British Journal of Pharmacology (1997) 122, 1725–1731

Influence of olfactory bulbectomy and subsequent imipramine treatment on 5-hydroxytryptaminergic presynapses in the rat frontal cortex: behavioural correlates

*Gisela Grecksch, Dan Zhou, Christian Franke, †Ulrike Schröder, †Bernhard Sabel, *Axel Becker & Gerald Huether

Psychiatric Clinic of the University of Göttingen, *Pharmacological Institute of the University of Magdeburg and †Psychological Institute of Medical Psychology of the University of Magdeburg, Germany

1 Alterations of 5-hydroxytryptaminergic mechanisms are thought to play a special role in the pathogenesis of depression and antidepressant treatments are assumed to restore these changes.

2 We have used one of the most reliable models of depression, the olfactory bulbectomized rat to study the long term consequences of this manipulation and of subchronic imipramine treatment on two parameters of 5-hydroxytryptaminergic presynapses, 5-hydroxytryptamine (5-HT) transporter density and tryptophan hydroxylase apoenzyme concentration, in the frontal cortex as well as on active avoidance learning several weeks after bulbectomy.

3 The B_{max} value of [^{3}H]-paroxetine binding and the concentration of the 5-HT synthesizing enzyme were both significantly elevated in the frontal cortex of bulbectomized rats compared to sham-operated controls.

4 Imipramine treatment, either by daily injections or by subcutaneous implantation of slow release imipramine-containing polymers reduced the elevated tryptophan hydroxylase apoenzyme levels in the frontal cortex of bulbectomized, but not of sham-operated control rats and restored the deficient learning performance of bulbectomized rats.

5 Both effects were more pronounced after continuous drug administration by imipramine-releasing polymers compared to daily i.p. injections.

6 These findings indicate that bulbectomy leads to a compensatory 5-hydroxytryptaminergic hyperinnervation of the frontal cortex. Chronic antidepressant treatment seems to attenuate the increased output of the 5-hydroxytryptaminergic projections in the frontal cortex through the destabilization of the rate limiting enzyme of 5-HT synthesis of the 5-hydroxytryptaminergic nerve endings in this brain region.

Keywords: Bulbectomy; frontal cortex; 5-HT transporter; tryptophan hydroxylase; depression; antidepressants; imipramine; active avoidance learning

Introduction

Alterations of the central 5-hydroxytryptaminergic (5-HT) system are generally believed to play a special role in the pathogenesis of depression and chronic antidepressant treatments are assumed to somehow restore these changes. This view is supported by altered CSF-levels of 5-hydroxyindole acetic acid (5-HIAA) in depressive patients (van Praag, 1984; Meltzer & Lowry, 1987), by 5-HT transporter and receptor changes in *postmortem* brain samples (Yates *et al*., 1990; Leake *et al*., 1991), by the relapse of depressive symptomatology in remitted patients after acute tryptophan-depletion (Delgado *et al*., 1990), by the rather consistent down-regulation of 5-HT_2-receptors associated with antidepressant treatments and by the antidepressant action of selective 5-HT drugs such as 5-HT_{1A} agonists or selective 5-HT reuptake inhibitors (for reviews see Briley, 1993; Blier & de Montigny, 1994). Several aspects of these neurochemical impairments and drug actions have been confirmed in numerous experimental studies with different animal models of depression (Jesberger & Richardson, 1985; Willner, 1989; Richardson, 1991), but a consistent picture of the role of the 5-HT system and its derangement in the brain of depressive patients is still elusive. This is at least partially due to the fact that many of the models employed fail to simulate the human disease conditions closely enough. Ideally, an animal model should induce significant and stable alterations in normal brain functions which are associated with measurable behavioural and neurochemical changes and which are normalized only by chronic, but not by acute, administration of clinically efficacious antidepressants (Jesberger & Richardson, 1985). The olfactory bulbectomized rat is one of the few models to which these criteria apply. Bilateral destruction of the olfactory bulbs in the rat induces a number of behavioural changes, including increased locomotor activity and deficits in active and passive avoidance tasks (Cairncross *et al*., 1979; Leonard & Tuite, 1981; van Riezen & Leonard, 1990). These behavioural impairments are rather selectively normalized by chronic not by acute administration of many different typical and atypical antidepressants (Leonard *et al*., 1989; Richardson, 1991). The time course of this behavioural normalization was found to coincide with restoration of biochemical parameters, such as decreased 5-HT_2 receptor density in frontal cortex of bulbectomized rats (Gurevic *et al*., 1993; Song & Leonard, 1994; Mudunkotuwa & Horton, 1996).

This impressive selectivity of the olfactory bulbectomy model for drugs with antidepressant actions in patients and the remarkable specificity of the action of these drugs on behavioural and biochemical parameters suggest that the analysis of the neurochemical impairments seen in olfactory bulbectomized rats, of their responses to antidepressant treatment and their relationship to the treatment related behavioural changes, may be a useful strategy to uncover the neurobiological substrates of human major depressive disorder. It is one of the most interesting features of this model that removal of the olfactory bulbs will produce not only a disruption of local

[1]Author for correspondence at: Psychiatric Clinic, University Göttingen, von Siebold-Str. 5, 37075 Göttingen, Germany.

neuronal circuitry but also initiate a sequence of secondary compensatory structural and functional alterations in brain areas which are far remote from the lesion (Jesberger & Richardson, 1988). These lesion-induced reorganization processes in limbic and cortical areas, rather than the anosmia *per se*, appear to be responsible for the secondary, late appearing behavioural abnormalities seen in bulbectomized rats (Tiffany *et al*., 1979; Jesberger & Richardson, 1988; van Riezen & Leonard, 1990). Antidepressant treatments must therefore be assumed to restore these aberrant behaviours through the long-term attenuation of the impact of this newly formed connectivity on global information processing in the brain.

