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Autoradiographic Distribution of Cholinergic Muscarinic Receptors and Serotonin$_2$ Receptors in Olfactory Bulbectomized (OB) rats after Chronic Treatment with Mianserin and Desipramine

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Bilateral removal of the olfactory bulbs in rats produces a behavioural abnormality that is defined by hyperactivity in the open-field test. This abnormality may be related to depression since these behavioural effects can be attenuated by antidepressant drugs. Moreover, changes in the cholinergic and serotonergic system may be involved in the pathogenesis of depression. Thus, muscarinic cholinergic and serotonin$_2$ receptors were measured by quantitative autoradiography after the bilateral removal of the olfactory bulbs from the rat. In OB rats, muscarinic receptor density was decreased in several brain regions including the amygdaloid cortex, the basal ganglia, hippocampus, hypothalamus, cortex and olfactory regions. Serotonin$_2$ receptors were increased in all cortical regions, in the hippocampus and the thalamus. When OB rats were treated chronically for 35 days with mianserin (5 mg/kg i.p.) or desipramine (7·5 mg/kg i.p.) the behavioural hyperactivity was reversed and muscarinic receptor density was increased in the hippocampus and cortical regions while serotonin$_2$ receptors were normalized. The results are consistent with a cholinergic and serotonin involvement in depressive illness and suggest that the cholinergic and serotonergic modulatory properties of antidepressant drugs may contribute to their therapeutic effectiveness.

KEY WORDS—Olfactory bulbectomy, open field behaviour, antidepressant, quantitative autoradiography, muscarinic cholinergic receptors, serotonin$_2$ receptors.

INTRODUCTION

Bilateral removal of the olfactory bulbs in the rat is associated with changes in exploratory behaviour and passive avoidance that are largely attenuated by chronic but not acute treatment with antidepressant drugs (Leonard and Tuite, 1981; Jancsar and Leonard, 1981a,b, 1983). The results thus far published (Van Riezen and Leonard, 1990) reveal that the olfactory bulbectomized (OB) rat is a useful model for the selection of potential antidepressant drugs.

Bilateral olfactory bulbectomy is reported to result in a number of neurochemical alterations in central neurotransmitter function; decreases in noradrenaline, dopamine, serotonin, acetylcholine, aspartate and glutamate (Pohorecky *et al.*, 1969a,b; King and Cairncross, 1974; Yoshimura *et al.*, 1974; Cairncross *et al.*, 1975; Harvey *et al.*, 1975; Neckers *et al.*, 1975; Nakamura and Nakamura, 1979).

The cholinergic system in the brain has been implicated in the manifestation of muricide behaviour in the OB rat (Miczek and Barry, 1976; Yoshimura and Ueki, 1977).

Butler *et al.* (1988a) have shown that platelets from bulbectomized rats show a deficit in serotonin transport similar to that found in depressed patients, (Butler and Leonard, 1988b), and that following chronic antidepressant treatment the uptake of [^{3}H]-serotonin returns to sham operated control values.

Change in neurotransmitter function in the OB rat have been largely confined to HPLC or fluorimetric analysis of discrete brain areas. The purpose of the present study was to examine the effects of bilateral olfactory bulbectomy on the densities of muscarinic cholinergic receptors and serotonin$_2$ receptors in several regions of the rat brain using quantitative autoradiography. An additional purpose of the study was to evaluate the effects of chronic treatment with mianserin (5 mg/kg i.p.) and desipramine (7·5 mg/kg i.p.) on the densities of muscarinic cholinergic and serotonin$_2$ receptors in sham and OB treated rats.

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MATERIALS AND METHODS

Male Sprague-Dawley rats with a mean body weight of 280–320 g at the time of surgery, were used. They were housed four per cage, food and water were available ad libitum and maintained on a 12-h (8.00 a.m.–8.00 p.m.) light/12-h (8.00 p.m.–8.00 a.m.) dark cycle. Bilateral olfactory bulbectomy was performed under tribromoethanol anaesthesia as described by Van Riezen and Leonard (1990). Sham-operated animals were treated in the same way but although the dura overlying the bulbs was removed, the bulbs were left intact. Following recovery from surgery (14 days), drugs were prepared in physiological saline and administered once daily (10.00 h) by intraperitoneal injection for 35 days. The experiment consisted of three sham-operated groups (saline treated, mianserin (5 mg/kg) treated and desipramine 7·5 mg/kg) and three groups of bulbectomized animals injected with saline, or mianserin or desipramine. Commencing at 9.00 h on the 15th day of drug treatment, the animals were placed singly in the open-field apparatus for 3 min (Gray and Lalljee, 1974). Ambulation (number of squares crossed), rearing (fore-paws raised from the floor of the apparatus), grooming and defaecation (number of faecal boli deposited) were recorded for a 3-min period of observation. All observations were performed in a quiet room between 9.00 and 13.00 h. The apparatus was illuminated by a 60 W bulb positioned 45 cm above the centre of the base.

Twenty four hours after the last administration of drugs, animals were sacrificed by decapitation 35 days after the onset of drug treatment, their brains were rapidly removed, blocked and frozen on dry ice powder and stored at −70°C until use. The frozen brains were cut into 20 μm thick coronal sections at −15°C, then thaw-mounted on chrome alum/gelatin coated glass slides.

Autoradiography of cholinergic muscarinic receptors was performed by the method described by Yamamura and Snyder (1974). The tissue sections were incubated for 2 h at 25°C in phosphate buffered saline (0·9 per cent NaCl and 15 mM Na_2HPO_4) pH 7·4 with 1 nM [^{3}H] quinuclidinyl benzilate (QNB) as ligand. Non-specific binding was defined as the labelling in the presence of 1 μM atropine, a muscarinic antagonist.

The sections were apposed against Amersham [^{3}H] Hyperfilm for two weeks (Earley *et al.*, 1989). Autoradiography of 5-HT_2 serotonin receptors on sections was performed using [^{3}H] ketanserin at a concentration of 1 nM. Non-specific binding was determined in the presence of 1 μM mianserin. Two 20-min wash periods in ice-cold buffer followed the incubation. Slides were then dipped in ice-cold water to remove buffer salts, dried on a 50°C hot plate and the sections were apposed against tritium-sensitive film (Amersham [^{3}H]-Hyperfilm) for 8 weeks (Biegon *et al.*, 1986; Gross-Isseroff *et al.*, 1990a,b). Because the relationship between the signal in the film and the control of radioactivity in the specimen is not a simple one, the quantitative interpretation of autoradiograms required the utilization of autoradiographic standards with the brain sections.

Individual films were coexposed to commercially available (Amersham) tritiated standards. Films were developed and fixed with Kodak developer and fixer.

Tritium brain-mash standards

Tritium brain-mash standards were made by thoroughly mixing one half of a rat forebrain with 0·5–48 μCi of [^{3}H]leucine (53 Ci/mmol, New England Nuclear) in 0·01 N HCl (1 μCi/μl). Brain tissue was mixed with [^{3}H]leucine in a polyethylene tube with a Teflon-coated spatula to minimize tissue adherence to glass or metal. The brain mash was transferred to conical shaped aluminium-foil moulds, approximately 1 cm in height and 0·5 cm in base diameter, and frozen in powdered dry ice. After removal of the aluminium-foil, the frozen brain-mash standards were mounted onto cryostat chucks and cut into 20 μm thick sections at −15°C. At intervals through the frozen brain-mash, representative sections were collected with forceps and were homogenized in 200 μl of double distilled water. Triplicate 20 μl aliquots at the homogenized sections were used either for scintillation counting or for protein measurements according to the method of Bradford (1976). The external standard ratio method was used to correct scintillation measurements for quench and efficiency. The remaining sections were thaw-mounted onto subbed glass slides, dried briefly on a 50°C slide warmer and exposed against Amersham [^{3}H]-Hyperfilm at 23°C in X-ray film cassettes as described (Rainbow *et al.*, 1982a). Exposure times were 2 weeks and 8 weeks respectively.

