 5 9	21*	22 9 ± 2 5	29 1 ± 1 7	27*
Superficial grey layer of the superior colliculus	72 6 ± 6 7	98 4 ± 5 2	35‡	94 2 ± 5 7	115 7 ± 5 0	23†	51 1 ± 7 5	69 5 ± 2 2	36*
Superior colliculus	38 8 ± 2 5	50 0 ± 4 6	29†	48 5 ± 2 5	58 4 ± 3 9	21*	29 0 ± 3 3	36 4 ± 1 9	26*
Inferior colliculus	33 7 ± 1 3	40 3 ± 1 8	20	36 3 ± 1 7	40 0 ± 5 7	10	—	—	—
Dorsal raphe nucleus	130 0 ± 20 4	53 5 ± 6 4	−59*	107 3 ± 12 5	41 0 ± 16 2	−62*	—	—	—
Choroid Plexus									
Lateral ventricle	138 6 ± 7 3	180 8 ± 18 8	30*	—	—	—	—	—	—
Third ventricle	149 0 ± 13 7	177 4 ± 18 0	19	—	—	—	—	—	—

Animals were sacrificed 5 weeks after injections of vehicle or 5,7-DHT into the midbrain raphe nuclei Serial brain sections of each rat were incubated with either 2 nM [^{3}H]5-HT, 14 nM [^{3}H]eltoprazine, or 2 nM [^{3}H]5-HT in the presence of DOI and 8-OH-DPAT Autoradiograms were quantified using a Vidas image analysis system The amount of each ligand bound in the rat brain regions was determined by converting optical density measurements to fmol/mg tissue equivalent using ^{3}H-standard microscales (Amersham) Specific binding values are the mean ± S D of average measurements from n rats, with multiple readings per brain region in each rat Control (n = 5) and 5,7-DHT (n = 3)-treated rats were compared for significant differences using the two-tailed Student's *t*-test *$p<0.05$, †$p<0.01$, ‡$p<0.001$ — = not determined The differences in binding data are given by Δ%

sites. Again, in none of the regions studied a reduction in 5-HT$_{1B}$ binding was found.

Following 5,7-DHT treatment, the binding of [^{3}H]5-HT was also increased in choroid plexus of the lateral and third ventricles (30% $p=0.02$ and 19% $p=0.10$, respectively). These regions almost exclusively contain 5-HT$_{1C}$ sites (48,75)

DISCUSSION

Injections of 5,7-DHT into the dorsal and median raphe nuclei of male TMD-S3 rats induced alterations in aggressive behaviour, affected the antiaggressive activity of eltoprazine and changed the radioligand binding to 5-HT$_1$ receptor subtypes in several brain regions. We will discuss these effects separately as well as in relation to each other

Behavioural Effects of 5,7-DHT Lesions

The finding that depletion of central 5-HT resulted in a decrease of offensive aggressive behaviour of resident rats confronted with an intruder in their home cage suggests a stimulatory influence of 5-HT on offensive aggression The decrease in aggression, however, was not very impressive as it only reached significance in one out of two experiments. Besides, in both experiments, 5,7-DHT-treated animals still spent a fair amount of time (approx. 15% of the total test time) on offense The limited behavioural changes following the lesions are rather unexpected when seen in relation to the severe reductions in 5-HT and 5-HIAA concentrations in frontal cortical brain tissue This may either suggest that the facilitatory role of 5-HT in the regulation of offense is of minor importance, or that this role is largely fulfilled by spared serotonergic projections, which have been reported to exhibit an increased tryptophan hydroxylase activity (59) A third possibility, discussed below, involves a more complex mechanism in which both stimulatory and inhibitory processes are at work

It is unlikely that the 5,7-DHT-induced reduction in offense is caused by sedation or a general disturbance of motoric processes, since social interest for intruders was completely unaffected and a modest increase in exploration concurrently occurred. Moreover, in experiments where rats were tested for their predatory behaviour, we (data not shown) and many others [for reviews, see (36) and (52)] have found that the incidence of mouse-killing behaviour was increased by 5,7-DHT treatment. These observations indicate different roles for serotonin in intermale offensive aggression and mouse-killing behaviour

