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The effect of serotonin depletion on motor activity habituation, and [^{3}H]muscimol binding in the rat hippocampus

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Abstract

The effect of serotonin depletion (*p*-chlorophenylalanine pretreatment) on habituation of exploratory motor activity, and on cortical and hippocampal [^{3}H]muscimol binding in vitro, was examined in rats. It appeared that the very strong decrease in serotonin concentration abolished motor habituation in the open field and decreased [^{3}H]muscimol binding to cortical and hippocampal brain slices. The $GABA_A$ receptor down-regulation was due to a decrease in the apparent affinity of the radioligand for the receptors. *p*-Chlorophenylalanine-induced biochemical changes were selective and most probably secondary to serotonin depletion, as the serotonin synthesis inhibitor did not displace [^{3}H]muscimol from its binding sites in neural membranes taken from the occipital cortex. It is concluded that there is a functional interaction between brain serotonin and GABA (γ-aminobutyric acid) systems, both at behavioral and biochemical levels, that is involved in the motor activity habituation process.

Keywords: *p*-Chlorophenylalanine; [^{3}H]Muscimol binding; Autoradiography; Locomotor activity; Habituation; Hippocampus, rat

1. Introduction

It has been suggested that novel stimuli exert a bidirectional influence on animal behavior. Novelty can be stressful, particularly when it is inescapable, and simultaneously rewarding (Bardo et al., 1996). Both phenomena, stress reaction and a novelty-induced arousal, are interrelated, as shown by the self-administration of corticosterone in rats (Dellu et al., 1996). The involvement of emotions and some neurotransmitter systems in these processes is evidenced by the fact that diazepam attenuates novelty-induced dopamine release in the medial prefrontal cortex of rats (Feenstra et al., 1995). Benzodiazepines and other anxiolytic drugs are also known to decrease the suppressive influence of novelty on behavior of rodents in the open field, elevated plus maze, and the black and white two compartment box (Sanchez, 1995; Stefański et al., 1993). Similar behavioral effects are seen after repeated exposure of animals to the same experimental conditions, i.e., when the degree of novelty declines on repeated daily testing in the same environment. It is noteworthy, that habituation, called desensitization or implosive behavioral therapy, is used also in the clinic for the treatment of phobias (Goldenberg, 1977; O'Leary and Wilson, 1987).

Given these facts, the aim of the present experiment was to describe and analyze the changes in motor activity of unhabituated rats subjected to repeated daily testing in the open field test. The behavior of control animals was compared with that of serotonin-lesioned rats for the following reasons: (i) it is known that serotonin depletion interferes with the processes of stimulus control (Soubrie, 1986), (ii) behavioral and physiological challenges produce, in a manner proportional to their arousing potencies, increases in extracellular serotonin levels in the rat forebrain (Rueter and Jacobs, 1996), (iii) inhibition of serotonergic neurons activity by benzodiazepines or $5\text{-}HT_{1A}$ receptor agonists is suggested to play a role in the anti-