Generation of tritium standard curve

After development and fixation as described (Rainbow *et al.*, 1982b), the optical densities of the stan-

dard autoradiograms were plotted against their tritium concentration per mg protein.

Autoradiograms were analysed with the aid of an IBM-PC based computerized image analysis system with a PC-vision digitizing board (Image Technology Inc.) and customized software (Isseroff and Lancet, 1985). Anatomical structures to be measured were defined by referring to the histologically stained cresyl violet section which generated the measured autoradiogram (Paxinos and Watson, 1986). Amersham standards containing varying amounts of radioactivity which were co-exposed with the experimental tissue were analysed mathematically and the quantity of radioactivity in the standard was related to the optical density or grain density in the autoradiogram. The software program (Anat Biegon, Israel) uses mathematical functions that adequately describe the relationship; length of exposure of the autoradiograms and the range of standards used. Standard curves derived from the tritiated standards and brain-mash standards were used to convert gray level readings from the autoradiograms into equivalents of pmol [^{3}H] QNB and fmol [^{3}H] ketanserin specifically bound per mg of protein by cross-reference to brain-mash standards containing known amounts of radioactivity and protein per section. Non-specific binding was subtracted from total binding to obtain specific binding.

ANALYSES OF RESULTS

Open-field data were analysed using the Mann-Whitney *U* test. Median values in all tests were chosen for statistical testing. In evaluating all comparisons two-tailed tests of significance were used.

Receptor densities were analysed by one-way analysis of variance using SPSS routines on measurements obtained from $N = 10$ animals per group. Values are expressed as mean pmol of [^{3}H]QNB and mean fmol of [^{3}H] ketanserin specifically bound per mg of protein ± S.D. A total of 20 brain sections were analysed in an anterior to posterior direction per individual rat brain. Up to 20–30 measurements were taken for each rat brain region and included measurements for both the left and right hemispheres. A mean value for receptor density, including left and right hemispheres, was then obtained for each animal. Ten animals were analysed per group. To determine overall treatment effects, data were subjected to an analysis of variance procedure (Winer, 1962). If a significant overall effect was observed, Duncan's test was applied to determine statistical difference between groups, for which the level of significance was fixed at $p < 0{\cdot}01$ without making allowances for the multiple areas tested.

RESULTS

Effects of mianserin and desipramine on the behaviour of OB rats in the open field apparatus

Bilateral olfactory bulbectomy resulted in a hypermotility in the stressful environment of the 'open-field'. The increased ambulation scores were attenuated by chronic administration of mianserin (5 mg/kg i.p.) and desipramine (7·5 mg/kg i.p.). None of the drugs had a significant effect on the behaviour of the sham-operated animals. No consistent changes were found in the grooming or defaecation scores and therefore the results are excluded from Figure 1.

The distribution of muscarinic cholinergic and serotonin receptors after bilateral olfactory bulbectomy and the effects of chronic mianserin and desipramine treatments

The autoradiographic localization of [^{3}H]QNB binding is shown in Tables 1 and 2. In this autoradiographic study the distribution of [^{3}H]QNB was examined in several brain regions. When observing the effects of olfactory bulbectomy on receptor density a significant decrease was observed in several brain regions (Table 2 and Plate 1). However, the receptor loss was more marked in some regions than in others. A significant decrease in density was found in the central amygdaloid and medial amygdaloid nuclei; the frontal cortex and parietal motor cortex layers i–iv; the CA_1-CA_4 fields of the hippocampus; the ventrolateral thalamic nuclei; the nucleus of the horizontal limb of the diagonal band of Broca, the substantia innominata and the olfactory tubercles.

In sham mianserin treated rats (Table 1), a down-regulation of cholinergic receptor density was observed in the following regions; the basolateral and central amygdaloid nuclei, frontal and parietal motor cortex layer i–ii, and in the nucleus of the horizontal limb of the diagonal band of broca.

Following chronic mianserin treatment (Table 3 and Plate 2) an increase in receptor density was found in the following brain regions in OB rats; CA1-P and CA2-P pyramidal cell layers of the hippocampus and the ventrolateral thalamic nuclei.

Following chronic desipramine administration

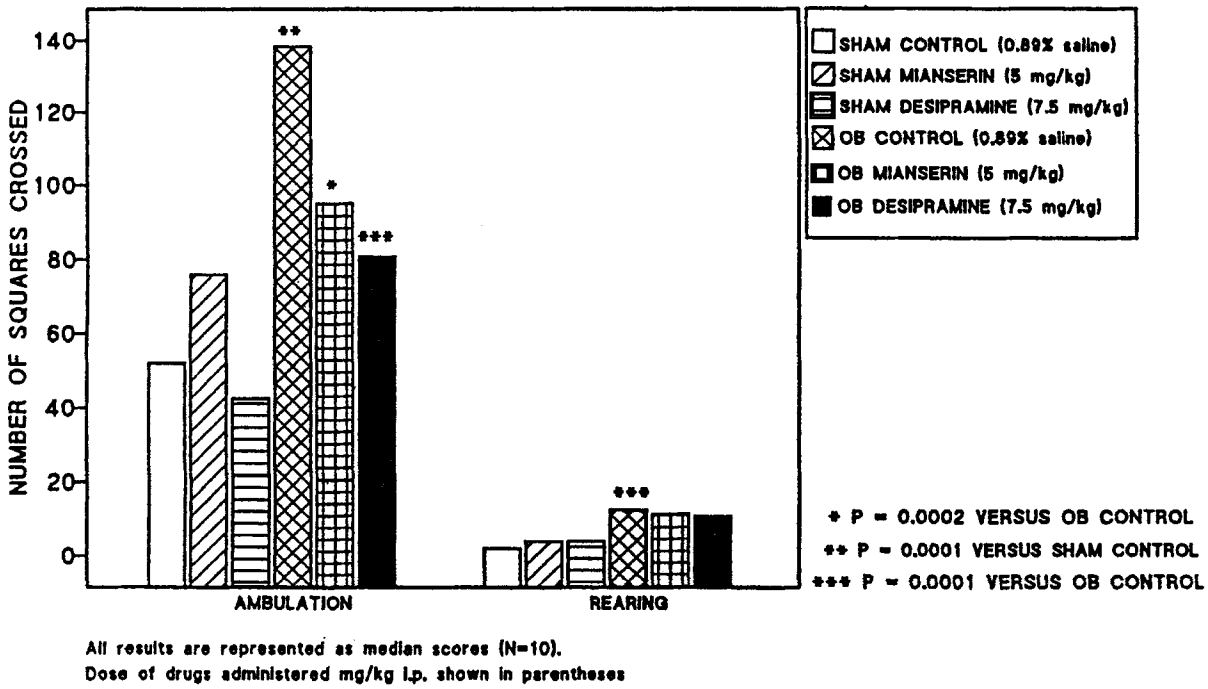

Figure 1. The effects of mianserin and desipramine on the ambulation (number of squares crossed) and rearing scores of the olfactory bulbectomized (OB) rat in the "open field" apparatus over a 3-min period of observation.

to sham-operated animals (Table 1) a decrease in receptor binding for [^{3}H]QNB was found in the basolateral, central amygdaloid and medial amygdaloid nuclei; the lateral part of the caudate putamen and striatal streak region of the basal ganglia, the frontal cortex and parietal motor cortex layers i–iv, the arcuate nucleus of the hypothalamus, the ventral pallidum and the olfactory tubercles.

When OB animals were treated chronically with desipramine no change in receptor density was found in the amygdaloid cortex (Table 2). In the basal ganglia, an increase in density was found in the caudate putamen, the frontal cortex, the CA_1 and CA_2 pyramidal cell layers, the CA_1 and CA_2 oriens cell layers and the CA_1 and CA_2 molecular layers of the hippocampus.

The distribution of [^{3}H]ketanserin binding in sham and OB rats and the effects of chronic mianserin and desipramine treatments are shown in Tables 3 and 4 and Plates 3 and 4.