Several observations suggest that an impairment of the central 5-HT system may play a special role in the behavioural changes caused by bulbectomy and that the restoration of certain impairments of the central 5-HT system is closely related to the behavioural normalization seen after chronic antidepressant treatments. Firstly, many of the biochemical and behavioural effects seen after surgical ablation of the bulbs are likewise produced by the selective destruction of its 5-hydroxytryptaminergic innervation by intrabulb injections of 5, 6-dihydroxytryptamine (Cairncross *et al*., 1977). Secondly, several behavioural changes seen in bulbectomized rats have been attributed to an imbalance between the central 5-hydroxytryptaminergic and noradrenergic systems (for review see Cairncross *et al*., 1979). Thirdly, chronic administration of selective 5-HT reuptake inhibitors has been shown to attenuate or reverse the impairments in behaviour, neurotransmitters and immune functions in bulbectomized rats (Song & Leonard, 1994). Finally, the time course of normalization of altered behavioural responsiveness in bulbectomized rats caused by chronic desipramine administration coincides with the down-regulation of 5-HT_{2A} receptors, but not of β-adrenoceptors in the frontal cortex (Mudunkotuwa & Horton, 1996).

Until now, research on this and other animal models of depression has almost exclusively been focused on changes of postsynaptic 5-HT receptors. Very little is presently known about possible alterations of presynaptic parameters of 5-HT neurotransmission. This is a serious flaw since usually such changes will precede and act as triggers for the up- or down-regulation of postsynaptic 5-HT receptors. The current lack of knowledge about the alterations of 5-HT presynaptic mechanisms imposes a serious limitation to the interpretation of postsynaptic changes of receptor densities or intracellular signal transduction mechanisms. The need of such an investigation is underlined by an increasing number of studies demonstrating an amazing degree of structural plasticity of the 5-HT innervation pattern in the adult brain (for reviews see Azmitia & Whitaker-Azmitia, 1991; Jacobs & Azmitia, 1992). In the ascending 5-HT projections originating in the pontine and midbrain raphe nuclei, extensive collaborative sprouting and regeneration phenomena have been observed after lesions all along the course of the medial forebrain bundle (Bjorklund *et al*., 1981; Ueda & Kawata, 1994). The reinnervation of projection fields after the selective destruction of 5-HT efferences is a rather slow process and may even lead to a 5-HT hyperinnervation of certain regions (Bjorklund *et al*., 1973; Zhou *et al*., 1995). Early histofluorescent studies of indoleamine accumulation in different brain regions revealed an increased indoleamine fluorescence in the olfactory tubercle and the piriform cortex 5 weeks after olfactory bulbectomy in mice (Garris *et al*., 1984). This finding, together with our recent observation of a simultaneous increase of the density of 5-HT transporters and of tryptophan hydroxylase apoenzyme concentrations in the frontal cortex of bulbectomized rats (Zhou *et al*., 1997), suggests that one of the slow, secondary events associated with the reorganization of neuronal circuits is a compensatory 5-HT hyperinnervation of anterior cortical regions.

The present study was performed in order to assess the influence of long-term antidepressant treatment on two specific parameters of 5-HT presynapses, [^{3}H]-paroxetine binding to the 5-HT transporter and tryptophan hydroxylase apoenzyme concentrations, in the frontal cortex of bulbectomized rats. These biochemical studies were combined with a behavioural analysis of the effects of a subchronic antidepressant treatment on the learning deficit of these bulbectomized rats in a one-way active avoidance task, the pole jumping test. Imipramine, one of the most thoroughly studied and most widely medicated antidepressants was chosen for this study and chronically applied either by daily injections or by subcutaneous implants of slow-release imipramine-containing polymers.

Methods

Animals

For all experiments male Wistar rats were used (Mol:Wist/Shoe-strain, Moellegard Breeding Center, Germany). The animals were housed in groups of five rats per cage under controlled laboratory conditions (temperature 20 $\pm$ 2°C, relative air humidity 55–60%, LD 12 : 12, light on at 06 h 00 min) with free access to complete standard diet (Altromin 1326) and tap water.

All animal experiments were conducted in accordance to the requirements of the National Act on the Use of Experimental Animals in Germany and approved by the 'Tierschutzkommission des Landes Sachsen-Anhalt'.

Bulbectomy

Bilateral olfactory bulbectomy was performed as described by O'Connor & Leonard (1986). Briefly, rats were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.). The animals were fixed in a stereotactic instrument and a skin incision was made to expose the skull overlying the bulbs. Two 2 mm diameter holes were drilled above the bulbs (6.5 mm anterior to bregma and 2 mm on both sides) and both holes were joined to a slotted hole. The olfactory bulbs were cut and removed by aspiration by use of a deflected pipette. The resulting spaces were filled with haemostatic sponges and the skin closed by tissue adhesive. Sham-operated rats were treated in the same way, including piercing of the dura mater but their bulbs were left intact.

The rats were bulbectomized at the age of 7 weeks (body weight 220–280 g). After seven weeks, when the behavioural experiments were performed, the body weights were 350–450 g. There were no differences in body weights between bulbectomized and control animals. At the end of the experiments, when the brains were removed for neurochemical analysis, each brain was checked to ensure that the olfactory bulbs had been completely removed and that there was no damage to the frontal cortex.

Imipramine treatment

Acute administration In order to assess the influence of acute imipramine administration on performance in the active avoidance task, imipramine was injected one hour before each training session (20 mg kg^{-1}, i.p., in saline, 10 ml kg^{-1} body weight). Control animals received saline injections

Subchronic administration The subchronic treatment with imipramine or saline was started 6–7 weeks after bulbectomy and exactly 11 days before the learning experiments. Imipramine was administered either by daily i.p. injections (3.75 mg kg^{-1} body weight in saline) or by subcutaneous slow-release implants. For this purpose, ethylene vinyl acetate copolymers containing 50% (wt/wt) imipramine (length: 40 mm, diameter: 5 mm) were prepared as described elsewhere (Sabel *et al*., 1990). These polymers released about 3.75 mg imipramine kg^{-1} body weight per day, as measured spectrophotometrically before their implantation and after their removal at the end of the treatment period of 17 days. Imipramine-containing or empty polymers were implanted under the neck skin and removed at the end of the experiment (one day after the last training session) under anaesthesia.

The following treatment groups were formed: (1) sham-operated animals with blank-polymers and daily i.p. injections of saline (Bulb-C/Sal). (2) bulbectomized animals with blank-polymers and daily i.p. injections of saline (Bulb/Sal). (3) bulbectomized animals with blank polymers and daily i.p. injections of 3.75 mg kg^{-1} imipramine (Bulb/IMI-I). (4) bulbectomized animals with polymer implants releasing 3.75 mg imipramine kg^{-1} body weight per day and daily i.p. injection of saline (Bulb/IMI-P).