Previous studies concerning the effects of 5-HT depletion on territorial aggression have not resulted in a coherent picture Combined 5,7-DHT lesions into dorsal and median raphe nuclei of resident rats have been described to be ineffective (15), whereas Vergnes et al. (67) showed an increase in offensive aggression of PCPA-treated rats tested in a comparable resident-intruder paradigm Resident-offensive aggression in mice, on the other hand, has recently been reported to be reduced by PCPA treatment (9) Methodological differences in the manner of 5-HT depletion, the housing of the residential animals and the time period between depletion and behavioural testing may underlie some of the conflicting data.

One important aspect we would specifically like to mention concerns differences in basal aggression levels of the residents We used TMD-S3 rats that spent between 100 and 300 s per 10-min session on offensive aggression, whereas Vergnes et al. (67), whose results contrast with ours, used Wistar rats that displayed a relatively low level of offensive behaviours (mean duration of offense was less than 10 s per 8-min session) The frequency of offensive postures was also much higher in our residents as compared to those in other studies (15, 16, 67, 68) Furthermore, we found that the 5,7-DHT-induced decrease in offensive aggression only reached significance in the group of animals that displayed relatively high levels of offensive behaviours. Thus it could be hypothesized that the behavioural outcome of manipulations that

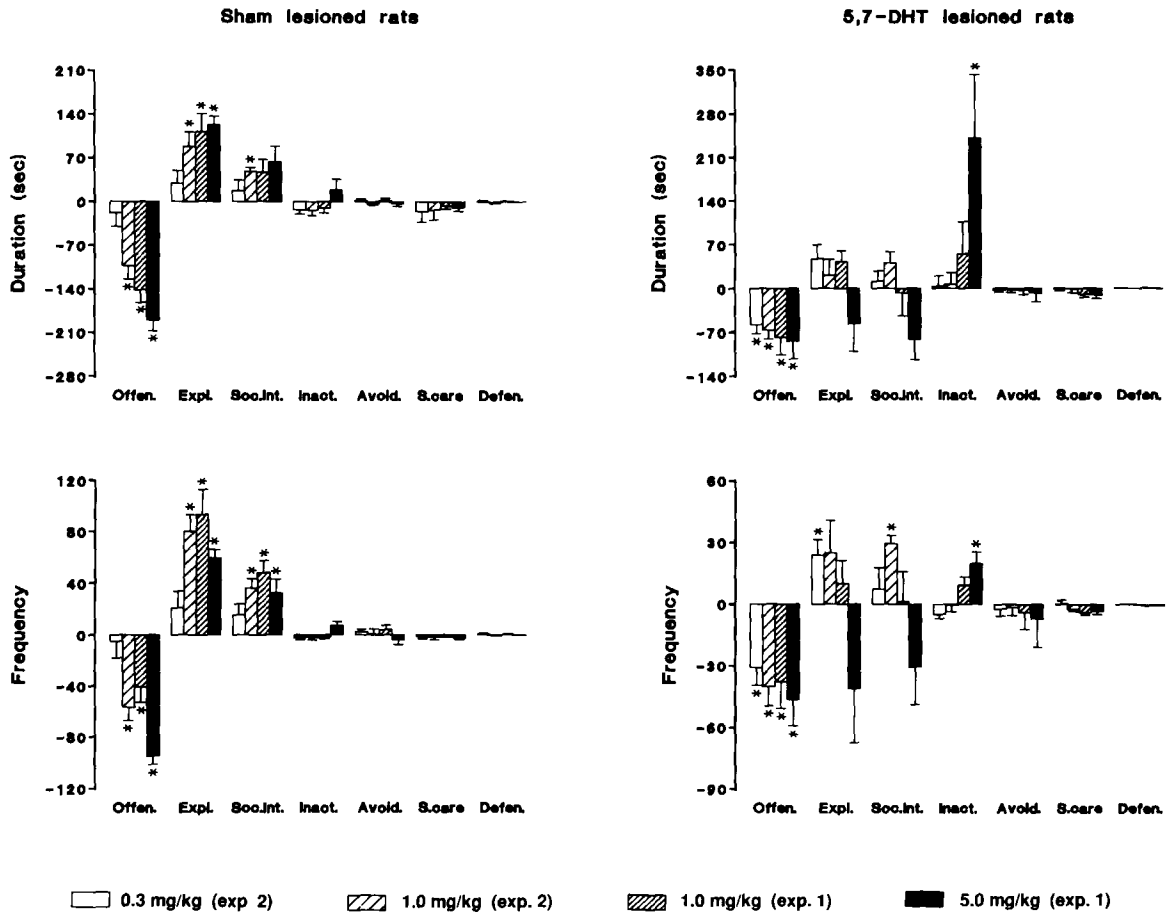

FIG 1 Behavioural effects of orally administered eltoprazine in sham- and 5,7-DHT-lesioned animals during a 10-min resident-intruder paradigm Statistical analyses were performed with the Wilcoxon matched-pairs test $*p<0$ 05 Data are presented as the delta of placebo values and they are the mean ± S.E M of n (see the Method section) rats For behavioural categories see the Method section

decrease the content of brain 5-HT depends on the strain and/or the "aggressive state" of the animals.