In OB saline treated rats, no change in 5-HT_2 receptor density was found in the amygdaloid nuclei, the basal ganglia, the hypothalamus, septum, and olfactory regions (Table 4). However, a significant increase in receptor density was observed in most cortical regions, the frontal cortex and the parietal motor cortical regions layers i–vi. Increased 5-HT_2 receptor density was also observed in the hippocampus, (pyramidal cell layers, CA_1 and CA_3, dentate gyrus granular layer) and in the thalamus (laterodorsal and mediodorsal thalamic nuclei).

In sham mianserin treated rats, a down regulation of 5-HT_2 receptor was found in all cortical regions (Table 3) including the anterior and posterior cingulate gyrus, the frontal cortex and the parietal motor cortex layers i–iv.

In OB mianserin treated rats, a normalization of receptor density was found in the cortex (frontal cortex, parietal motor cortex layers i–vi) and hippocampus ($CA_1$and CA_3 pyramidal cell layers and the dentate gyrus) (Table 4).

In sham desipramine treated rats a down regulation of 5-HT_2 receptors was observed in all cortical regions (anterior and posterior cingulate gyrus, frontal cortex and parietal motor cortical regions) (Table 3).

In OB desipramine treated rats a normalization of receptor density was observed in cortical, hippocampal and hypothalamic regions (Table 4).

Table 1. Autoradiography of cholinergic muscarinic receptor density in sham rats treated chronically (35 days) with mianserin and desipramine. The results are expressed as mean pmoles [^{3}H]QNB specifically bound per mg of protein ± S.D. ($N = 10$)

Region (abbreviation)			Treatment Groups		
			Sham control	Sham + Mianserin (5)	Sham + Desipramine (7.5)
Amygdala	BL	Basolateral amygdaloid nucleus	11·377 ± 2·13	12·838 ± 2·40	12·237 ± 1·69
	BM	Basolateral amygdaloid nucleus	10·203 ± 1·28	9·411 ± 0·66*	9·025 ± 0·46*
	CE	Central amygdaloid nucleus	9·972 ± 1·17	9·015 ± 0·39*	8·816 ± 0·34*
	LA	Lateral amygdaloid nucleus	10·262 ± 0·94	10·965 ± 1·46	10·127 ± 0·93
	ME	Medial amygdaloid nucleus	10·101 ± 1·36	9·158 ± 0·49	9·086 ± 0·77*
Basal ganglia	Acb	Accumbens	15·482 ± 2·60	15·378 ± 1·67	14·158 ± 2·04
	CPu	Caudate putamen	11·545 ± 2·60	11·261 ± 1·78	10·572 ± 1·54
	lcp	Lateral caudate putamen	16·143 ± 2·19	15·841 ± 2·32	14·095 ± 1·94*
	ss	Striatal streak	13·800 ± 2·24	13·901 ± 1·81	11·951 ± 0·21*
Cortex	Acg	Anterior cingulate gyrus	8·650 ± 0·63	8·651 ± 0·63	8·848 ± 0·69
	Fr	Frontal cortex	9·144 ± 0·42	8·943 ± 0·38*	8·815 ± 0·47*
	PAM	Frontoparietal motor	8·801 ± 0·78	8·612± 0·69	8·801 ± 0·31
	PAM1	Layers i–ii	10·213 ± 1·72	9·119 ± 0·46*	9·107 ± 0·67*
	PAM2	Layers iii—iv	7·974 ± 0·87	7·690 ± 0·80	7·694 ± 0·57
	PAM3	Layers v–vi	8·073 ± 0·83	8·002 ± 0·64	7·830 ± 0·64
	PAS-P	Parietal motor cortex posterior part	8·848 ± 1·21	8·113 ± 1·77*	8·285 ± 0·59
	PCG	Posterior cingulate gyrus	7·625 ± 0·78	8·006 ± 0·57	7·966 ± 0·42
Hippocampus	CA1-P	CA1 field, pyramidal	13·271 ± 1·66	13·793 ± 2·31	13·310 ± 1·97
	CA2-P	CA2 field, pyramidal	8·912 ± 0·54	9·033 ± 0·35	8·766 ± 0·39
	CA3-P	CA3 field, pyramidal	9·190 ± 0·92	9·155 ± 0·33	8·908 ± 0·39
	CA1–0	CA1 field, oriens layer	13·178 ± 1·69	13·622 ± 2·28	12·918 ± 2·04
	CA2–0	CA2 field, oriens layer	8·838 ± 0·61	9·053 ± 0·34	8·736 ± 0·47
	CA3–0	CA3 field, oriens layer	9·089 ± 0·74	9·167 ± 0·41	8·866 ± 0·49
	CA1-M	CA1 field, molecular layer	9·053 ± 0·44	8·902 ± 0·34	8·813 ± 0·49
	CA2-M	CA2 field, molecular layer	8·548 ± 0·71	8·685 ± 0·30	8·426 ± 0·48
	CA3-M	CA3 field, molecular layer	8·816 ± 0·64	8·820 ± 0·23	8·631 ± 0·54
	CA4	CA4 field	9·353 ± 0·52	9·359 ± 0·32	9·225 ± 0·37
	Dgg(U)	Dentate gyrus (upper) granular layer	10·967 ± 1·31	11·451 ± 1·79	10·288 ± 1·04
	Dgg(L)	Dentate gyrus (lower) granular layer	13·488 ± 1·78	14·041 ± 2·63	12·594 ± 1·69
Hypothalamus	ARC	Arcuate nucleus	8·794 ± 1·31	8·778 ± 0·37	8·434 ± 0·44*
	VMH	Ventromedial	8·660 ± 0·52	8·739 ± 0·31	8·496 ± 0·46
Septum	lsd	Lateral n.dorsal part	8·333 ± 0·27	8·335 ± 0·32	8·329 ± 0·46
	lsv	Lateral n. ventral part	8·350 ± 0·42	8·319 ± 0·34	8·179 ± 0·42
	MS	Medial n.	8·195 ± 0·42	8·429 ± 1·53	7·994 ± 0·88
Thalamus	LD	Laterodorsal	7·683 ± 0·85	7·919 ± 0·51	7·740 ± 0·43
	MD	Mediodorsal	7·849 ± 0·71	8·149 ± 0·46	7·896 ± 0·48
	VL	Ventrolateral	7·842 ± 1·00	7·373 ± 0·86	6·987 ± 1·01
Ventral forebrain nuclei	HDB	N. of the horizontal limb of the diagonal band	8·744 ± 0·19	8·451 ± 0·29*	8·452 ± 0·37
	SI	Substantia innominata	8·262 ± 0·54	8·147 ± 0·21	7·998 ± 0·41
	VDB	N. of the ventral limb of the diagonal band	8·515 ± 0·48	8·289 ± 0·41	8·381 ± 0·34
	VP	Ventral pallidum	8·137 ± 0·64	7·961 ± 0·26	7·997 ± 0·31*
Olfactory regions	TU	Olfactory tubercle	21·620 ± 2·62	19·952 ± 3·24	18·395 ± 0·54*
White matter	cc	Corpus callosum	1·330 ± 1·66	0·931 ± 1·28	0·976 ± 1·14*

The doses of mianserin and desipramine are in parentheses as mg/kg i.p.
* $p < 0.01$ versus sham control.