All treatments were continued until the last training session. Intraperitoneal imipramine injections were given one hour before the training sessions. Injections were finished before, and polymers were removed the day after the last training session.

Learning test: pole-jumping

Seven to eight weeks after surgery and 11 days after the start of the subchronic imipramine treatment, the learning performance of the animals was tested in a one-way active avoidance task, the pole jumping test. In this test, the rat had to learn to jump on a pole to avoid electric foot-shocks. The conditioned stimulus was a sound produced by a buzzer. The unconditioned stimulus was an electric foot-shock (pulsatile direct current with a frequency of 50 Hz, 10 ms pulse-width, intensity according to the individual susceptibility, maximum 0.8 mA) delivered through stainless steel rods covering the floor. The conditioning-unconditioned stimulus interval was 4 s. One trial was limited to 20 s when an animal failed to jump on the pole within this period. Each session consisted of 10 trials and was repeated over a period of five consecutive days. All sessions were performed during the light period of the 12 h light 12 h dark cycle at about the same time. Before the first session, the rats were allowed to explore the box for 5 min, on the following days 1 min was provided for exploration. The number of escapes (instrumental reactions $>$ 4 s, reaction time $<$ 20 s) and conditioned reactions (reaction time $<$ 4 s) were recorded for further evaluation.

Sample preparation and measurements of neurochemical parameters

One week after the discontinuation of the daily imipramine injections or removal of the implanted polymers, the rats were killed by decapitation. The brains were quickly removed, frozen in liquid nitrogen and stored at $-80^{\circ}C$ until further analysed.

The frontal cortices were dissected and processed as described recently (Huether *et al*., 1997). Briefly, tissues were homogenized and centrifuged to obtain cytoplasmatic supernatant samples for measurements of tryptophan hydroxylase apoenzyme concentrations. The pellets were repeatedly resuspended and centrifuged to prepare partially purified membrane samples for measurements of 5-HT transporter densities.

The concentration of the tryptophan hydroxylase apoenzyme in the frontal cortex of the differently treated rats were measured by a double sandwich ELISA, by use of a monoclonal antibody against tryptophan hydroxylase for specific binding and a polyclonal antiserum for detection. For the assesssment of treatment-related changes in 5-HT transporter densities, the B_{max} value of [^{3}H]-paroxetine binding was determined by a highly specific ligand binding assay (Huether *et al*., 1997).

Protein concentrations in ELISA and binding samples were measured by the method of Lowry *et al*., (1951).

Data analysis and statistics

To evaluate the learning performance of the animals, the repeated measures model was applied several times to test hierarchical hypotheses. The basis of statistical decision was a significance level of $P<0.05$. The calculations were carried out by SPSS/PC software (ANOVA and MANOVA).

The affinity and capacity parameters (K_D and B_{max} values) of [^{3}H]-paroxetine binding were derived from Scatchard plots of saturation isotherms of specific binding data measured over a concentration range of 0.05 to 1.00 nM by least squares regression analysis (NCSS 5.9 software of J.L. Hintze, Kaysville, Utah, U.S.A.), and the results of tryptophan hydroxylase ELISA were calculated by Biolinx 2.0 software (Dynatech laboratories Inc., Chantilly, Virginia, U.S.A.). Data are expressed as means $\pm$ s.d. Statistically significant differences were tested by one-way ANOVA followed by *post hoc t* test.

Results

Pole-jumping test

Sham-operated rats acquired the learning task rapidly (see Figures 1 and 2). However, the learning performance of bulbectomized animals was extremely low. They did not perform the conditioned reactions (see Figures 1 and 2b), and also the acquisition of the instrumental reaction was disturbed (see Figures 1 and 2a). The acute administration of imipramine at a relatively high dose (20 mg kg^{-1}, i.p.) before each training session caused an almost complete normalization of the instrumental reactions in bulbectomized rats. However, their learning performance of the conditioned reactions was not significantly improved and remained below the levels reached by sham-operated control rats (Figure 1b). The learning performance of control animals was significantly impaired by the acute imipramine injections.

Subchronic imipramine treatment of the bulbectomized rats caused a significant improvement of their learning performance which was more pronounced if the drug was continuously adminstered by the slow release imipramine containing polymers (Figure 2). The daily i.p. injection of imipramine (3.75 mg kg^{-1}) significantly ameliorated the poor learning performance of conditioned reactions (Figure 2b), but these animals still showed a lower learning performance if compared to control animals. If the same daily dose of imipramine was administered continuously by slow-release polymers, the bulbectomy induced learning deficit was completely abolished. Both administration schedules completely restored the instrumental reaction (see Figure 2a).

[^{3}H]-paroxetine binding and tryptophan hydroxylase apoenzyme assays

Specific binding of [^{3}H]-paroxetine to membrane preparations was saturable and of high affinity. At equilibrium, specific binding were presented by about 80% of total binding at a [^{3}H]-paroxetine concentration of 1.0 nM. The saturation curves were better fitted by a one-site rather than a two-site model, with Hill coefficients (n_H) very close to 1. Scatchard transformation of the binding data gave a single straight unbroken line, indicating a single apparent class of binding sites with no evidence of cooperation.

The K_D values of [^{3}H]-paroxetine binding to membrane preparations of the frontal cortex showed no difference between sham-operated and bulbectomized rats at 8 weeks after surgery. However, the density of 5-HT transporters, as indicated by the increased B_{max} values of [^{3}H]-paroxetine binding in the frontal cortex, was significantly higher in the bulbectomized compared to sham-operated control animals (Table 1). Subchronic imipramine treatment for 2 weeks, either by daily i.p. injections or by imipramine slow-release polymer implants had no effect of K_D values of [^{3}H]-paroxetine binding. Also, the B_{max} values measured in sham-operated control animals and the elevated B_{max} values seen in bulbectomized rats were not altered by the imipramine treatment (Table 1).