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neophobic effects of these drugs (Stefański et al., 1993; Płaźnik et al., 1994b). Also, changes in [^{3}H]muscimol binding have been studied in vitro and with autoradiography in several brain structures, including the hippocampus and occipital cortex of the control and serotonin-depleted animals. The role of the interaction between GABA (γ-aminobutyric acid) and serotonergic systems in the control of emotional behavior has been studied intensively over the last 2 decades. There are immunohistochemical and autoradiographic data indicating that GABA and serotonin can coexist in the same neurons (Harandi et al., 1987; Nanopoulos et al., 1982), and serotonergic axonal terminals directly contact hippocampal GABA interneurons (Freund et al., 1990; Halasy et al., 1992). It has been recently found with in situ hybridization and immunocytochemistry that more than 90% of 5-HT$_3$ receptor-expressing cells in the neocortex and hippocampus are GABAergic (Morales et al., 1996). As activation of this receptor type causes a rapid depolarization of neuronal membranes, it suggests that 5-HT can affect GABA release via 5-HT$_3$ receptors. In functional studies, it has been shown that the fear-evoking stimuli simultaneously enhance 5-HT release and decrease GABA release in the hippocampus and frontal cortex (File et al., 1993; Matsuo et al., 1996). Competitive and non-competitive GABA$_A$ receptor complex antagonists, bicuculine and picrotoxin, antagonize the anxiolytic-like effects of *p*-chlorophenylalanine and 5,7-dihydroxytryptamine-induced lesions of the serotonergic neurons in some animal models of anxiety (a modified Vogel's test and elevated plus maze), thus pointing at the GABA$_A$ receptors as the principal target of serotonergic denervation (Söderpalm and Engel, 1989, 1990, 1991). Finally, it has been recently reported that a subpopulation of very high-affinity sites for GABA is exclusively found in limbic nuclei, such as the amygdala and hippocampus (Onoe et al., 1996). Taken together, these data indicate, therefore, the possibility of the involvement of cortical and hippocampal serotonergic vs. GABA systems, in modifying the reaction of animals to acutely and repeatedly applied behavioral challenges. The present experiment was aimed at studying the parallel occurrence of adaptive changes in a behavioral response, and cortical and hippocampal GABA$_A$ receptors in serotonin-depleted animals.

2. Materials and methods

2.1. Animals

Male Wistar rats (200 ± 20 g), bought from a licensed breeder, were housed in standard laboratory conditions under a 12-h light/dark cycle (lights on at 0600 h), constant temperature (21 ± 2°C), and 70% humidity. The animals were kept in pairs in cages ($60 \times 30 \times 20$ cm), with free access to food and tap water.

2.2. Drugs and treatment

p-Chlorophenylalanine methyl ester hydrochloride (Sigma, St. Louis, MO, USA) was administered at the dose of 150 mg/kg (dissolved in 0.9% NaCl, 2 ml/kg, i.p.). Control rats received an appropriate volume of vehicle. *p*-Chlorophenylalanine or vehicle was administered to an appropriate group three times: on the 4th and the 3rd day before and on the 2nd day after the beginning of the open field tests immediately after testing of the animals on that day. The third dose of *p*-chlorophenylalanine was given to sustain changes in serotonin concentration during a 9-day long experiment. Each group contained 19–20 rats.

2.3. Open field test

The open field apparatuses used in this experiment consisted of two round arenas (80 cm diameter) with 30-cm high walls, each equipped symmetrically with three photocells mounted 80 cm apart and 4 cm above the floor level. The test was performed in a soundproof chamber under dim light and continuous white noise (65 dB) without previous habituation. General activity (number of photobeam interruptions) was scored automatically by a cumulative recorder for 15 min. The behavior of rats was also observed via closed-circuit television from an adjacent chamber. The open field sessions were performed for five consecutive days (Monday–Friday).

2.4. Biochemical analysis of monoamine concentrations

On the last testing day, i.e., at the time corresponding to the 3rd day after the last dose of the serotonin synthesis inhibitor, the rats were killed and biochemical analysis was performed. The brains were rapidly removed and the hippocampus was dissected bilaterally and frozen at -70°C. Serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline concentrations in the hippocampus were assayed by using a fully automated high-pressure liquid chromatography system (Shimadzu, Japan) with electrochemical detection and by using standard biochemical methods (Stefański et al., 1993).

2.5. Autoradiography

p-Chlorophenylalanine was administered twice, on the 4th and the 3rd day prior to decapitation. After decapitation the brains were rapidly removed and frozen in isopentane (-30 to -40°C) cooled with dry ice. A detailed description of the method for receptor autoradiography has been published (Kuhar and Unnerstall, 1990). Briefly, the whole brains were stored at -70°C. Coronal sections (10 μm) were cut on a cryostat at -20°C according to the atlas of the rat brain ($A = 2.6$ mm) (Pellegrino et al., 1967) and thaw-mounted onto gelatin-coated glass slides.