Table 2. Autoradiography of cholinergic muscarinic receptor density in sham and olfactory bulbectomized (OB) rats treated chronically (35 days) with mianserin and desipramine. The results are expressed as mean pmol [^{3}H]QNB specifically bound per mg of protein ± S.D. ($N = 10$)

Brain region (abbreviation)			Treatment groups			
			Sham control	OB Control	OB + Mianserin (5)	OB + Desipramine (7.5)
Amygdala	BL	Basolateral amygdaloid nucleus	11·377 ± 2·13	11·686 ± 1·40	11·55 ± 1·18	12·014 ± 0·98
	BM	Basomedial amygdaloid nucleus	10·203 ± 1·28	9·088 ± 0·99	9·016 ± 0·71	9·325 ± 1·01
	CE	Central amygdaloid nucleus	9·972 ± 1·17	8·727 ± 0·31*	8·753 ± 0·31	8·819 ± 0·19
	LA	Lateral amygdaloid nucleus	10·262 ± 0·94	9·751 ± 0·78	10·036 ± 0·89	9·928 ± 1·36
	ME	Medial amygdaloid nucleus	10·101 ± 1·36	8·880 ± 0·68*	8·933 ± 0·53	9·045 ± 0·33
Basal ganglia	Acb	Accumbens	15·482 ± 2·60	13·649 ± 1·76	12·965 ± 3·10	13·605 ± 2·37
	CPu	Caudate putamen	11·545 ± 2·60	9·901 ± 0·68	9·827 ± 1·43	11·605 ± 2·94#
	lcp	Lateral caudate putamen	16·143 ± 2·19	13·380 ± 1·87	13·591 ± 1·86	13·413 ± 2·60
	ss	Striatal streak	13·800 ± 2·24	11·394 ± 1·65	11·535 ± 2·17	12·023 ± 1·93
Cortex	Acg	Anterior cingulate gyrus	8·650 ± 0·63	8·530 ± 0·471	8·623 ± 0·62	8·519 ± 0·78
	Fr	Frontal cortex	9·144 ± 0·42	8·618 ± 0·36*	8·522 ± 0·44	8·895 ± 0·42#
	PAM	Frontoparietal motor	8·801 ± 0·78	8·640 ± 0·42	8·663 ± 0·44	8·816 ± 0·28
	PAM1	Layers i—ii	10·213 ± 1·72	8·903 ± 0·25*	8·823 ± 0·66	8·812 ± 0·39
	PAM2	Layers iii—iv	7·974 ± 0·87	7·466 ± 0·60*	7·408 ± 0·83	7·215 ± 0·80
	PAM3	Layers v—vi	8·073 ± 0·83	7·685 ± 0·62	7·697 ± 0·64	7·570 ± 0·64
	PAS-P	Parietal motor cortex posterior part	8·848 ± 1·21	8·008 ± 0·56	8·073 ± 0·49	8·482 ± 0·47
	PCG	Posterior cingulate gyrus	7·625 ± 0·78	7·742 ± 0·43	7·846 ± 0·44	8·129 ± 0·32#
Hippocampus	CA1-P	CA1 field, pyramidal	13·271 ± 1·66	11·354 ± 1·78*	12·582 ± 1·82#	12·583 ± 1·27#
	CA2-P	CA2 field, pyramidal	8·912 ± 0·54	8·373 ± 0·58*	8·640 ± 0·56#	8·708 ± 0·42#
	CA3-P	CA3 field, pyramidal	9·190 ± 0·92	8·533 ± 0·44*	8·678 ± 0·50	8·691 ± 0·34
	CA1-O	CA1 field, oriens layer	13·178 ± 1·69	11·480 ± 1·17*	11·439 ± 1·34	12·418 ± 1·18#
	CA2-O	CA2 field, oriens layer	8·838 ± 0·61	8·420 ± 0·47*	8·389 ± 1·40	8·629 ± 0·46#
	CA3-O	CA3 field, oriens layer	9·089 ± 0·74	8·441 ± 0·55*	8·569 ± 0·29	8·658 ± 0·34
	CA1-M	CA1 field, molecular layer	9·053 ± 0·44	8·877 ± 0·55*	8·602 ± 1·42	8·892 ± 0·31#
	CA2-M	CA2 field, molecular layer	8·548 ± 0·71	7·993 ± 0·59*	8·081 ± 0·48	8·285 ± 0·47#
	CA3-M	CA3 field, molecular layer	8·816 ± 0·64	8·377 ± 0·53*	8·237 ± 0·46	8·551 ± 0·39
	CA4	CA4 field	9·353 ± 0·52	8·941 ± 0·33*	9·005 ± 0·36	9·155 ± 0·31
	Dgg(U)	Dentate gyrus (upper) granular layer	10·967 ± 1·31	9·867 ± 0·69*	10·271 ± 1·12	9·973 ± 0·67
	Dgg(L)	Dentate gyrus (lower) granular layer	13·488 ± 1·78	12·187 ± 1·44	13·11 ± 2·23	12·122 ± 1·64
Hypothalamus	ARC	Arcuate nucleus	8·794 ± 1·31	8·483 ± 0·29	8·618 ± 0·35	8·556 ± 0·28
	VMH	Ventromedial	8·660 ± 0·52	8·494 ± 0·3	8·509 ± 0·36	8·584 ± 0·24
Septum	lsd	Lateral n. dorsal part	8·333 ± 0·27	7·892 ± 0·47	7·751 ± 0·76	8·336 ± 0·47
	lsv	Lateral n. ventral part	8·350 ± 0·42	7·888 ± 0·58	7·723 ± 0·79	8·092 ± 0·40
	MS	Medial n.	8·195 ± 0·42	8·154 ± 0·73	7·665 ± 0·20	7·897 ± 1·14
Thalamus	LD	Laterodorsal	7·683 ± 0·85	7·519 ± 0·72	7·311 ± 0·49	7·615 ± 0·34
	MD	Mediodorsal	7·849 ± 0·71	7·892 ± 0·51	7·765 ± 0·64	8·004 ± 0·37
	VL	Ventrolateral	7·842 ± 1·00	7·222 ± 0·86*	6·698 ± 1·80#	7·015 ± 0·87
Ventral forebrain nuclei	HDB	N. of the horizontal limb of the diagonal band	8·744 ± 0·19	8·423 ± 0·32*	8·254 ± 0·42	8·367 ± 0·39
	SI	Substantia innominata	8·262 ± 0·54	7·948 ± 0·49*	8·016 ± 0·34	7·961 ± 0·54
	VDB	N. of the ventral Limb of the diagonal band	8·515 ± 0·48	8·245 ± 0·52	7·867 ± 1·50	8·181 ± 0·50
	VP	Ventral pallidum	8·137 ± 0·64	7·680 ± 0·52*	7·864 ± 0·35	7·578 ± 0·40
Olfactory	TU	Olfactory tubercle	21·620 ± 2·62	17·883 ± 3·04*	17·796 ± 3·85	19·397 ± 5·24
White matter	cc	Corpus callosum	1·330 ± 1·66	0·945 ± 1·30	0·789 ± 1·09	0·870 ± 1·19

The doses of mianserin and desipramine are in parentheses as mg/kg i.p. *$p < 0.01$ versus sham control; #$p < 0.01$ versus OB control.

Table 3. Autoradiography of 5-HT_2 receptor density in sham rats treated chronically (35 days) with mianserin and desipramine. The results are expressed as mean fmoles [^{3}H] ketanserin specifically bound per mg of protein ± S.D. ($N = 10$)