The double sandwich ELISA procedure used for measurements of tryptophan hydroxylase apoenzyme concentrations in supernatants of brain homogenates, employed a specific

monoclonal antibody, was very sensitive (detection limit: 0.1 ng/well) and gave highly reproducible results (intraassay variance: < 5%, interassay variance: < 10%). In the frontal cortex of bulbectomized rats, a significant increase in the concentrations of tryptophan hydroxylase apoenzyme was observed (Table 2). In sham-operated control rats, subchronic imipramine treatment, either by daily injections or by implants, had no effect on this parameter. In bulbectomized rats, a significant reduction of the elevated tryptophan hydroxylase levels in the frontal cortex was noticed. This decline was somewhat more pronounced after the implantation of slow-release imipramine containing polymers compared to the daily administration by i.p. injections (Table 2).

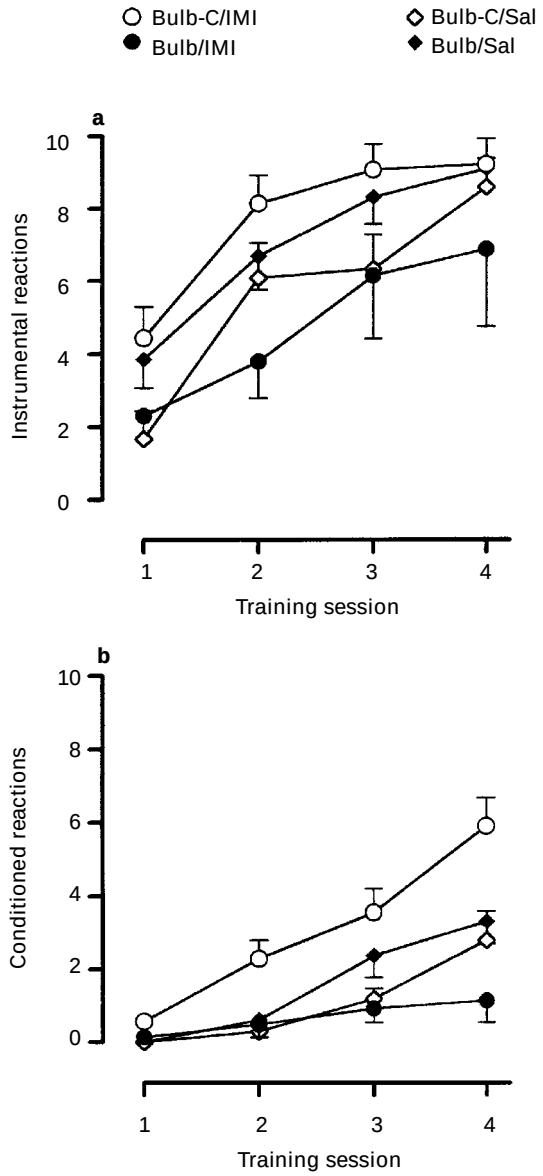

Figure 1 Effect of acute imipramine injections before each training session on pole jumping learning in four training sessions of bulbectomized and sham-operated rats. Experimental groups: Bulb-C/Sal = sham-operated control animals, saline injections ($n = 14$); Bulb/Sal = bulbectomy group, saline injections ($n = 14$); Bulb-C/IMI = sham operated control animals, imipramine injections of 20 mg kg^{-1} ($n = 12$); Bulb/IMI)-bulbectomy group, imipramine injections of 20 mg kg^{-1} ($n = 13$). (a) Instrumental reactions: Bulb/Sal versus Bulb-C/Sal F (1,26) = 14.20, $P < 0.001$; Bulb/Sal versus Bulb/IMI F (1,25) = 9.94, $P < 0.036$. (b) Conditioned reactions: Bulb/Sal versus Bulb-C/Sal F (1,26) = 14.20, $P < 0.001$; Bulb-C/Sal versus Bulb-C/IMI F (1,24) = 5.36, $P < 0.025$; Bulb/Sal versus Bulb/IMI F (1,25) = 3.23, NS; Bulb-C/Sal versus Bulb/IMI F (1,25) = 5.47, $P < 0.028$. Each point represents the mean and vertical lines indicate s.e.mean.

Discussion

Compared to other animal models, the olfactory bulbectomized rat appears to be a particularly attractive model for studying the neurobiological basis of depression and the mechanisms of action of antidepressant drugs. Bulbectomy produces stable impairments of neurochemical and