The concept of opposing serotonergic influences regulating offensive aggression is supported by studies employing relatively selective central 5,7-DHT lesions. File et al (16) reported that 5,7-DHT injection into the median raphe nucleus led to an increase in territorial aggression, whereas they found this behaviour to be decreased (though in a nonspecific manner) in rats with dorsal raphe-lesions A differential serotonergic involvement may also be present at the level of the serotonergic projection areas, as bilateral injections of 5,7-DHT into the amygdaloid complex have been shown to reduce dominance behaviour of residents (17), while similar injections into the lateral hypothalamus have resulted in an enhancement of offensive aggression (68) Thus granting that a complex system exists which engages both facilitatory and inhibitory influences of serotonin on offensive behaviour, one may indeed expect offense to be increased (67), decreased (this paper) or to be unaffected (15) following relatively complete central 5-HT depletions.

Behavioural Effects of Eltoprazine in Control and 5,7-DHT-Lesioned Rats

Previous behavioural studies have shown that eltoprazine inhibits offensive aggression in various animal models (44–46). The drug selectively acts at 5-HT$_1$ recognition sites, as has been demonstrated by radioligand binding (45), autoradiographic (58), electrophysiological (29) and neurochemical studies (55). In the present study, we confirm the effects of eltoprazine on the behaviour of resident male rats in a territorial situation In sham-operated animals, eltoprazine dose-dependently decreased offensive aggressive behaviour. Exploration and social interest were concomitantly increased indicating that the reduction in offense did not result from nonspecific sedatory effects of eltoprazine.

The finding that 5,7-DHT treatment does not prevent the specific antiaggressive effects of eltoprazine indicates that these behavioural actions are mediated by postsynaptic 5-HT$_1$ receptors. However, a noteworthy difference was observed with regard to the efficacy of eltoprazine to inhibit offensive aggression in 5,7-DHT as compared to sham-operated animals. A relative low dose (0.3 mg/kg) significantly reduced duration and frequency of offense behaviour in 5,7-DHT-treated rats, whereas this dose did not induce behavioural changes in sham rats, suggesting a denervation-induced supersensitivity Furthermore, whereas in sham rats the reduction in offense was always specific (without concomitant reductions in other social and nonsocial behaviours), in the 5,7-DHT-lesioned rats, the highest dose of eltoprazine (5 mg/kg) also inhibited exploration and social interest concurrent with a significant increase in inactivity. Such effects of eltoprazine