Region (abbreviation)			Treatment Groups Sham Control	Sham+ Mianserin (5) [^{3}H] Ketanserin	Sham+ Desipramine (7.5) receptor density
Amygdala	BL	Basolateral amygdaloid nucleus	111·7 ± 45·87	124·0 ± 14·99	116·6 ± 25·76
	BM	Basolateral amygdaloid nucleus	110·05 ± 41·42	125·8 ± 20·71	110·7 ± 22·12
	CE	Central amygdaloid nucleus	109·1 ± 41·35	121·0 ± 8·43	117·3 ± 23·18
	LA	Lateral amygdaloid nucleus	104·7 ± 41·62	114·2 ± 9·9	113·5 ± 21·85
	ME	Medial amygdaloid nucleus	115·4 ± 49·55	130·5 ± 17·11	125·9 ± 27·01
Basal ganglia	Acb	Accumbens	228·3 ± 48·67	171·4 ± 39·07	190·4 ± 59·07
	CPu	Caudate putamen	173·7 ± 48·12	161·4 ± 26·35	162·6 ± 35·85
Cortex	Acg	Anterior cingulate gyrus	127·7 ± 34·83	89·0 ± 25·47*	87·0 ± 33·66*
	FR	Frontal cortex	122·9 ± 27·75	61·6 ± 37·12*	97·4 ± 42·52*
	PAM1	Layers i—ii	129·7 ± 29·08	64·9 ± 21·96*	92·4 ± 35·58*
	PAM2	Layers iii—iv	159·2 ± 59·47	63·0 ± 21·01*	102·4 ± 46·12*
	PAM3	Layers v—vi	117·2 ± 45·56	57·5 ± 19·87*	85·4 ± 38·67*
	PCG	Posterior cingulate gyrus	46·3 ± 12·16	56·6 ± 17·30	56·8 ± 20·05
Hippocampus	CA1-P	Pyramidal cell layer	49·7 ± 29·25	56·5 ± 23·41	63·1 ± 18·75
	CA3-P	Pyramidal cell layer	64·4 ± 26·50	66·4 ± 24·12	75·5 ± 9·98
	Dgg(U)	Dentate gyrus (upper) granular layer	62·1 ± 32·47	72·6 ± 14·28	79·1 ± 11·22
	Dgm(L)	Dentate gyrus (lower) molecular layer	62·1 ± 34·44	77·1 ± 17·18	83·6 ± 14·52
Hypothalamus	ARC	Arcuate nucleus	170·8 ± 44·17	175·8 ± 22·63	173·7 ± 21·35
	VMH	Ventromedial	151·1 ± 45·56	169·2 ±13·72	151·9 ± 27·52
Septum	MS	Medial nucleus	129·5 ± 62·76	123·3 ± 22·39	110·7 ± 30·32
Thalamus	LD	Laterodorsal	72·0 ± 43·28	89·7 ± 14·02	71·9 ± 22·38
	MD	Mediodorsal	93·0 ± 33·08	111·1 ± 13·37	108·4 ± 21·48
	VL	Ventrolateral	79·8 ± 45·13	97·3 ± 15·21	87·9 ± 19·17
Olfactory regions regions	TU	Olfactory tubercle	244·1 ± 71·82	187·0 ± 48·37	191·1 ± 54·51
White matter	cc	Corpus callosum	15·7 ± 14·17	11·2 ± 3·06	15·5 ± 17·30

The doses of mianserin and desipramine are in parentheses as mg/kg i.p.
* $p < 0.01$ versus sham control.

DISCUSSION

The present study sought to establish the distribution of [^{3}H]QNB muscarinic cholinergic receptors and 5-HT_2 receptors in sham and OB rats and analyse the neuroanatomical effects of chronic mianserin and desipramine on these receptors in both sham and OB rats. Olfactory bulbectomy resulted in a hyperactivity in the 'open-field' which was significantly attenuated by chronic administration of mianserin and desipramine. Bulbectomy was associated with a decrease in the density of muscarinic cholinergic receptors and an increased density of 5-HT_2 receptors in specific brain regions. Several studies have reported that bilateral olfactory bulbectomy caused an increase of noradrenaline in the hypothalamus. Iwasaki *et al.* (1986) have reported that desipramine, which blocks the reuptake of noradrenaline into nerve endings, inhibited muricidal behaviour in the OB rat and this effect was potentiated by chronic antidepressant treatment. Other pharmacological studies on the neurochemical mechanisms mediating mouse killing behaviour suggest that cholinergic systems in the brain participate in the manifestation of the behaviour (Miczek and Barry, 1976; Yoshimura and Ueki, 1977). Furthermore, Yoshimura (1980) has postulated that the release of acetylcholine (ACh) and activation of cholinergic mechanism play an important role in the regulation of mouse killing behaviour. In the present experiment, therefore, we examined

Table 4. Autoradiography of 5-HT_2 receptor density in sham and OB rats treated chronically (35 days) with mianserin and desipramine. The results are expressed as mean fmoles [^{3}H] ketanserin specifically bound per mg of protein ± S.D. ($N = 10$)

Region (abbreviation)			Treatment Groups [^{3}] Ketanserin receptor density Sham Control	OB Control	OB+ Mianserin (5)	OB+ Desipramine (7.5)
Amygdala	BL	Basolateral amygdaloid nucleus	111·7 ± 45·87	129·0 ± 33·33	116·3 ± 33·94	114·1 ± 18·09
	BM	Basolateral amygdaloid nucleus	110·05 ± 41·42	131·7 ± 30·57	115·2 ± 36·24	108·2 ± 27·06
	CE	Central amygdaloid nucleus	109·1 ± 41·35	131·7 ± 32·51	121·3 ± 35·73	102·6 ± 17·32
	LA	Lateral amygdaloid nucleus	104·7 ± 41·62	123·9 ± 34·33	117·7 ± 36·02	106·2 ± 21·12
	ME	Medial amygdaloid nucleus	115·4 ± 49·55	138·6± 32·16	142·0 ± 38·53	125·2 ± 23·24
Basal ganglia	Acb	Accumbens	228·3 ± 48·67	198·5 ± 53·10	188·2 ± 17·46	186·4 ± 63·93
	CPu	Caudate putamen	173·7 ± 48·12	170·0 ± 50·94	165·6 ± 37·03	162·4 ± 61·54
Cortex	Acg	Anterior cingulate gyrus	127·7 ± 34·83	189·0 ± 25·4*	86·2 ± 18·50#	104·3 ± 27·83
	Fr	Frontal cortex	122·9 ± 27·75	201·3 ± 21·32*	86·9 ± 21·81#	104·1 ± 40·44#
	PAM1	Layers i—ii	129·7 ± 29·08	196·9 ± 22·95*	75·1 ± 12·61#	95·1 ± 34·73#
	PAM2	Layers iii—iv	159·2 ± 59·47	181·8 ± 21·71*	55·2 ± 18·00#	96·5 ± 36·99#
	PAM3	Layers v—vi	117·2 ± 45·56	196·4 ± 29·63*	82·4 ± 22·80#	73·3 ± 38·67#
	PCG	Posterior cingulate gyrus	46·3 ± 12·16	76·6 ± 19·30	70·3 ± 21·51	59·1 ± 15·08
Hippocampus	CA1	Pyramidal cell layer	49·7 ± 29·25	79·08 ± 18·46*	52·0 ± 32·76#	53·0 ± 11·13#
	CA111	Pyramidal cell layer	64·4 ± 26·50	82·3 ± 20·63*	61·1 ± 30·00#	63·2 ± 17·59#
	Dgg(U)	Dentate gyrus (upper) granular layer	62·1 ± 32·47	92·9 ± 18·20*	69·7 ± 31·82#	63·1± 11·05#
	Dgm(L)	Dentate gyrus (lower) molecular layer	62·1 ± 34·44	89·2 ± 15·88*	69·4 ± 28·17#	67·6 ± 13·03#
Hypothalamus	ARC	Arcuate nucleus	170·8 ± 44·17	192·3 ± 32·8	177·0 ± 20·42	157·0 ± 27·78#
	VMH	Ventromedial	151·11 ± 45·56	177·8 ± 30·55	162·6± 24·11	147·8 ± 34·17#
Septum	MS	Medial nucleus	129·5 ± 62·76	123·5 ± 34·76	117·5 ± 31·77	110·0 ± 49·20
Thalamus	LD	Laterodorsal	72·0 ± 43·28	98·3 ± 25·67*	100·9 ± 22·70	70·3 ± 16·50#
	MD	Mediodorsal	93·0 ± 33·08	114·1 ± 19·29*	115·0 ± 19·03	92·4 ± 23·55#
	VL	Ventrolateral	79·8 ± 45·13	100·3 ± 25·83	100·1 ± 27·82	76·4 ± 13·78#
Olfactory regions	TU	Olfactory tubercle	244·1 ± 71·82	250·1 ± 43·37	194·3 ± 47·33	209·1 ± 73·14
White matter	cc	Corpus callosum	15·7 ± 14·17	11·43 ± 3·66	15·7 ± 8·98	11·1 ± 4·80

The doses of mianserin and desipramine are in parentheses as mg/kg i.p.
* $p < 0.01$ versus Sham Control.
# $p < 0.01$ versus OB control.

whether changes in the densities of the muscarinic cholinergic and 5-HT_2 receptors were normalized by chronic antidepressant treatment. The present study suggests that a major effect of olfactory bulbectomy in several regions of the rat brain is to alter muscarinic and serotonin$_2$ binding sites.The limbic region examined either receive efferent or contribute to centrifugal input to the olfactory bulbs. The olfactory tubercle and the anterior olfactory nucleus in the olfactory peduncle, which receive olfactory efferents, send projections to the hypothalamus (Haberly and Price, 1978). In addition, it has been reported that the hypothalamus and amygdala have reciprocal connections (Cowan *et al.*, 1965) and that there are complex efferent projections from various limbic cortical areas to the amygdala (Veening, 1978).