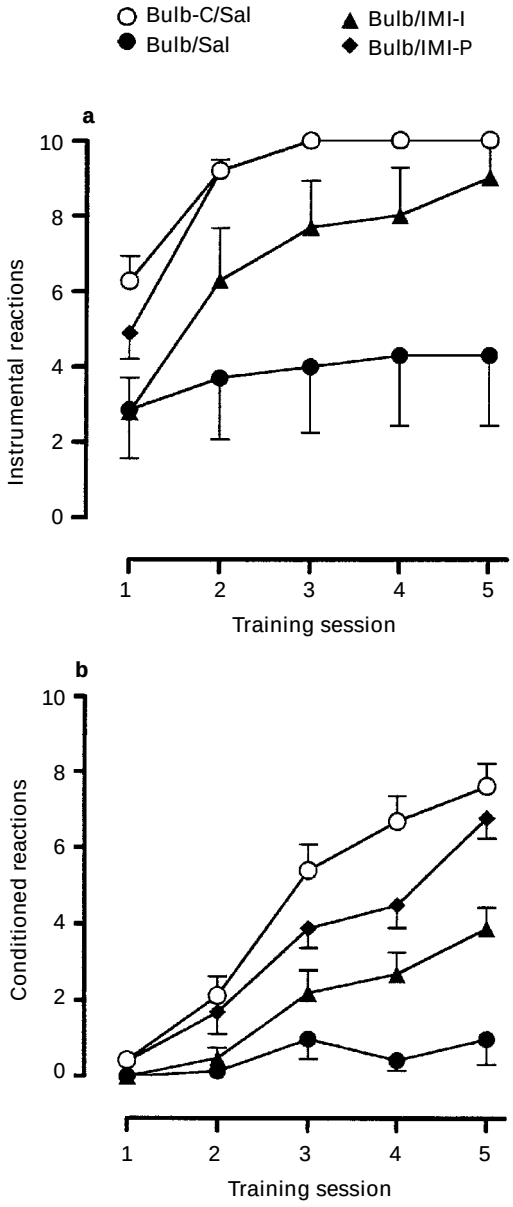

Figure 2 Effects of subchronic imipramine administration on pole jumping learning in five training sessions of bulbectomized and sham-operated control rats. Experimental groups: Bulb-C/Sal = sham-operated control rats, saline injections, blanck polymers ($n = 14$); Bulb/Sal = bulbectomized rats, saline injections, blanck polymers ($n = 7$); Bulb/IMI-I = bulbectomized rats, subchronic daily injections of 3.75 mg kg^{-1} imipramine, implantation of blanck polymers ($n = 10$); Bulb/IMI-P = bulbectomized rats, implantation of imipramine releasing polymers (3.75 mg kg^{-1} per day), daily saline injections ($n = 10$). (a) Instrumental reactions: Bulb/Sal versus Bulb-C/Sal F (1,19) = 17.02, $P < 0.001$; Bulb/Sal versus Bulb/IMI-P P (1,15) = 10.99, $P < 0.005$; Bulb-C/Sal versus Bulb/IMI-P F (1,22) = 1.11, NS; Bulb-C/Sal versus Bulb/IMI-I F (1,22) = 6.8, $P < 0.016$. (b) Conditioned reactions: Bulb/Sal versus Bulb-C/Sal F (1,19) = 32.05, $P < 0.001$; Bulb/Sal versus Bulb/IMI-I F (1,15) = 12.06; $P < 0.003$; Bulb/Sal versus Bulb/IMI-P F (1,15) = 36.43, $P < 0.001$; Bulb-C/Sal versus Bulb/IMI-I F (1,22) = 20.05, $P < 0.001$; Bulb-C/Sal versus Bulb/IMI-P F (1,22) = 2.67, NS; Bulb/IMI-I versus Bulb/IMI-P F (1,18) = 15.31, $P < 0.001$. Each point represents the mean and vertical lines indicate s.e.mean.

Table 1 Effect of chronic imipramine treatment on the density of 5-HT transporters and on tryptophan hydroxylase apoenzyme concentration in the frontal cortex of bulbectomized rats

Treatment group	*B_{max} value of [^{3}H]-paroxetine binding* (fmol mg^{-1} protein)	*Tryptophan hydroxylase apoenzyme concentration* (ng mg^{-1} protein)
Bulb-C/Sal	1109.0 ± 170.5	2.86 ± 0.37
Bulb-/Sal	1430.2 ± 287.8	4.07 ± 0.19
Bulb/IMI-I	1388.2 ± 170.9	3.46 ± 0.43
Bulb/IMI-P	1362.4 ± 122.6	2.85 ± 0.31

Treatment groups: Bulb = bulbectomy group, Bulb C = sham-operated control rats, IMI-I = subchronic daily injections of imipramine in a dose of 3.75 mg kg^{-1} and implantation of blank polymers, IMI-P = implantation of imipramine releasing polymers, 3.75 mg kg^{-1} per day, and daily saline injections, and Sal = daily saline injection. Values represent the mean ± s.d. of 6 rats per group. The densities of [^{3}H]-paroxetine binding sites were measured by ligand binding assays performed in triplicate as described in Methods. The significance of the differences was investigated by one-way ANOVA followed by *post hoc t* test. Significantly different values of the density of 5-HT transporter were found in the frontal cortex between Bulb-C and Bulb groups ($P < 0.05$). Imipramine treatment had no effect on this parameter. Significantly different values of tryptophan hydroxylase apoenzyme concentration were found between Bulb-C/Sal and Bulb/Sal ($P < 0.01$), between Bulb/Sal Bulb/IMI-I ($P < 0.05$), as well as between Bulb/Sal and Bulb/IMI-P groups ($P < 0.01$).

neuroendocrine mechanisms which are associated with measurable behavioural changes and which show striking similarities to disturbances observed in people with major depression (Jesberger & Richardson, 1986; Leonard, *et al*. 1989; Richardson, 1991). Bulbectomized animals display a remarkable selectivity to drugs with antidepressant activity in man (Richardson, 1991). Most of the bulbectomy-induced symptoms are alleviated by chronic but not by acute antidepressant treatment, and the time course of the actions of antidepressants in bulbectomized animals is reasonably similar to the time course of their clinical actions in depressive patients (Broekkamp *et al*., 1980; Joly & Sander, 1986).

The results of our behavioural studies in bulbectomized and imipramine-treated rats confirm the findings of other groups, showing that bulbectomy leads to severe impairments in several learning tasks which are not reserved by acute but almost completely normalized following long-term treatment with different typical and atypical antidepressant drugs (for reviews see Leonard & Tuite, 1981; Jesberger & Richardson, 1986; Leonard *et al*., 1989). In the present study, the learning deficit was validated by the pole-jumping test. Interestingly, the continuous administration of imipramine via slow release polymer implants was more effective in correcting this deficit than the daily administration by i.p. injections. Likewise, also the continuous administration of antidepressants to severely depressed patients via permanent i.v. catheters was shown to cause greater improvements in depression scores at lower dosages, and therefore with fewer side effects, if compared to daily oral intake (Adler *et al*., 1997).

Several attempts have been made by other groups to relate the behavioural alterations and their normalization by antidepressant treatments to alterations at the level of transmitter metabolism and receptor expression in the brain of bulbectomized rats. Apart from some isolated studies on alterations of γ-aminobutyric acid (GABA)ergic (Jancsar & Leonard, 1984) and cholinergic (Broekkamp *et al*., 1986) mechanisms, the majority of changes was found in the noradrenergic and the 5-HT systems. Interestingly, the alterations were restricted to certain brain regions, such as the amygdala, hippocampus hypothalamus, or cerebral cortex. Many of these changes, e.g. the increased density of β-adrenoceptors in the hippocampus (Jesberger & Richardson, 1986), the up-regulated 5-HT$_2$-receptor density in the frontal cortex (Gurevich *et al*., 1993) or regional changes of 5-HT and noradrenaline contents as well as their metabolites (for review see Song & Leonard, 1995) were found to be reversed by chronic antidepressant treatments.