Sections were stored at −20°C until assay (1 to 2 days). Slides were preincubated in 50 mM Tris–citrate buffer (pH 7.1) for 20 min at 4°C to remove endogenous competitors. After being dried, they were incubated in the same buffer as used for the preincubation but supplemented with 10 nM [^{3}H]muscimol (19.1 Ci/mmol, Amersham) for 40 min at 4°C. Non-specific binding was determined in the presence of 0.2 mM GABA. The sections were then washed in the cold buffer for 1 min and quickly in–out dipped in distilled water. The slides were dried under a cold stream of air, placed in X-ray cassettes and exposed to tritium-sensitive film ([^{3}H]Hyperfilm, Amersham) at 4°C together with standards ([^{3}H]microscale, Amersham). After exposure for 6 weeks, the films were developed with a Kodak LX-24 developer for 5–7 min, fixed, washed, and dried. The autoradiogram was placed in a white light transilluminator (Sigma) to measure the densitometrically determined optical density values. Quantitative analysis of the autoradiogram was performed with an image analysis system (Analytical Imaging Station, Imaging Research, St. Catharines, Canada). For each film the best fit of the film densities produced by radioactive Amersham standards to a 4th-degree polynomial was generated by the computer as a standard curve. Subsequently, this standard curve was used to convert optical densities produced by selected brain regions into amount of radioligand bound (nCi/mg tissue). [^{3}H]Muscimol non-specific binding was negligible. Each group contained eight animals.

2.6. In vitro binding

p-Chlorophenylalanine was administered twice, on the 4th and the 3rd day prior to decapitation. Membranes were taken from the occipital cortex (three animals in each experimental group) prepared essentially according to the method of Enna and Snyder (1975). Briefly, cortex tissue was homogenized immediately after dissection in 15 volumes of cold 0.32 M sucrose and centrifuged at $1000 \times g$ for 20 min at 4°C. The supernatant was decanted and centrifuged at $20\,000 \times g$ for 20 min at 4°C. The resulting pellet was suspended in 15 volumes of cold distilled H_2O and sonicated with a Virsonic apparatus at 40% power output two times 15 s with a 15-s break in between. This suspension was then centrifuged at $8000 \times g$ for 20 min at 4°C. The supernatant with a white buffy layer was decanted and centrifuged at $48\,000 \times g$ for 20 min. The pellet was resuspended in cold distilled water and recentrifuged. Before running binding assays, the pellet was kept frozen at −20°C for at least 18 h. On the day of analysis, the pellet was suspended in 0.05 M Tris–citrate buffer, pH 7.1, containing 0.05% Triton X-100 and incubated for 30 min at 36°C. Following incubation, the suspension was centrifuged 25 min at $48\,000 \times g$, and the pellet was suspended in about 5 volumes of distilled water (as related to the initial tissue weight) for protein analysis with the method of Lowry et al. (1951). Then an equal volume of 0.1 M Tris–citrate buffer, pH 7.1, was added, resulting in final protein concentration of 0.5–0.8 mg/ml.

Binding assays were performed with [^{3}H]muscimol (Amersham, 19.1 Ci/mmol). Assay conditions were taken from the work of Hetmar and Nielsen (1982). The incubation mixture contained in a final volume of 0.3 ml 0.05–0.08 mg of membrane protein in 0.05 M Tris–citrate buffer, pH 7.1, to which increasing concentrations (0.05 to 45 nM) of [^{3}H]muscimol were added. Determinations of non-specific binding were carried out in the presence of 10^{-3} M GABA. Incubation was performed for 30 min in 96-well titer plates at 0–4°C and was terminated by harvesting membranes with a Filtermate 196 harvester (Packard) onto UniFilter 96 microplate with a bonded GF/B filter. The filter plate was washed three times with cold distilled water and left to dry overnight. Next, 25 μl of Microscint-O scintillator was added to each filter well and bound radioactivity was measured in a Packard 'Topcount' Microplate Scintillation Counter. Each measurement of specific and non-specific binding was done in triplicate and the results were transformed into a Scatchard plot by means of the EBDA-LIGAND program. The same assay conditions were used in the *p*-chlorophenylalanine-displacement experiment.