Fundamentally, all the cholinergic input to the olfactory bulbs is centrifugal in origin, and contributing areas include the piriform cortex, the nucleus of the horizontal limb of the diagonal band which is found adjacent to the olfactory tubercle and the rostral part of the hypothalamus (Wenk *et al.*, 1977). Thus, destruction of cholinergic cell bodies after bulbectomy, may be responsible for the decrease in cholinergic density which was observed. Hirsch (1980) reported in mice that cholinergic ligand binding in limbic structures was altered by bilateral olfactory bulbectomy and he proposed that the olfactory bulbs produce an inhibitory influence on limbic cholinergic receptors. On the other hand Millan *et al.* (1986) published that olfactory bulbectomized rats are less sensitive to pilocarpine-induced convulsions. In rats, bulbectomy is followed by a deficit in acquisition of a passive avoidance task and in active avoidance learning (Cairncross and King, 1971; Sieck and Gordon, 1972; Thomas, 1973; Sieck *et al.*, 1974).

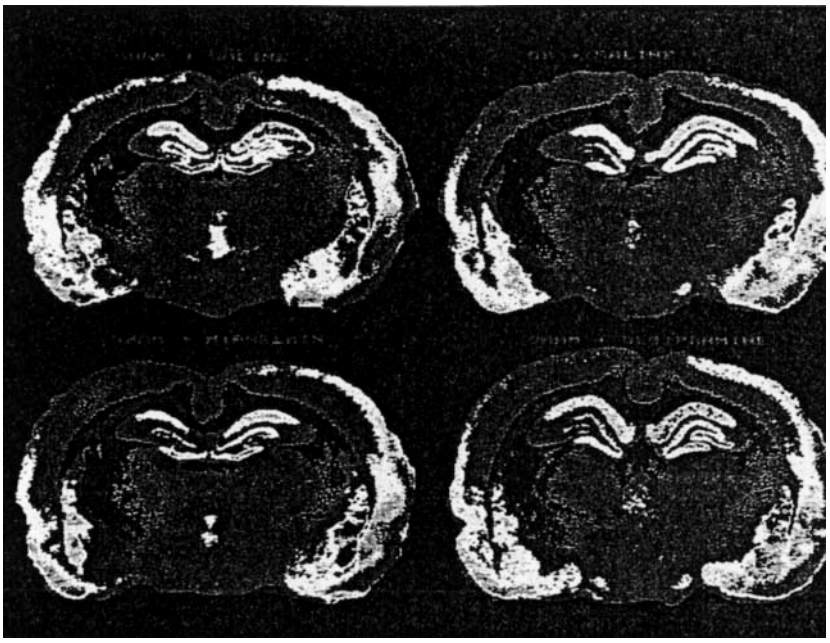

Plate 1. Pseudocoloured images of [^{3}H]QNB binding in brain sections from sham animals at the level of the anterior hippocampus. The top left and top right sections are taken from sham and OB saline treated rats. The bottom left and bottom right are representative sections from sham mianserin treated and sham desipramine treated rats. The colours represent the density of binding using the rainbow spectrum (from purple, low, to red, high)

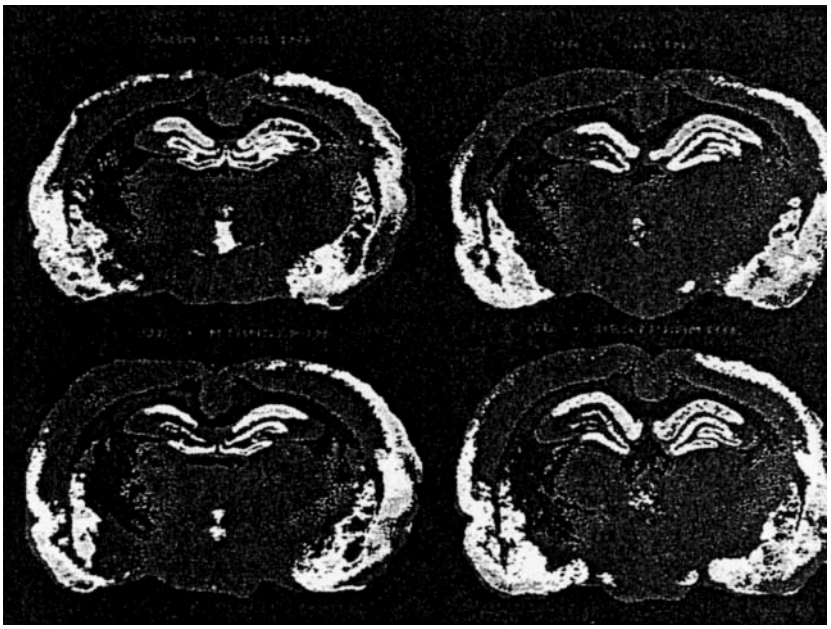

Plate 2. Pseudocoloured images of [^{3}H]QNB binding in brain sections from olfactory bulbectomized (OB) animals at the level of the anterior hippocampus. The top left and top right sections are taken from sham and OB saline treated rats. The bottom left and bottom right are representative sections from OB mianserin treated and OB desipramine treated rats. The colours represent the density of binding using the rainbow spectrum (from purple, low, to red, high)

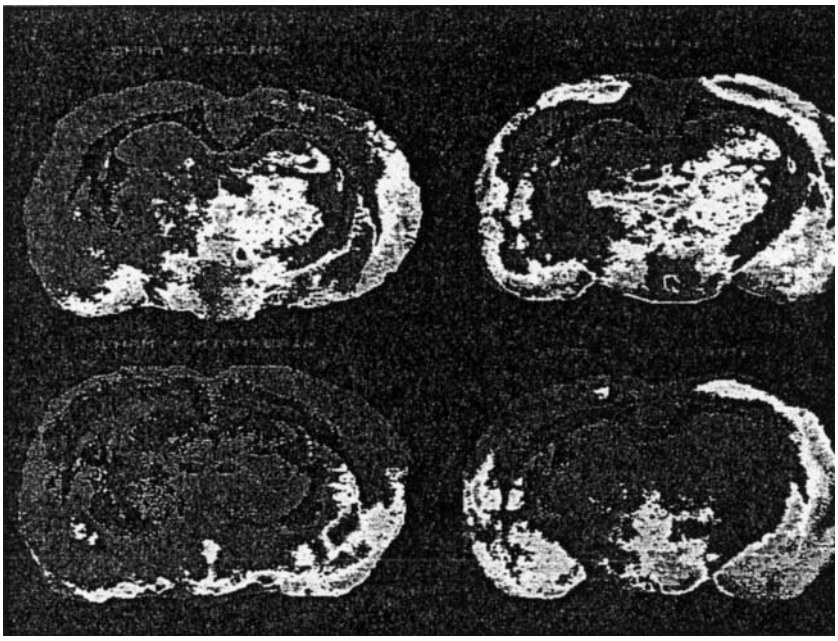

Plate 3. Pseudocoloured images of [^{3}H]Ketanserin binding in brain sections from sham animals at the level of the anterior hippocampus. The top left and top right sections are taken from sham and OB saline treated rats. The bottom left and bottom right are representative sections from sham mianserin treated and sham desipramine treated rats. The colours represent the density of binding using the rainbow spectrum (from purple, low, to red, high)