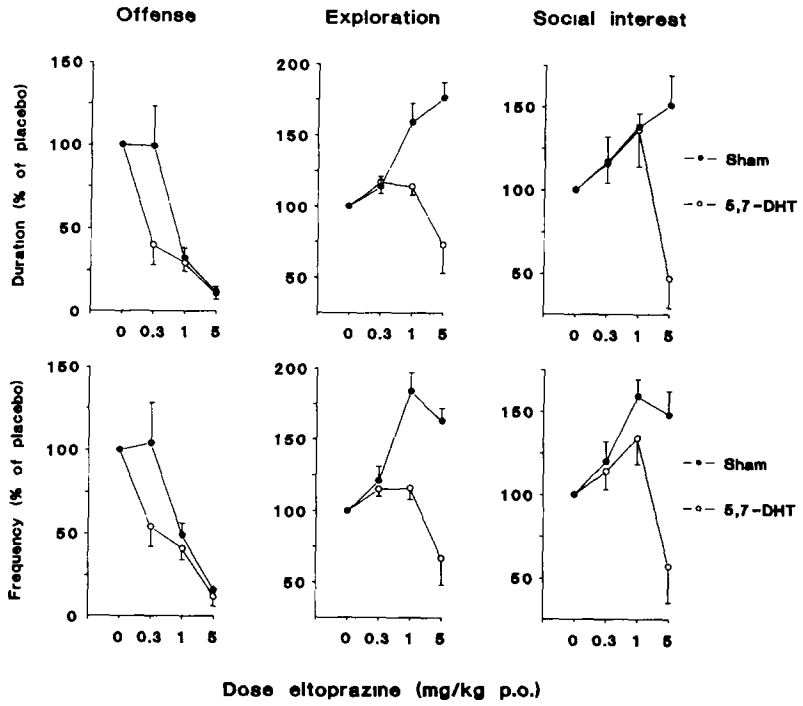

FIG 2 Effects of eltoprazine on offense, exploration and social interest of sham- and 5,7-DHT-lesioned rats in a resident-intruder aggression test Data are expressed as percentage of placebo values and they are the mean ± S E M of n rats from two experiments (see the Method section)

have only been observed in normal rats at very high oral doses (unpublished results). The fact that in 5-HT-depleted rats sedation occurs at a lower dose can possibly be related to a general increase in sensitivity of the central serotonergic system. Evidence for supersensitivity to serotonergic compounds following depletion of central 5-HT has also been provided by a study employing the predatory aggression paradigm (38).

As 5,7-DHT treatment moderately reduced offensive aggression, suggesting that the role of 5-HT in this type of behaviour is stimulatory rather than inhibitory, it can be hypothesized that the antiaggressive effects of eltoprazine are due to an antagonistic action on postsynaptic 5-HT_1 sites. Indeed, antagonistic properties of eltoprazine have been found on the 5-HT_{1A}-mediated hyperpolarization of pyramidal neurons in the rat hippocampus (29) and on the 5-HT_{1C}-mediated phosphoinositide hydrolysis in the pig choroid plexus (55). However, the eventual mode of action is probably much more complex as there is also evidence that eltoprazine acts as a (partial) agonist both on the 5-HT_{1A}-mediated adenylate cyclase activity in rat hippocampus and on the 5-HT_{1B}-regulated 5-HT release in rat cerebral cortex (45,55). So the outcome of the actions of eltoprazine may either be agonistic or antagonistic depending on the 5-HT_1 receptor subtype(s) involved and the relative occupation of these receptors by endogenous 5-HT.