2.7. Statistical analysis

The data are shown as means ± S.E.M. Data involving one control group and one treated group were analyzed by using Student's *t*-test for independent samples. Within group statistical analysis was performed with the help of Student's *t*-test for dependent samples. Data involving multiple comparisons were analyzed by one-way analysis of variance, followed by Newman–Keuls post hoc test.

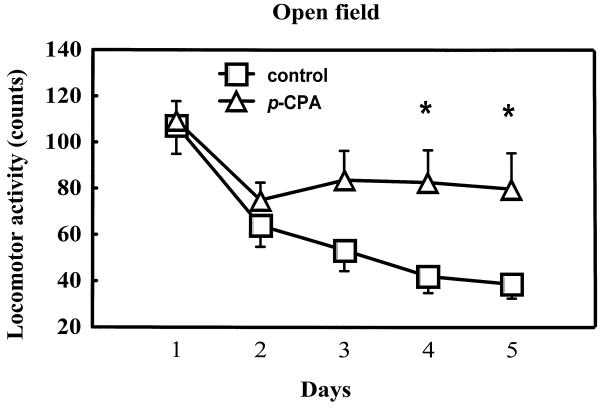

Fig. 1. Locomotor activity of *p*-chlorophenylalanine-treated (*p*-CPA) and control rats in five consecutive daily sessions in the open field test. The number of rats in each experimental group varied from 19 to 20. Data are shown as means ± S.E.M. * Differs from control. * $P < 0.05$.

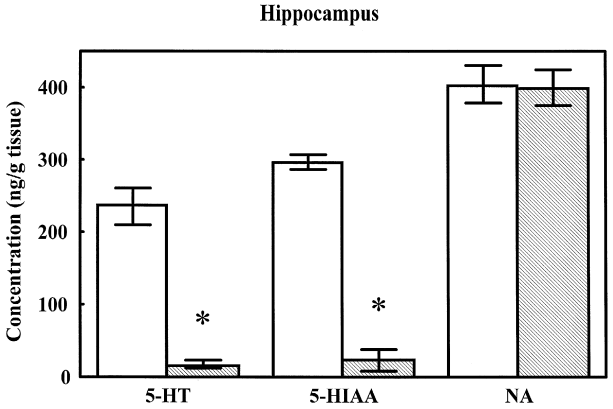

Fig. 2. The effect of *p*-chlorophenylalanine on monoamines (serotonin, 5-HT; noradrenaline, NA) and 5-HIAA concentrations in the hippocampus, studied on the day corresponding to the last day of the open field test. Each bar represents the mean ± S.E.M. The number of *p*-chlorophenylalanine-administered and control animals was 7. * Differs from control. * $P < 0.01$).

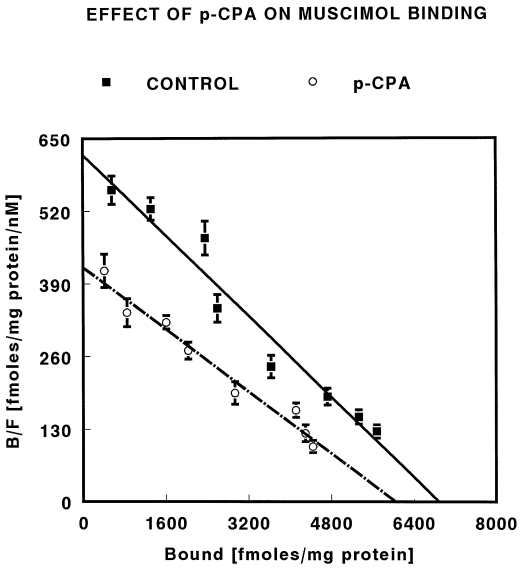

Fig. 3. Representative Scatchard plot of [^{3}H]muscimol binding to the occipital cortex. Individual points represent means ± S.E.M., obtained from triplicate determinations. The lines represent EBDA-LIGAND computer fit to data.

The confidence limit of $P < 0.05$ was considered statistically significant.