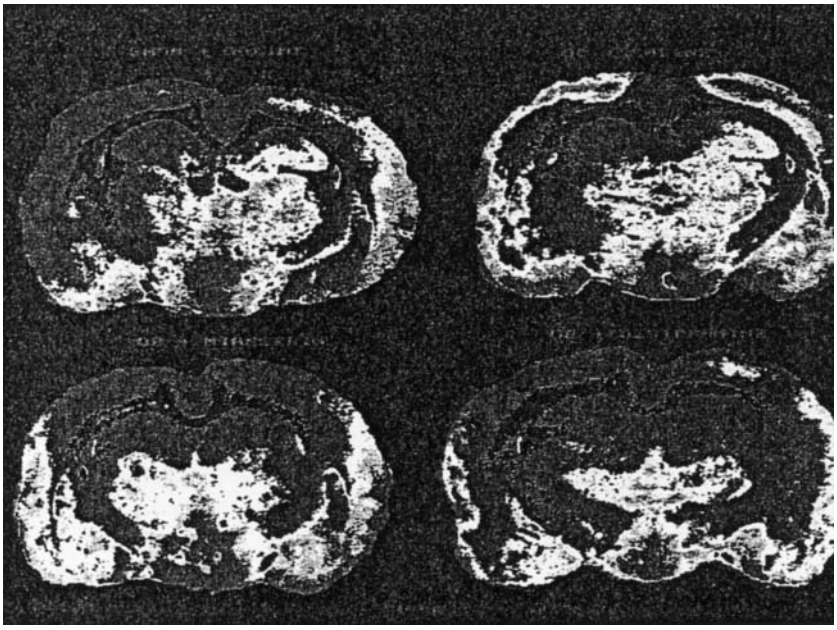

Plate 4. Pseudocoloured images of [^{3}H]Ketanserin binding in brain sections from olfactory bulbectomized (OB) animals at the level of the anterior hippocampus. The top left and top right sections are taken from sham and OB saline treated rats. The bottom left and bottom right are representative sections from OB mianserin treated and OB desipramine treated rats. The colours represent the density of binding using the rainbow spectrum (from purple, low, to red, high)

Because bulbectomy has been associated with several neurochemical changes in brain structures, it is not surprising that functions of the septo-hippocampal system are impaired. Hall and Macrides (1983) reported that olfactory bulbectomized rats showed marked deterioration in preoperatively trained behaviour in the eight-arm radial maze.

Thus, in the present study, cholinergic deafferentation may lead, by an unknown mechanism, to an up-regulation of 5-HT_2 receptors on target cells.

The relevant clinical importance of 5-HT_2 receptor binding is well known. The rate of serotonin uptake into platelets is less in depressed patients than normal controls (Toumisto *et al.*, 1979) and returns to normal following successful treatment with antidepressant drugs (Healy *et al.*, 1983). Butler *et al.* (1988b) have shown that platelets from bulbectomized rats show a deficit in serotonin transport similar to that of depressed patients and that, following chronic treatment with either desipramine or sertraline, the uptake of [^{3}H] serotonin returned to levels found in sham-operated animals.

In a study of patients with unipolar affective disorder, Biegon *et al.* (1987) have reported an increased concentration of 5-HT_2 receptors on platelet membranes which decreased to control levels following treatment with amitriptyline and trazodone.

The serotonergic innervation of the rat olfactory bulb arises mainly from dorsal and median raphe projections (McLean and Shipley, 1987). Olfactory bulbectomy therefore will disrupt the centrifugal fibres running towards the bulbs and cause retrograde degeneration.

Cairncross *et al.* (1979, 1981) showed that 5,7-dihydroxytryptamine, which selectively destroys serotonergic neurons, produced the behavioural changes similar to those present in the OB rat, and which could be reversed by chronic treatment with amitryptiline or mianserin. These results suggest that lesions of the serotonergic system play a pivotal role in producing behavioural deficits seen in the OB rat. Muramatsu *et al.* (1988) have reported that 5-HT exerts an inhibitory action on ACh release in the rat hippocampus, an effect which is mediated by the localization of 5-HT_2 receptors on cholinergic neurons derived from the medial septum. In the area of depression, several established compounds have been identified as 5-HT_2 antagonists e.g. mianserin and teciptiline (unselective) or trazodone (selective) (Pinder and Wieringa, 1993). However, the effect of 5-HT_2 agonists and antagonists on the regulation of the 5-HT_2 receptor is not fully elucidated.

In the present study, chronic antidepressant treatment caused a down regulation of 5-HT_2 receptors in sham animals and a normalization of receptor density in cortical regions of the OB animals. Roth and Ciaranello (1991) demonstrated that chronic mianserin failed to affect 5-HT_2 mRNA levels in rat brain, although it reduced the number of 5-HT_2 receptors. A possible explanation for the increase in 5-HT_2 receptor density in cortical and hippocampal regions of OB animals may be as a consequence of denervation. It has been proposed that one effect of bilateral olfactory bulbectomy is a stimulation of limbic cholinergic activity possibly due to sprouting (Gilad and Reis, 1979). It would appear from the present study that upregulation of 5-HT_2 receptors, and down regulation of muscarinic cholinergic receptors, might underlie the behavioural deficits elicited by bulbectomy. One is tempted to relate recovery in receptor function following chronic antidepressant treatment with the improvement in 'open-field' performance of OB rats. However, it would appear that more complex neurotransmitter and receptor interrelationships take place which warrants further investigation. It would be of interest to determine the effects of olfactory bulbectomy on M_1 and M_2 receptor sub-types.

REFERENCES

Biegon, A., Kargman, S., Snyder, L. and McEwen, B. (1986). Characterization and localization of serotonin receptors in human brain postmortem. *Brain Research*, **363**, 91–98.

Biegon, A., Weizman, A., Karp, L., Ram, A., Tiano, S. and Wolff, M. (1987). Serotonin 5-HT_2 receptor binding on blood platelets—a peripheral marker for depression? *Life Science*, **41**, 2485–2492.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye-binding, *Analytical Biochemistry*, **72**, 248–254.

Butler, J., Tannian, M. and Leonard, B. E. (1988a). The chronic effects of desipramine and sertraline on platelet and synaptosomal 5-HT uptake in olfactory bulbectomized rats. *Progress in Neurobiology Psychopharmacology* and *Biological Psychiatry*, **12**, 585–594.

Butler, J. and Leonard, B. E. (1988b). The platelet serotonergic system in depression and following sertraline treatment. *International Clinical Psychopharmacology*, **3**, 343–347.

Cairncross, K. D. and King, M. G. (1971). Facilitation of avoidance learning in anosmic rats by amitriptyline.

Proceedings of the Australian Physiol. Pharmac. Society, **2**, 1–2.

Cairncross, K. D., Schofield, S. P. M. and Bassett, J. R. (1975). Endogenous norepinephrine levels following bilateral olfactory bulb ablation. *Pharmacology Biochemistry and Behavior*, **3**, 425–427.

Cairncross, K. D., Cox, B., Forster, C. and Wren, A. T. (1979). Olfactory projection systems, drugs and behaviour: a review. *Psychoneuroendocrinology*, **4**, 253–272.

Cairncross, K. D., Cox, B., Forster, C. and Schnieden, H. (1981). The ability of 5·6 DHT injected into dorsal raphe projections to mimic surgical bulbectomy. *Proceedings of the Australian Society of Clinical Experimental Pharmacology*, **15**, 147.

Cowan, W. M., Raisman, G. and Powell, T. P. S. (1965). The connections of the amygdala. *Journal of Neurology and Neurosurgery Psychiatry*, **28**, 137–191.

Earley, B., Biegon, A. and Leonard, B. E. (1989). Quantitative auto-radiographic analysis of muscarinic receptors and quantitative histochemistry of acetylcholinesterase in the rat brain after trimethyltin intoxication. *Neurochemistry International*, **15**, 475–483.

Gilad, G. M. and Reis, D. J. (1979). Transneuronal effects of olfactory bulb removal on choline acetyltransferase and glutamic acid decarboxylase activities in the olfactory tubercle. *Brain Research*, **178**, 185–190.

Gray, J. A. and Lalljee, B. (1974). Sex differences in emotional behaviour in the rat: correlation between the 'open field' defaecation and active avoidance. *Animal Behaviour*, **22**, 856–861.

Gross-Isseroff, R., Salama, D., Israeli, M. and Biegon, A. (1990a). Autoradiographic analysis of [^{3}H]ketanserin binding in the human brain postmortem: effect of suicide. *Brain Research*, **507**, 208–215.

