Delayed effects of antidepressant drugs in rats

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The present study has addressed the question of what is more important for the occurrence of adaptive changes observed in the organism treated with antidepressant drugs: a daily dosing of the drug or the period of time necessary for the plastic events to develop. Here, we report on the effects of desipramine given to rats acutely (and tested following 2 drug-free weeks) as when the drug was administered repeatedly, on behavior in the forced swim test (i.e. significant shortening of immobility time by ca. 60%) and on the binding of [^{3}H]CGP12177 to β-adrenergic receptors in the rat brain cortex (significant decrease of the binding by ca. 15%). Additionally, using the procedure of the repeated forced swim test (six times over 21 days), we show that the shortening of immobility time induced by a single dose of imipramine persisted throughout the whole experimental period and was similar to that seen in a group of animals treated repeatedly with the drug. Also, the effects of citalopram on immobility and climbing were similar after acute treatment and delayed testing to those seen after repeated drug exposure. The results obtained in the present study may question some conclusions that are usually drawn from the behavioral and, especially, biochemical studies concerning the need for repeated treatment with antidepressant drugs to induce various adaptive changes in the brain, which are thought to be responsible for the therapeutic efficacy of these drugs. *Behavioural Pharmacology* 17:641–649 © 2006 Lippincott Williams & Wilkins.

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Introduction

Depression is a clinically and biologically heterogeneous disorder, manifested at psychological, behavioral and physiological levels, and not easy to mimic in laboratory animals. The mechanism of action of antidepressant drugs (ADs) is not fully understood, despite the well-known pharmacological effects of acute administration of these drugs. ADs, however, need to be administered for a few weeks [or at least as has been recently shown by Katz *et al.* (2004) for a few days] to achieve therapeutic efficacy. It has been widely accepted that perturbed monoaminergic transmission is causally implicated in depressive states (Blier, 2003; Manji *et al.*, 2003). A plethora of experimental data, as well as monoamine depletion studies in patients, have convincingly demonstrated the importance of functionally competent monoaminergic pathways for combating depressive states, and all currently used treatments of depression restore the activity of monoaminergic pathways (Delgado, 2000; Pacher *et al.*, 2001; Millan, 2004). The temporal mismatch between the rapid elevations in extracellular levels of monoamines induced by ADs and their slow onset of action reflects the initiation of adaptive changes, starting as alterations in receptor density and intracellular signaling, and changes in synaptic transmission, which in turn lead to phosphorylation of transcription factors and ultimately to changes in gene expression, modification of neuronal architecture and neurogenesis (Duman *et al.*, 1997; Kempermann and Kronenberg, 2003; Holoubek *et al.*, 2004).

In preclinical research, the ADs are typically administered to laboratory animals (mainly rodents) and then the behavioral and biochemical alterations are assessed in comparison with a vehicle-receiving group. Some of these changes have been postulated to be important for antidepressant action. Among them, the downregulation of β-adrenergic receptor (β-AR) density and the desensitization of β-AR coupled with adenylate cyclase system observed in the frontal cortex and/or hippocampus following repeated administration of ADs (Sulser *et al.*, 1978) are widely accepted as a common biochemical effect of chronic treatment with ADs. Of all the behavioral procedures used in preclinical research of depression, the forced swim test (FST) is the most widely used (Lucki, 1997; Porsolt, 2000; Cryan *et al.*, 2002). Although this test can by no means be regarded as a rodent model of human depression, it certainly is – as Cryan *et al.* (2005) have stated recently – 'an objective marker for a behavioral state associated with depression'. The test is based on the observation that rodents, following initial escape-oriented movements, develop an

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immobile posture in an inescapable cylinder filled with water. Following antidepressant administration, the animals will actively persist engaging in escape-oriented behaviors for a longer time than after vehicle treatment. The FST detects the antidepressant-like effects of drugs after acute administration (typically three doses in rats), although the same shortening of immobility time is observed following repeated treatment (Borsini and Meli, 1988). Out of various classes of ADs tested so far, desipramine (DMI, a noradrenaline reuptake inhibitor), administered in conventional way, appeared more effective following chronic treatment (Borsini and Meli, 1988). More recently, Cryan *et al.* (2004) have shown that the effects of antidepressants from different chemical classes with distinct mechanisms of action were augmented following chronic administration for 14 days (in comparison with a 3-day treatment), especially when given at low doses. Drug administration via osmotic minipump (in contrast to conventional, peripheral administration), however, was employed in that study.

The present study has addressed the question of what is more important for the occurrence of the adaptive changes observed in the organism treated with ADs: a daily dosing of the drug or the period of time necessary for the plastic events to develop. Chiodo and Antelman (1980) were the first to investigate this question, on the basis of their studies of the attenuation of the ability of apomorphine to reduce the firing of the dopaminergic neurons in the rat substantia nigra. Interestingly, the effect was not only induced by repeated antidepressant treatment, but also by a single dosing followed by drug-free period. In line with these findings, we have shown, in behavioral studies, that the subsensitivity of presynaptic dopamine D_2 receptors was observed not only after repeated administration of various ADs, but also after a single dose of these drugs followed by two drug-free weeks (Dziedzicka-Wasylewska and Rogóż, 1997). Similar effects were observed behaviorally at the level of α_2-adrenergic and dopamine D_3 receptors (Dziedzicka-Wasylewska *et al.*, 1999). Antelman *et al.* (1997) later coined the term 'time-dependent sensitization' to describe effects that grow with the passage of time following acute drug treatment or a single stressor.