Only recently, the first attempts were made to relate directly to the time course of such neurochemical changes to the behavioural changes seen in bulbectomized and antidepressant treated animals. The behavioural normalization of desipramine-treated bulbectomized rats was found to coincide with the down-regulation of 5-HT$_2$-receptors in the frontal cortex but not with the down-regulation of β-adrenoceptors in this or any other brain region (Mudunkotuwa & Horton, 1996). This finding, in conjunction with our previous demonstrations of an elevated 5-HT transporter density, tryptophan hydroxylase apoenzyme concentration and higher 5-HIAA/5-HT-ratio in the frontal cortex (and no other brain regions) of bulbectomized rats (Zhou *et al*., 1997), suggests that a local and specific alteration of 5-HT neurotransmission in the frontal cortex may be related to the long-lasting changes in behavioural responsiveness caused by bulbectomy. The striking parallelism seen in the present study between the effectiveness of the two different schedules of imipramine treatment employed, on the normalization of active avoidance learning and the decline of tryptophan hydroxylase levels in the frontal cortex, supports the view that the behavioural normalization of antidepressant treated bulbectomized rats is related to a restoration of aberrant 5-HT mechanisms in the frontal cortex.

Most likely the concomitant elevation of all three specific markers of 5-HT presynapses indicates an increased 5-HT innervation density in the frontal cortex of bulbectomized rats. Due to the predominantly inhibitory, constraining effects of the 5-HT afferences on cortical information processing (Jacobs & Azmitia, 1992; Spoont, 1992), such an increased 5-HT innervation density must be expected to suppress the general neuronal activity, and therefore the metabolic rate and blood flow in frontal cortical areas. In bulbectomized rats, no information about alterations of these parameters is presently available. However, it is an interesting corollary that a selective diminution of local blood flow and cerebral metabolic rate in anterior cortical areas are among the most consistent findings of PET and SPECT studies in depressive patients (Bench *et al*., 1993; Drevets *et al*., 1994). It has also been found that this frontal hypoperfusion is restored by antidepressant treatment (Mayberg *et al*., 1994).

In our animal model, subchronic imipramine treatment reduced the bulbectomy-induced elevation of tryptophan hydroxylase in the frontal cortex, but had no effect on the level of this enzyme in the frontal cortex, of sham-operated controls. The selective action of subchronic imipramine treatment on the level of expression of the 5-HT synthesizing enzyme only in the 5-HT hyperinnervated, but not on the normally innervated, frontal cortex is the most interesting finding of this investigation. If a local imbalance in 5-HT output between functionally different brain regions causes an impaired balance in global neuronal activity patterns between such regions (and, consequently, characteristic alterations in behavioral reactivity), an effective treatment should act to restore this secondarily disturbed balance through the restoration of the original balance of the regional 5-HT output patterns. Such an effect may be achieved by different means. Drugs which act to down-regulate (tricyclic antidepressants) or to block 5-HT$_2$-receptors (mianserin) will automatically have a greater influence on 5-HT-mediated effects in regions where these receptors are most abundantly expressed, i.e. the frontal cortex in rats (Leysen *et al*., 1982; Peroutka & Snyder, 1983). The local down-regulation of tryptophan hydroxylase in the frontal cortex of bulbectomized rats by chronic imipramine treatment suggests the

existence of an additional or alternative mechanism by which a disturbed balance in 5-HT output patterns may be restored, namely by a reduced synthesis, and therefore release, of 5-HT by 5-hydroxytryptaminergic presynapses. Since chronic antidepressant treatments do not affect the m-RNA content of this enzyme (Spurlock *et al*., 1994), and since the down-regulation of this enzyme is only seen in the frontal cortex of bulbectomized rats, a global influence of the imipramine treatment on the expression of this enzyme is not very likely. Rather, the subchronic imipramine treatment must be assumed to compromise the stability and to shorten the half-life of the enzyme selectively in 5-HT presynapses in the frontal cortex of bulbectomized rats. This strange effect may have a rather simple explanation, if we consider that this cytoplasmatic enzyme is particularly vulnerable to proteolytic damage and that it is normally protected by its substrate, tryptophan (Neckers *et al*., 1977; Kuhn *et al*., 1980; Vitto & Mandell, 1981). The drug-induced blockade of 5-HT reuptake depletes presynaptic 5-HT stores and causes a massive stimulation of 5-HT synthesis. The intrasynaptic availability of tryptophan declines and the stability of the enzyme becomes increasingly governed by the supply of tryptophan from external sources, i.e. the blood. Poorly perfused brain regions (the 5-HT hyperinnervated frontal cortex of bulbectomized rats or anterior cortical regions of depressive patients) are less efficiently supplied by tryptophan and, therefore, the enzyme is less efficiently protected against proteolytic degradation. As the levels of tryptophan hydroxylase fall, less 5-HT is released and consequently the regional imbalance in 5-HT output patterns is gradually restored and, therefore, the behavioural disturbance is normalized.

Further studies are needed in order to assess the validity of these assumptions. However, the heuristic value of this concept is quite remarkable. It may explain some unexpected clinical observations, e.g. that tryptophan availability has long been noticed to be a critical factor in the aetiology of depression and in the responsiveness to (certain) antidepressant treatments (Moller, 1987; Cowen *et al*., 1987), and that successfully treated remitted patients are extremely vulnerable to a sudden decline of their tryptophan supply (Delgado *et al*., 1994). Our demonstration that both bulbectomy and the subsequent antidepressant treatment affect presynaptic mechanisms, involved in the regulation of 5-HT output in the frontal cortex, raises serious concerns over the interpretation of many earlier findings of alterations of postsynaptic 5-HT receptor densities in this and other animal models of depression (Gurevich *et al*., 1993; Berendsen, 1995; Mudunkotuwa & Horton, 1996). It is possible that such postsynaptic 5-HT receptor changes are triggered by an altered presynaptic 5-HT output and therefore are of a secondary nature. Finally, even though the observed changes in 5-hydroxytryptaminergic mechanisms appear to be closely associated with the learning deficit in bulbectomized rats and its reversal by subchronic antidepressant treatment, other transmitter systems may be affected as well. In particular, the possible changes in noradrenergic mechanisms are of interest in this respect and deserve a more thorough investigation in this animal model of depression.

This research was supported by a grant of the Deutsche Forschungsgemeinschaft (Hu 351/9-2) and of Eli-Lilly Int. Fdn.

References

ADLER, L., LEHMANN, K., KUNERT, H.-J., HOFFMANN, G., ISSINGER, J., BÖKE, J., HUETHER, G. & RÜTHER, E. (1997). On the problems of switching from intravenous to oral administration in drug treatment of endogenous depressen. A placebo-controlled double-blind trial with doxepin. *Pharmacopsychiatry*, **30,** 1–8.