Gross-Isseroff, R., Salama, D., Israeli, M. and Biegon, A. (1990b). Autoradiographic analysis of age-dependent changes in serotonin 5-HT_2 receptors of the human brain postmortem. *Brain Research*, **519**, 223–227.

Haberly, L. B. and Price, J. L. (1978). Association and commissural fiber systems of the olfactory cortex of the rat. II. Systems originating in the olfactory peduncle. *Journal of Comprehensive Neurology*, **178**, 711–740.

Hall, R. D., and Macrides, F. (1983). Olfactory bulbectomy impairs the rat's radial maze behaviour. *Physiology and Behaviour*, **30**, 797–803.

Harvey, J. A., Scholfield, C. N., Graham, Jr., L. T. and Aprison, M. H. (1975). Putative transmitters in denervated olfactory cortex. *Journal of Neurochemistry*, **24**, 445–449.

Healy, D., Carney, P. A. and Leonard, B. E. (1983). Monamine-related markers of depression: Changes following treatment. *Journal of Psychiatric Research*, **17**, 251–260.

Hirsch, J. D. (1980). Opiate and muscarinic ligand binding in five limbic areas after bilateral olfactory bulbectomy. *Brain Research*, **198**, 271–283.

Isseroff, A. and Lancet, D. (1985). An inexpensive microcomputer-based image analysis system: novel applications to quantitative autoradiography. *Journal of Neuroscience Methods*, **12**, 265–275.

Iwasaki, K., Fujiwara, M., Shibata, S. and Ueki, S. (1986). Changes in brain catecholamine levels following olfactory bulbectomy and the effect of acute and chronic administration of desipramine in rats. *Pharmacology Biochemistry and Behaviour*, **24**, 1715–1719.

Janscar, S. and Leonard, B. E. (1981a). Effects of some antidepressants and other psychotropic drugs on the behavior of olfactory bulbectomized rats in the open field. *Irish Journal of Medical Science*, **150**, 93–94.

Janscar, S. and Leonard, B. E. (1981b). The effects of antidepressant drugs on conditioned taste aversion learning in the olfactory bulbectomized rat. *Neuropharmacology*, **20**, 1341–1345.

Janscar, S. and Leonard, B. E. (1983). The olfactory bulbectomized rat as a model of depression. In: *Frontiers in Neuropsychiatric Research*, Usdin, E., Goldstein, M., Friedhoff, A. J. and Georgotas, A. (Eds), Macmillan, New York, pp. 357–372.

King, M. G. and Cairncross, K. D. (1974). Effects of olfactory bulb section on brain noradrenaline, corticosterone and conditioning in the rat. *Pharmacology Biochemistry and Behavior*, **2**, 347–353.

Leonard, B. E. and Tuite, M. (1981). Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat. *International Review of Neurobiology*, **22**, 251–286.

McLean, J. H. and Shipley, M. T. (1987). Serotonergic afferents to the rat olfactory bulb: II. Changes in fiber distribution during development. *The Journal of Neuroscience*, **7**(10), 3029–3039.

Miczek, K. A. and Barry, H. (1976). III. Pharmacology of sex and aggression. In: *Behavioral Pharmacology*, Glick, S. D. and Goldfarb, J. (Eds), Mosby, St. Louis, pp. 176–257.

Millan, J. H., Patel, S. and Meldrum, B. S. (1986). Olfactory bulbectomy protects against pilocarpine induced motor limbic seizures in rats. *Brain Research*, **398**, 204–206.

Muramatsu, M., Tamaki-Ohashi, J., Usuki, C., Araki, H. and Aihara, H. (1988). Serotonin-2 receptor-mediated regulation of release of acetylcholine by minaprine in cholinergic nerve terminal of hippocampus of the rat. *Neuropharmacology*, **27**, 603–609.

Nakamura, K. and Nakamura, K. (1979). Changes in brain stem monoamine turnover after surgical and chemical denervation of the olfactory system. In: *Chemical Tools in Catecholamine Research*, Vol. 1, Jonsson, G., Malmfors, T. and Sachs, C. (Eds), North-Holland Publ., Amsterdam, pp. 285–290.

Neckers, L. M., Zarrow, M. X., Myers, M. M. and Denenberg, V. H. (1975). Influence of olfactory bulbectomy and the serotonergic system upon inter-male aggression and maternal behavior in the mouse. *Pharmacology Biochemistry and Behavior*, **3**, 545–550.

Paxinos, G. and Watson, C. (1986). *The Rat Brain in Stereotaxic Co-ordinates*. Academic Press, New York.

Pinder, R. M. and Wieringa, J. H. (1993). Third-generation antidepressants. *Medicinal Research Reviews*, **13**, 259–325.

Pohorecky, L. A., Larin, F. and Wurtman, R. J. (1969a). Mechanisms of changes in brain norepinephrine levels following olfactory bulb lesions. *Life Science*, **8**, 1309–1317.

Pohorecky, L. A., Zigmond, M. J., Heimer, L. and Wurtman, R. J. (1969b). Olfactory bulb removal: effects on brain norepinephrine. *Proceedings of the National Academy Science USA*, **62**, 1052–1055.

Rainbow, T. C., Biegon, A. and Bleisch, W. V. (1982a). Quantitative autoradiography of neurochemicals. *Life Science*, **30**, 1769–1774.

Rainbow, T. C., Bleisch, W. U., Biegon, A. and McEwen, B. S. (1982b). Quantitative densitometry of neurotransmitter receptors. *Journal Neuroscience Methods*, **5**, 127–138.

Roth, B. L. and Ciaranello, R. D. (1991). Chronic mianserin treatment decreases 5-HT_2 receptor binding without altering 5-HT_2 receptor mRNA levels. *European Journal of Pharmacology*, **207**, 169–172.

Sieck, M. H. and Gordon, B. L. (1972). Selective olfactory bulb lesions: reactivity changes and avoidance learning in rats. *Physiology and Behaviour*, **9**, 545–552.

Sieck, M. H., Baumback, H. D., Gordon, B. L. and Turner, J. F. (1974). Changes in spontaneous, odor modulated and shock induced behavioural patterns following direct olfactory lesions. *Physiology and Behaviour*, **13**, 427–439.

Thomas, J. B. (1973). Some behavioural effects of olfactory bulb damage in the rat. *Journal of Comprehensive Physiology and Psychology*, **83**, 140–148.

Toumisto, J., Tukianen, E. and Ahlfors, U. G. (1979). Decreased uptake of 5-hydroxytryptamine in blood platelets from patients with endogenous depression. *Psychopharmacology*, **65**, 141–147.

Van Riezen, H. and Leonard, B. E. (1990). Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacology Therapeutics*, **47**, 21–34.

Veening, J. G. (1978). Cortical afferents of the amygdaloid complex in the rat: an HRP study. *Neuroscience Letters*, **8**, 191–195.

Wenk, H., Meyer, U. and Bigl, V. (1977). Centrifugal cholinergic connections in the olfactory systems of rats. *Neuroscience*, **2**, 797–800.

Winer, B. J. (1962). *Statistical Principles in Experimental Design*. McGraw-Hill, New York.

Yamamura, H. I. and Snyder, S. H. (1974). Muscarinic cholinergic binding in rat brain. *Proceedings of the National Academy of Science USA*, **71**, 1725–1729.

Yoshimura, H., Gomita, Y. and Ueki, S. (1974). Changes in acetylcholine content in rat brain after bilateral bulbectomy in relation to mouse-killing behavior. *Pharmacology Biochemistry and Behavior*, **2**, 703–705.

Yoshimura, H. and Ueki, S. (1977). Biochemical correlates in mouse killing behavior of the rat: Prolonged isolation and brain cholinergic function. *Pharmacology Biochemistry and Behavior*, **6**, 193–196.

Yoshimura, H. (1980). Regional changes in brain cholinergic enzyme activities after bilateral olfactory bulbectomy in relation to mouse-killing behaviour by rats. *Pharmacology Biochemistry and Behavior*, **15**, 517–520.