When considering an antagonistic action of eltoprazine, one possible explanation for the increased antiaggressive activity of eltoprazine following 5-HT depletion may be a decreased availability of the endogenous agonist 5-HT Another possibility, discussed below, is the development of postsynaptic hypersensitivity which, on the other hand, more likely reflects agonistic influences of eltoprazine. Taken together, it may well be that the complex serotonergic regulation of offensive aggression is modulated in more than one way by eltoprazine

Effects of 5,7-DHT Lesions on 5-HT_1 Receptor Binding

The present autoradiographic study demonstrates striking effects of 5,7-DHT treatment on 5-HT_1 receptor binding in brains from TMD-S3 rats previously used as residents in behavioural experiments. The 5,7-DHT-induced reduction in [^{3}H]8-OH-DPAT binding in dorsal and median raphe nuclei corroborates with findings by others (32, 66, 72), indicating dense localization of 5-HT_{1A} binding sites on serotonergic cell bodies in these two nuclei. No evidence was found for presynaptic localization of either 5-HT_1, 5-HT_{1A} or 5-HT_{1B} binding sites in any of the other brain areas, although it is generally accepted that the presynaptic autoreceptors on serotonergic terminals are of the 5-HT_{1B} type (14,37).

In contrast, many regions in fore- and midbrain (Table 3 and Fig. 3) displayed an increase in both [^{3}H]5-HT and [^{3}H]-eltoprazine binding in 5,7-DHT-treated animals. This increase in 5-HT_1 receptor binding was present in regions primarily containing 5-HT_{1A} sites (e.g., lateral septum and dentate gyrus), in regions strongly enriched in 5-HT_{1B} sites (e.g., basal ganglia and subiculum) and in choroid plexus which mainly comprises 5-HT_{1C} sites (48,75). Selective labelling with [^{3}H]8-OH-DPAT or [^{3}H]5-HT in the presence of 8-OH-DPAT and DOI [a 5-HT_{1C}/5-HT_2 selective compound (26)] demonstrated that binding to 5-HT_{1A} and 5-HT_{1B} receptor subtypes was indeed enhanced in many brain areas of 5,7-DHT-lesioned rats. The methodology used in the present study, however, does not allow to elucidate whether the increase in binding of the tritiated serotonergic ligands is due to an increase in receptor number and/or receptor affinity.