3. Results

In the open field test, control rats showed clear-cut and progressive habituation of exploratory motor activity, their motility scores being the lowest on the 4th and 5th day of testing (Fig. 1). The behavior of *p*-chlorophenylalanine-pretreated animals was different, statistical analysis revealed significant differences across all testing days ($F_{4,72} = 2.89$, $P < 0.05$). Within group comparisons showed statistically significant differences on the 2nd and 3rd day, compared to the 1st day. On the 4th ($t = 2.6$, $df = 37$, $P < 0.05$) and 5th day ($t = 2.5$, $df = 37$, $P < 0.05$), serotonin-depleted animals were significantly more active than control rats (Fig. 1).

p-Chlorophenylalanine caused potent and selective depletion of hippocampal serotonin (8% of control group values) and 5-HIAA acid (8% of control group values) concentrations 3 days after the last dose of the serotonin synthesis inhibitor, i.e., at the time corresponding to the 5th day of behavioral testing (Fig. 2). No significant changes in noradrenaline levels were observed (Fig. 2).

Autoradiography of [^{3}H]muscimol binding showed a statistically significant decrease in specific radioligand binding to the $GABA_A$ receptors in the hippocampal dentate gyrus, by about 27% (Table 1) ($t = 3.74$, $P < 0.01$). A similar significant [^{3}H]muscimol down-regulation was found in the occipital cortex (by 22%, $t = 4.37$, $P < 0.001$) (Table 1). In the other examined brain structures (CA-3 region of the hippocampal formation, the geniculate nucleus and entorhinal cortex) changes in [^{3}H]muscimol binding were very much the same in both direction and magnitude (Table 1). The only exception was the substantia nigra (taken as a whole), which was characterized by a lower saturation with [^{3}H]muscimol and the lack of differences in isotope binding after *p*-chlorophenylalanine ($P = 0.82$) (Table 1).

Scatchard analysis of in vitro [^{3}H]muscimol binding to the membranes obtained from the occipital cortex in the

Table 1
Specific binding of [^{3}H]muscimol to the $GABA_A$ receptors in different brain structures after pretreatment of rats with *p*-chlorophenylalanine (*p*-CPA)

	Control	*p*-CPA	*P*	% Ratio
Occipital cortex	13.95 ± 0.56	10.84 ± 0.43	0.0006	(−) 22.3
Dentate gyrus	12.25 ± 0.74	9.00 ± 0.46	0.002	(−) 26.5
CA-3 area	5.82 ± 0.32	4.37 ± 0.26	0.004	(−) 24.9
Entorhinal cortex	11.10 ± 0.55	8.36 ± 0.32	0.001	(−) 24.7
Geniculate nucleus	15.78 ± 0.53	11.56 ± 0.41	0.0001	(−) 26.7
Substantia nigra	4.53 ± 0.47	4.36 ± 0.54	0.82	(−) 3.8

The data are shown as means ± S.E.M. in nCi/mg tissue. The number of rats in each experimental group was seven to eight.

Table 2
B_{max} and K_d of [^{3}H]muscimol binding to cortical membranes of control and *p*-chlorophenylalanine-pretreated rats (*p*-CPA)

	Occipital cortex	
	K_d (nM)	B_{max} (fmol/mg protein)
Control	9.62 ± 0.63	7050 ± 550
p-CPA	12.84 ± 0.98	6360 ± 350
% Ratio	(+) 33.5%	(−) 9.8%

The data obtained from three Scatchard plots of three separate animals in each experimental group are shown as means ± S.E.M. For more details see Section 2.