AZMITIA, E.C. & WHITAKER-AZMITIA, P.M. (1991). Awakening the sleeping giant: anatomy and plasticity of the brain serotonergic system. *J. Clin. Psychia.*, **52,** 4–16.

BENCH, C.J., FRISTON, K.J., BROWN, R.G., FRACKOWIAK, R.S. & DOLAN, R.J. (1993). Regional cerebral blood flow in depression measured by positron emission tomography, the relationship with clinical dimensions. *Psychol. Med.*, **23,** 579–590.

BERENDSEN, H.H.G. (1995). Interactions between 5-HT receptor subtypes: Is a disturbed receptor balance contributing to the symptomatology of depression in humans. *Pharmacol. Ther.*, **66,** 17–37.

BJORKLUND, A., NOBIN, A., & STENEVI, U. (1973). Regeneration of central serotonin neurons after axonal degeneration induced by 5,6-dihydroxytryptoamine. *Brain Res.* , **50,** 214–220.

BJORKLUND, A., WIKLUND, L., & DESCARRIES, L. (1981). Regeneration and plasticity of central serotonergic neurons: A review. *J. Physiol.*, **77,** 247–255.

BLIER, P. & DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci.*, **15,** 220–226.

BRILEY, C. (1993). Neurobiological mechanisms involved in antidepressant therapies. *Clin. Neuropharmacol.*, **16,** 387–400.

BROEKKAMP, C.L., GARRIGUO, D. & LLOYD, K.G. (1980). Serotonin-mimetic and antidepressant drugs on passive avoidance learning by olfactory bulbectomized rats. *Pharmacol. Biochem. Behav.*, **13,** 643–646.

BROEKKAMP, C.L., O'CONNOR, W.T., TONNAER, J.A.D.M., RLIK, H.W. & VAN DELFT, A.M.L. (1986). Corticosteron, cholineacetyl transferase and noradrenaline levels in olfactory bulbectomized rats in relation to changes in passive avoidence acquisition and open field activity. *Physiol. Behav.*, **37,** 429–434.

CAIRNCROSS, K.D., COX, B., FORSTER, C., & WREN, A. (1977). The ability of local injection of 6-OHDA, 5,6-DHT and 5,7-DHT into the olfactory bulbs, to mimic the effects of bilateral bulbectomy in the rat. *Br. J. Pharmacol.*, **61,** 145P–146P.

CAIRNCROSS, K.D., COX, B., FORSTER, C. & WREN, A.F. (1979). Olfactory projection systems, drugs and behavior: A review. *Psychoneuroendocrinology*, **4,** 253–272.

COWEN, P.J., PARRY-BILLINGS, M. & NEWSHOLME, E.A. (1989). Decreased plasma tryptophan levels in major depression. *J. Aff. Dis.*, **16,** 27–31.

DELGADO, P.L., CHARNEY, D.S., PRICE, L.H., AGHAJANIAN, G.K., LANDIS, H. & HENINGER, G.R. (1990). Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch. Gen. Psychiat.*, **47,** 411–418.

DELGADO, P.L., PRICE, L.H., MILLER, H.L., SALOMON, R.M., AGHAJANIAN, G.K., HENINGER, G.R. & CHARNEY, D.S. (1994). Serotonin and the neurobiology of depression. Effects of tryptophan depletion in drug-free depressed patients. *Arch. Gen. Psychiat.*, **51,** 865–874.

DREVETS, W.C., VIDEEN, T.O., PRICE, J.L., PRESKOM, S.H., CARMICHAEL, S.T. & RAICHLE, M.E. (1994). A functional anatomical study of unipolar depression. *J. Neurosci.*, **12,** 3628–3641.

GARRIS, D.R., CHAMBERLAIN, J.K. & DA VANZO, J.P. (1984). Histofluorescent identification of indoleamine-concentrating brain loci associated with intraspecies, reflexive biting and locomotor behavior in olfactory-bulbectomized mice. *Brain Res.*, **294,** 385–389.

GUREVICH, E.V., ALEKSANDROVA, I.A., OTMAKHOVA, N.A., KATKOV, Y.A., NESTEROVA, I.V. & BOBKOVA, N. (1993). Effects of bulbectomy and subsequent antidepressant treatment on brain 5-HT_2 and 5-HT_{1A} receptors in mice. *Pharmacol. Biochem. Behav.*, **45,** 65–70.

HUETHER, G., ZHOU, D. & RÜTHER, E. (1997). Long-term modulation of presynaptic 5-HT output; experimentally induced changes in cortical 5-HT transporter density, tryptophan hydroxylase content and 5-HT innervation density. *J. Neural Transm.*, (in press).

JACOBS, B.L. & AZMITIA, E.C. (1992). Structure and function of the brain serotonin system. *Physiol. Rev.*, **72,** 165–216.

JANCSAR, S. & LEONARD, B.E. (1984). Changes in neurotransmitter metabolism following olfactory bulbectomy in the rat. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **8,** 263–269.

JESBERGER, J.A. & RICHARDSON, J.S. (1985). Animal models of depression: parallels and correlates to severe depression in humans. *Biol. Psychiat*., **20,** 764–784.

JESBERGER, J.A. & RICHARDSON, J.S. (1986). Differential effects of antidepressant drugs on [^{3}H]-dihydroalprenolol and [^{3}H]-imipramine ligand recognition sites in olfactory bulbectomized and sham-lesioned rats. *Gen. Pharmacol*., **17,** 293–307.

JESBERGER, J.A. & RICHARDSON, J.S. (1986). Effect of antidepressant drugs on the behavior of olfactory bulbectomized and sham-operated rats. *Behav. Neurosci*., **100,** 256–274.

JESBERGER, J.A. & RICHARDSON, J.S. (1988). Brain output dysregulation induced by olfactory bulbectomy: an approximation in the rat of major depressive disorder in humans? *Intern. J. Neurosci*., **38,** 241–265.

JOLY, D. & SANDER, A.J. (1986). The effect of fluoxetine and zimelidine on the behavior of olfactory bulbectomized rats. *Pharmacol. Biochem. Behav*., **24,** 199–204.

KUHN, D.M., RUSKIN, B., & LOVENBERG, W. (1980). Tryptophan hydroxylase. *J. Biol. Chem*., **255,** 4137–4143.