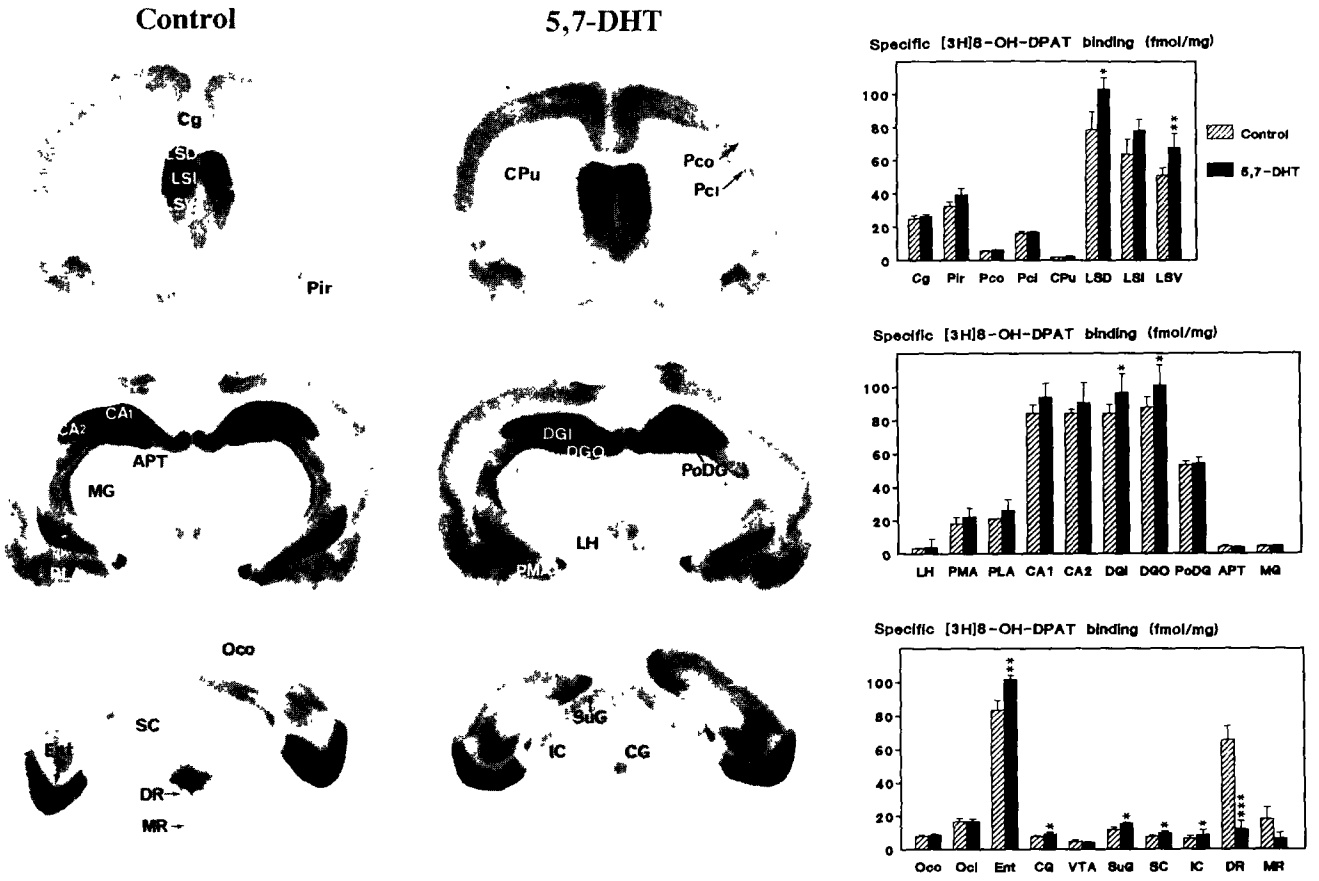

FIG 3 The images presented are bright-field photomicrographs of autoradiograms generated from coronal brain sections (20 μM) incubated with 0 5 nM [^{3}H]8-OH-DPAT at 3 representative levels. Autoradiograms were quantified and data were analysed as described in the Method section (see also legend to Table 3) APT, anterior pretectal area, CA$_1$ and CA$_2$, hippocampal regions, Cg, cingulate cortex, CG, central grey, CPu, caudate putamen, DGI, dentate gyrus (inner blade), DGO, dentate gyrus (outer blade), DR, dorsal raphe nucleus, Ent, entorhinal cortex, IC, inferior colliculus, LH, lateral hypothalamus, LSD, lateral septum (dorsal part), LSI, lateral septum (intermediate part), LSV, lateral septum (ventral part), MG, medial geniculate nucleus, MR, median raphe nucleus, Oci, occipital cortex (lamina IV–VI), Oco, occipital cortex (lamina I–III), Pci, parietal cortex (lamina IV–VI), Pco, parietal cortex (lamina I–III), Pir, piriform cortex, PLA, posterolateral cortical amygdaloid nucleus, PMA, posteromedial cortical amygdaloid nucleus, PoDG, dentate gyrus (polymorph layer), SC, superior colliculus, SuG, superficial grey layer of the superior colliculus, VTA, ventral tegmental area

While centrally administered 5,7-DHT has been reported to induce 5-HT$_1$ receptor upregulations in hippocampus (39), cerebral cortex (54), midbrain (53), substantia nigra (71), hypothalamus (20), caudate putamen (41) and recently in spinal cord (10), it has also been found not to alter (51) or even to induce regional reductions (19,66) in 5-HT$_1$ receptor binding. Differences in the exact location and size of the 5,7-DHT lesions and consequently the degree of 5-HT depletion in distinct brain regions may underlie some of the conflicting observations. Also the time period between lesion and decapitation seems to be important as 5-HT$_1$ receptor increases have been shown to follow the reductions in 5-HT content with some delay (10,39), and to disappear again after a longer time span (20,53). Notwithstanding the fact that we used a relatively long interval between 5,7-DHT injections and decapitations, the levels of 5-HT and 5-HIAA were still less than 12% of controls. This suggests that the lesions were rather thorough and may, therefore, have resulted in a significant overall upregulation of 5-HT$_1$ recognition sites in projection areas of the dorsal and median raphe nuclei.