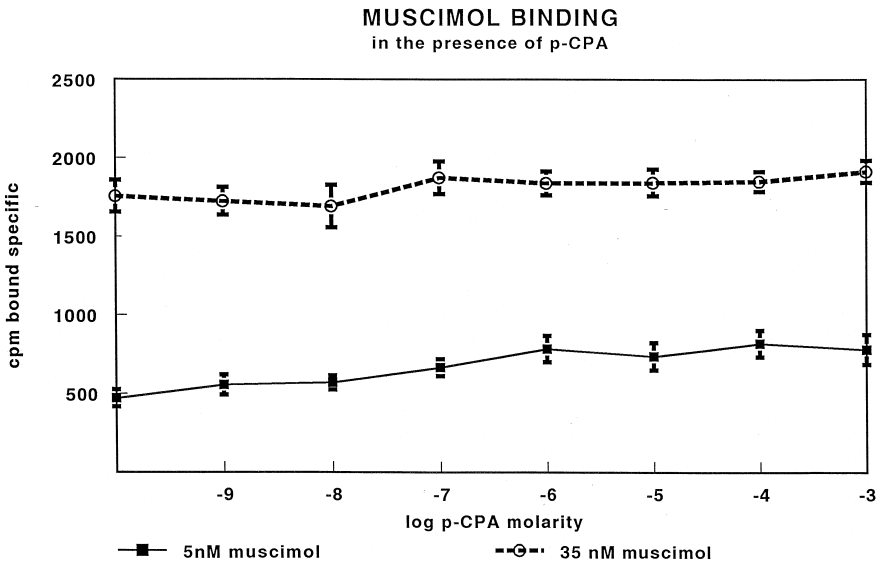

Fig. 4. In vitro displacement of [^{3}H]muscimol specific binding from occipital cortex membranes by *p*-chlorophenylalanine. The data are shown as means ± S.E.M. Ordinate, specifically bound radioactivity (cpm) after administration of [^{3}H]muscimol at two concentrations (5 and 35 nM). Abscissa, concentration of *p*-chlorophenylalanine in a logarithmic scale (10^{-9} to 10^{-3} M). Individual points represent means obtained from four separate animals, each of which was determined in triplicate.

p-chlorophenylalanine-treated rats showed that muscimol bound to a single population of recognition sites (Fig. 3). In the occipital cortex, the K_d of [^{3}H]muscimol in brain membranes of *p*-chlorophenylalanine-pretreated animals was increased by 33.5% and the B_{max} of [^{3}H]muscimol was reduced by almost 10%. The B_{max} and K_d values obtained by linear regression are summarized in Table 2.

Analysis of in vitro displacement of 5 and 35 nM [^{3}H]muscimol specific binding from occipital cortex membranes by *p*-chlorophenylalanine revealed no evident effect of the serotonin synthesis inhibitor over a wide range of concentrations (Fig. 4).

4. Discussion

The most interesting finding of the present study was that serotonin depletion significantly interfered with the process of habituation to a novel environment. It concomitantly decreased [^{3}H]muscimol binding in the dentate gyrus of the hippocampus. A 30% decrease in [^{3}H]muscimol binding to cortical membranes, although evident from the Scatchard plot, might not appear too impressive by itself. However, it correlates very closely with the effects observed by autoradiography in the occipital cortex and the dentate gyrus of the hippocampal formation (22% and 27% reduction of binding, respectively). Indeed, very similar changes in [^{3}H]muscimol binding after *p*-chlorophenylalanine were found with help of autoradiography in other brain structures (the geniculate nucleus and hippocampal CA3 region) as well. This finding indicates a uniform pattern of GABA–serotonin interaction in the rat forebrain. For these reasons it seemed justified to compare the autoradiography and receptor binding data obtained from different cortical areas of the brain. The only exception was the substantia nigra, in which there was much lower radioligand binding. This may indicate that changes in the $GABA_A$ receptors after serotonin depletion are localized mostly in the forebrain cortical and limbic structures; however, more brain areas ought to be analyzed to give a definite answer. Given that autoradiography slices were incubated at half of the saturating concentration of muscimol, such a correlation appears to indicate that it is the change in apparent affinity which is the principal cause of [^{3}H]muscimol binding down-regulation. Because *p*-chlorophenylalanine administered at the same dose (150 mg/kg, i.p.) twice and three times resulted in a selective and quantitatively identical pattern of serotonin decrease in the rat hippocampus and cortical areas (Płaźnik et al., 1994a; Płaźnik et al., data submitted for publication), it was possible to compare directly the biochemical and autoradiographical data in the present experiment. It is also noteworthy that biochemical analysis of monoamines concentration and autoradiography were performed on the 3rd day after the last dose of the serotonin synthesis inhibitor. Thus, in both instances the time of examination and the magnitude of the decrease in serotonin concentration were virtually identical. To ensure that residual *p*-chlorophenylalanine did not affect the binding assay, it was tested whether [^{3}H]muscimol specific binding was displaced from cortical membranes by various concentrations of the serotonin synthesis inhibitor. It appeared that *p*-chlorophenylalanine applied in a wide range of concentrations did not significantly decrease the binding of [^{3}H]muscimol, examined at concentration similar to and higher than that studied in the autoradiography experiment. Thus, this excludes

the possibility of a direct, chemically based mechanism of *p*-chlorophenylalanine-induced effects, and it points at a functional interaction between serotonin and GABA systems as the most plausible causal factor.