LEAKE, A., FAIRBAIRN, A.F., McKEITH, I.G. & FERRIER, I.N. (1991). Studies on the serotonin uptake binding sit in major depressive disorder and control post-mortem brain: neurochemical and clinical correlates. *Psychiat. Res*., **39,** 155–156.

LEONARD, B.E. & TUITE, M. (1981). Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat. *Int. Rev. Neurobiol*., **22,** 251–286.

LEONARD, B.E., BUTLER, J., O'NEILL, B. & O'CONNOR, W.T. (1989). Bilaterially olfactory bulbectomized rat model of depression. In *New Directions in Affective Disorders*. ed. Lerer, B. & Gershon, S. pp. 67–70. New York: Springer-Verlag.

LEYSEN, J.E., NIEMEGEERS, J.E., VANNUETEN, J.M. & B. LADURON, P.M. (1982). [^{3}H] Ketanserin, a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution, and functional role. *Mol. Pharmacol*., **21,** 301–314.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., **193,** 265–275.

MAYBERG, H.S., LEWIS P.J., REGENOLD, W. & WAGNER, H.N.J.R. (1994). Paralimbic hypoperfusion in unipolar depression. *J. Nucl. Med*., **35,** 929–934.

MELTZER, H.Y. & LOWRY, M.T. (1987). The serotonin hypothesis of depression. In *Psychopharmacology: The Third Generation of Progress*. ed. Meltzer, H.Y. pp. 513–526. New York: Raven Press.

MØLLER, S. (1987). Tryptophan and tyrosine ratios to neutral amino acids in depressed patients in regard to K_m: relation to efficacy of antidepressant treatments. In *Amino Acid Availability and Brain Function in Health and Disease*., ed. Huether, G. pp. 355–362. NATO ASI-Series H, Vol. 20, Springer-Verlag.

MUDUNKOTUWA, N.T. & HORTON, R.W. (1996). Desipramine administration in the olfactory bulbectomized rat: changes in brain β-adrenoceptor and 5-HT$_{2A}$ binding sites and their relationship to behavior. *Br. J. Pharmacol*., **117,** 1481–1486.

NECKERS, L.M., BIGGIO, G., MOJA, E. & MEEK, L. (1977). Modulation of brain tryptophan hydroxylase activity by brain tryptophan content. *J. Pharmacol. Exp. Ther*., **201,** 110–116.

O'CONNOR, W.T. & LEONARD, B.E. (1986). Effect of chronic administration of the 6-aza analogue of mianserin (Org. 3770) and its enantiomers on behaviour and changes in noradrenaline metabolism of olfactory bulbectomized rats in the 'open field' apparatus. *Neuropharmacology*, **25,** 267–270.

PEROUTKA, S.J. & SNYDER, S.H. (1983). Multiple serotonin receptors and their physiological significance. *Fedn. Proc*., **42,** 213–217.

RICHARDSON, J.S. (1991). Animal models of depression reflect changing views on the essence and etiology of depressive disorders in humans. *Prog. Neuro-Psychopharmacol. Biol. Psychiat*., **15,** 199–204.

SABEL, B.A., DOMINIAK, P., HAEUSER, W., DURING, M.A. & FREESE, A. (1990). Levodopa delivery from controlled-release polymer matrix: delivery for more than 600 days in vitro and 225 days for elevated plasma levels after subcutaneous implantation in rats. *J. Pharmacol. Exp. Ther*., **255,** 914–922.

SONG, C. & LEONARD, B.E. (1994). Serotonin reuptake inhibitors reverse the impairments in behaviour, neurotransmitter and immune functions in the olfactory bulbectomized rat. *Human Psychopharmacol*., **9,** 135–146.

SONG C. & LEONARD, B.E. (1995). The effect of olfactory bulbectomy in the rat, alone or in combination with antidepressants and endogenous factors, on immune function. *Human Psychopharmacol*., **10,** 7–18.

SPOONT, M.R. (1992). Modulatory role of serotonin in neural information processing: implications for human psychopathology. *Psychol. Bull*., **112,** 330–350.

SPURLOCK, BUCKLAND, P., O'DONOVAN, M. & MCGUFFIN, P. (1994). Lack of effect of antidepressant drugs on the levels of m-RNA encoding serotonergic receptors synthetic enzymes and 5-HT-transporter. *Neuropharmacology*, **33,** 433–440.

TIFFANY, P.B., MOLLENAUER, S., PLOTNIK, R. & WHITE, M. (1979). Olfactory bulbectomy: emotional behaviour and defence responses in the rat. *Physiol. Behav*., **22,** 311–317.

UEDA, S., & KAWATA, M. (1994). Regeneration of serotonergic immunoreactive fibers in the brain of 5,6-dihydroxytryptamine treated rat. *J. Hirnforsch*., **35,** 159–180.

VAN PRAAG, H.M. (1984). Depression, suicide, and serotonin metabolism in the brain. In *Neurobiology of Mood Disorders*, Vol. 1. ed. Post, R.M. & Ballenger, J.C. pp. 601–618. Baltimore: Williams & Wilkins.

VAN RIEZEN, H., & LEONARD, B.E. (1990). Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol. Ther*., **74,** 21–34.

VITTO, A., & MANDELL, A.J. (1981). Stability properties of activated tryptophan hydroxylase from rat midbrain. *J. Neurochem*., **37,** 601–607.

WILLNER, P. (1989). Animal models of depression: a review. *Pharmacol. Ther*., **45,** 425–455.

YATES, M., LEAKE, J. & FERRIER, I.N. (1990). 5-HT$_2$ receptor changes in major depression. *Biol. Psychiat*., **27,** 489–496.

ZHOU, F.C., AZMITIA, E.C. & BLEDSOE, S. (1995). Rapid serotonergic fiber sprouting in response to ibotenic acid lesion in the seriatum and hippocampus. *Dev. Brain Res*., **84,** 85–98.

ZHOU, D., GRECKSCH, G., BECKER, A., FRANKE, C., PILZ, J. & HUETHER, G. (1997). Serotonergic hyperinnervation of the frontal cortex in animal model of depression, the bulbectomized rat. *Eur. Arch. Clin. Neurosci*. (in press).

(Received May 6, 1997
Revised September 2, 1997
Accepted September 9, 1997)