Although 5,7-DHT treatment produced an increase in binding of tritiated serotonergic ligands in almost all brain regions, differences in the extent of the increase were present. For instance, the binding to 5-HT$_{1A}$ sites in lateral septum and to 5-HT$_{1B}$ sites in basal ganglia was enhanced with approximately 20 to 50%, whereas the increase in binding to such sites in parietal and occipital cortex was around 10% and often not significant It is unlikely that these differences are caused by unbalanced lesions of dorsal and median raphe nuclei, as projection regions from the dorsal raphe (e.g., striatum and amygdala) and from the median raphe nucleus (e.g., septum and hippocampus) (4) both displayed marked increases in 5-HT$_1$ receptor binding Perhaps regional differences in 5-HT activity (6,56) or the degree in which the various regions are innervated by serotonergic neurons (4,60) underlie some of the differences in 5-HT$_1$ receptor upregulation following 5,7-DHT lesions of the raphe nuclei.

The failure to indicate the presence of presynaptic binding sites (i.e., a reduction in binding to 5-HT$_{1B}$ sites) in any of the serotonergic projection regions can be explained by one or more of the following reasons. Firstly, radioligand binding may not be the appropriate method when postsynaptic occurring 5-HT$_1$ receptor

sites largely outnumber the ones that are located presynaptically Secondly, the observed increase in postsynaptic binding sites may have masked a decrease in presynaptic sites On the one hand, this does not seem to be very convincing since studies investigating [^{3}H]5-HT binding at a time when upregulation is less likely to occur (3 to 6 days after 5,7-DHT treatment), revealed no (10, 39, 53) or regionally limited (19) reductions in 5-HT_1 recognition sites On the other hand, one should bear in mind that the increase in [^{3}H]5-HT binding does not have to be purely neuronal, but may partly be due to proliferation of astroglial cells which also contain [^{3}H]5-HT binding sites (18, 25, 73)

Denervation-induced supersensitivity of 5-HT_1 receptors has not only been demonstrated by receptor binding studies, but also by investigations measuring neurochemical and behavioural responses. In fact, 5,7-DHT treatment has been reported to sensitise the 5-HT-stimulated adenylate cyclase activity in the rat hippocampus (5), which may be linked to 5-HT_{1A} receptors (33,57), and to potentiate the 5-HT_{1C}-mediated phosphoinositide hydrolysis response in rat choroid plexus (12). These results correspond very well with the increase in [^{3}H]5-HT binding we observed in both the hippocampus and the choroid plexus of 5-HT-depleted animals. Potentiation of 8-OH-DPAT-induced hypothermia (24,28), RU 24969-induced hyperlocomotion (40,62) and 1-5-HTP-induced flat body posture (11) are examples of either PCPA or 5,7-DHT-induced behavioural supersensitivity, supposedly mediated by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptors, respectively. These observations, together with the overall upregulation in 5-HT_1 receptor binding, suggest that these three 5-HT_1 receptor subtypes receive a tonic serotonergic input

Combining the autoradiographic data with the behavioural effects of eltoprazine, it is tempting to speculate that the 5,7-DHT-induced supersensitivity of postsynaptic 5-HT_1 receptors is underlying the increased efficacy of eltoprazine to reduce offensive aggression. Unfortunately, the rather nonselective upregulation of 5-HT_1 binding sites and the fact that eltoprazine binds to 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes makes it impossible to elucidate from the present study at which anatomical sites or receptor subtypes eltoprazine primarily acts. The septal area may be of particular interest as it displays a very high upregulation of [^{3}H]eltoprazine binding sites and lesioning this area usually decreases intraspecific aggression [for review see (2)]. However, further investigations, such as local injections of eltoprazine in specific rat brain regions, are mandatory to get a better insight into the neuronal substrates involved in the modulation of offensive aggressive behaviour and the sites at which eltoprazine operates.

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