The influence of serotonin depletion on motor activity is still an open question even though many data on this topic have been published over the last 3 decades (cf. Dringenberg et al., 1995). In the classic paper by Steigrad et al. (1978) it was found that *p*-chlorophenylalanine changed circadian rhythms of motor activity in rats: daytime activity decreased, whereas nighttime activity increased. This, however, was shown after administration of a very high dose of the serotonin synthesis inhibitor (100 mg/kg daily over 12 days). In the paper by Dringenberg et al. (1995) the most potent changes in the spontaneous motor activity of rats were observed after 500 mg/kg of *p*-chlorophenylalanine given as a bolus, and at the dose of 2×500 mg/kg over two consecutive days. These rats had to be kept on a special diet to prevent a 50% drop in body weight, they had reduced water and food intake and their fur was discoloured. Apparently, such high doses of *p*-chlorophenylalanine produced general toxic effects even if no obvious sensory deficits were present. It should be underlined that this dosage of *p*-chlorophenylalanine was much higher than that used in our study (2×150 mg/kg over 2 days or 3×150 mg/kg over 6 days). In our experiments we never observed such pronounced changes in the animals' appearance and physical form, and the doses of *p*-chlorophenylalanine we used appeared sufficient to cause an almost total depletion of hippocampal 5-HT and 5-HIAA. Irrespective of the regimen of serotonin synthesis inhibitor administration, quantitatively and qualitatively the pattern of changes in 5-HT and 5-HIAA concentrations in the rat forebrain were very similar, the relative differences being less than 5% (Płaźnik et al., 1994a; Płaźnik et al., data submitted for publication). Importantly, another method of a very selective and deep serotonin reduction (5,7-dihydroxytryptamine, i.c.v.) also did not affect the spontaneous motor activity of rats (Płaźnik et al., 1997). Recently, it was found that very high doses of *p*-chlorophenylalanine (200, 400 and 800 mg/kg, i.p., on days 1, 2, 4 and 6 of the experiment) produced, in a dose-dependent way, a reduction in both monoaminergic afferents and non-monoaminergic synapses in the cerebral cortex (up to 50% after the dose of 800 mg of *p*-chlorophenylalanine) (Chen et al., 1994). Thus, it seems that the dosage of serotonin synthesis inhibitor is a crucial factor in producing its behavioral effects, with higher doses being much less selective in this respect.

Serotonin is known to be released in rat forebrain structures by many behavioral and physiological challenges (Rueter and Jacobs, 1996). It may serve to attenuate the activity of neural processes called into play by divergent arousing stimuli, thus contributing to the processes of habituation. Accordingly, lesions to the serotonergic neurons have been reported to disinhibit many central activities, ranging from pain perception and sleep/waking cycle to aggressive and sexual behaviors (Płaźnik et al., 1994a,b; Puciłowski et al., 1985; Soubrie, 1986). As habituation most probably reflects a diminished reactivity of animals to both aversive and reinforcing stimuli, it can be further inferred that the disinhibitory influence of the serotonergic neurotoxin on rat motor behavior arises from its non-specific interference with the habituation processes. However, these mechanisms may not be affected in a balanced way. One line of reasoning might assume that the decrease in hippocampal [^{3}H]muscimol binding after *p*-chlorophenylalanine is an adaptive reaction, secondary to the disinhibition of activity of a local population of GABAergic neurons after serotonergic lesion (Söderpalm et al., 1992). The underlying mechanism may involve removal of an inhibitory influence of hippocampal serotonergic innervation on GABA interneurons activity, which normally occurs via 5-HT$_{1A}$ receptor-induced hyperpolarization of cell membranes (Freund et al., 1990; Halasy et al., 1992; Kia et al., 1996). This explanation supports the anti-emotional interpretation of the *p*-chlorophenylalanine-induced behavioral effect. Accordingly, there are many papers documenting a negative coupling between brain serotonin and GABA systems in the control of animal behavior. For example, *p*-chlorophenylalanine and 5,7-dihydroxytryptamine produced a clear-cut anti-conflict effect in a modified Vogel's test and in the elevated plus maze (Söderpalm and Engel, 1989, 1990, 1991). The anti-conflict effect of *p*-chlorophenylalanine was completely counteracted by both flumazenil and bicuculine, the GABA$_A$-receptor complex antagonists, at doses that did not alter behavior per se. These results clearly suggested enhanced transmission at the GABA$_A$/benzodiazepine receptor after functional lesion of serotonergic neurons.

However, GABA$_A$ receptor down-regulation might explain the lack of habituation of exploratory activity in serotonin-lesioned rats, if one also assumes an important role of the hippocampal GABA$_A$/benzodiazepine receptor complex in the processes of selective attention and stimulus control. Accordingly, benzodiazepines and other positive allosteric modulators of the GABA$_A$ receptor complex are known to accelerate the process of habituation to different categories of environmental stimuli. They can also mimic the behavioral changes that occur in the adaptive processes that accompany repeated exposure to the same behavioral challenge (Borros et al., 1994). Interestingly, in a recently published paper, Gruen et al. (1996) found a highly significant negative correlation between novelty-associated locomotion in rats and specific binding of the GABA$_A$ receptor antagonist, [^{3}H]SR95531 [2-(3′-carboxy-2′-propyl)-3-amino-6-*p*-methoxy phenylpyridazinum bromide], in the cingulate and prefrontal cortices and in the ventral pallidum. As in the present paper, there was a 46.9% decrease in [^{3}H]SR95531 binding to the prefrontal cortex that correlated with an almost 2-fold increase in novelty-induced locomotion in rats. Thus, these data corre-

spond very closely with the present findings of a parallel increase in motility and a decrease in [^{3}H]muscimol binding in the hippocampus, entorhinal and occipital cortices after *p*-chlorophenylalanine. Enhanced ambulatory behavior ought to interfere, in an obvious way, with the exploratory motor activity habituation. Furthermore, it is possible that the brain dopaminergic system is involved in the behavioral expression of the interaction between serotonin and GABA systems. Novelty is known to increase in vivo cortical and accumbal dopamine release (see Section 1), and $GABA_A$ receptor ligands have been demonstrated to alter dopamine release in the striatum and nucleus accumbens (Gruen et al., 1992; Tanganelli et al., 1994). Thus, facilitated dopamine release secondary to changes in $GABA_A$ receptor function after *p*-chlorophenylalanine could add to the effects of novelty, resulting in a more potent behavioral stimulation and interference with the processes of motor activity habituation. Accordingly, it has been found recently that 5,7-dihydroxytryptamine-induced serotonergic lesions significantly enhance the turnover rate of dopamine in the rat brainstem (Płaźnik et al., 1997).

In summary, the present data indicate that after serotonin depletion there is a simultaneous inhibition of habituation and a decrease in hippocampal [^{3}H]muscimol binding. Apparently, serotonin and GABA systems interplay in the process of habituation of exploratory motor behavior. The obtained results may also help to better understand the possible mechanisms of some symptoms of mental disorders related to disturbances in sensorimotor gating of irrelevant stimuli, e.g., attentional deficits in schizophrenics leading to the hyperarousal. Moreover, to our knowledge this is the first study to demonstrate that a highly significant correlation exists between *p*-chlorophenylalanine-induced serotonin depletion and changes in $GABA_A$ receptor binding in many brain areas. As *p*-chlorophenylalanine has been extensively used in experimental psychopharmacology over the last 2 decades, it seems that this finding may be important to the interpretation of many other experimental data as well.

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