Here, we report the effects of DMI given to rats acutely and tested following two drug-free weeks, in comparison with repeated administration, on the behavior in the FST and on the binding of [^{3}H]CGP12177 to β-AR in the rat brain cortex. Additionally, we describe the effects of a single dose of DMI, imipramine (IMI, a tricyclic antidepressant with similar potency to block both serotonin and noradrenaline transporters) and citalopram (CIT, a selective serotonin reuptake inhibitor) over a 21-day period when the animals were repeatedly examined in the FST.

Methods

Subjects

The experiments were carried out on male Wistar rats, ca. 80 days old, weighing 220–230 g; after 21 days of repeated drug administration, their weight increased to 280–320 g. The animals had free access to food and water before experiments and were kept at a constant room temperature (22 ± 1°C), under a 12-h light/dark cycle (lights on at 07.00 h). Experimental protocols were approved by the local Ethics Committee and met the guidelines of the responsible agency of the Institute of Pharmacology.

Drug administration

ADs were dissolved in saline and were administered intraperitoneally (i.p.) once daily for the indicated time periods. All animals were handled in the same manner. Control animals received vehicle for the whole experimental period, whereas repeatedly treated animals received the appropriate drug. The animals treated acutely with a drug received saline on all days following the appropriate drug administration. Using this experimental paradigm, we avoided the effect of the single i.p. injection, which inevitably, as a stressful event for an animal, might have masked or changed the actual effect of acute administration of the studied drug. Moreover, all groups of animals, treated acutely or repeatedly, were taken for behavioral experimentation or decapitated for biochemical assay at the same time.

Forced swim test

The FST was performed according to the original procedure described by Porsolt *et al.* (1977, 1978). Rats were placed in a cylinder (21 cm in diameter) filled with water (23–25°C) to a depth of 18 cm. To study the effects of CIT, the FST was modified, according to Detke and Lucki (1996). Namely, the cylinders were filled with greater water depth (30 cm) and other parameters, in addition to immobility, were estimated (climbing and swimming).

During the initial session, the rats were forced to swim for 15 min. Then, they were removed from water, dried with towels and placed in a warm enclosure, and later returned to their home cages. Each cylinder was emptied, cleaned and refilled with fresh water, before the next rat was placed in. This pretest was performed at the beginning of experiment 1 or 2 described below, before the administration of drugs.

Experiment 1

The rats were divided into groups, each of which consisted of eight animals. All were treated daily with i.p. injections. Two control groups received saline, the chronically treated group was given DMI (15 mg/kg) for 13 days and the 'withdrawn' group received a single dose of DMI (15 mg/kg) followed by 12 saline injections

(a better description of that experimental situation would be 'post-withdrawal DMI challenge' but for the sake of simplicity we use the term 'withdrawal'). At the end of the experiment, 'chronic', 'withdrawn' and one of 'control' groups were tested with the usual FST procedure; that is, rats were treated with three doses of DMI (10 mg/kg) at 24 (day 14), 5 and 1 h (day 15) before the test. The remaining control group received saline at the same time points. The table below presents the details of the treatment.

	1	2	3	…	12	13	14/15	15
Control	Sal	Sal	Sal	Sal	Sal	Sal	24,5,1 h sal	FST
Acute	Sal	Sal	Sal	Sal	Sal	Sal	24,5,1 h DMI 10 mg/kg	
Chronic	DMI	DMI	DMI	DMI	DMI	DMI		
Withdrawn	DMI	Sal	Sal	Sal	Sal	Sal		

Immobility was defined as cessation of limb movements, except minor movements necessary to keep the rat afloat. Immobility was measured during the 5-min session by a highly experienced observer.

Experiment 2

In this experiment, the effects of three drugs, DMI (15 mg/kg), IMI (15 mg/kg) and CIT (15 mg/kg), were studied over a period of 21 days. All animals were subjected to the pretest procedure of the FST as described above, then they were divided into groups. There were two groups of animals for each drug: one group treated acutely (i.p.) on day 1, followed by daily saline injections ('withdrawn') and the other group treated repeatedly with the appropriate drug ('chronic'). Control groups received daily saline injections. Each animal was subjected repeatedly to the FST, starting 5 h after the first injection. The next swimming session was after 24 h (and 5 h after the morning injections), and then on days 4, 7, 14 and 21. The timing of the experiment was held in such a manner that the swimming session was always 5 h after the injection of saline or the appropriate drug. These animals were not taken for biochemical assessments. The table below presents the details of the treatment.

	1		2		3	4		…
Control	Sal	FST	Sal	FST	Sal	Sal	FST	Sal
Chronic	Drug		Drug		Drug	drug		Drug
Withdrawn	Drug		Sal		Sal	Sal		Sal
	7		…	14		…	21	
Control	Sal	Chronic	Sal	Sal	FST	Sal	Sal	FST
Withdrawn	Drug		Drug	Drug		Drug	Drug	
FST	Sal		Sal	Sal		Sal	Sal	

For rats treated with IMI or DMI, immobility was defined as a cessation of limb movements, except for minor movements necessary to keep the rat afloat. Immobility was measured during the 5-min session by a highly experienced observer. As these two drugs are widely studied using the conventional FST procedure, we applied that procedure, so as to compare the obtained results with the data published so far.

On the other hand, for rats treated with CIT, a modified FST procedure was used, as selective serotonin reuptake inhibitors do not work in the FST performed in the conventional manner. Animals were forced to swim in the same opaque cylinders (21 cm in diameter) but filled with water (23–25°C) to a 30-cm depth. Each cylinder was emptied, cleaned and refilled with fresh water, before the next rat was placed in. The behavior of animals was videotaped using a videocamera placed above the cylinders. The quantification was by means of behavioral sampling; that is, the 5 min test session was divided into 60 5-s intervals. An experienced observer categorized the period as climbing, swimming or immobility on the basis of activity predominant in each interval.

Autoradiography of beta-adrenergic receptors

The rats tested in FST in experiment 1 were used for biochemical experiment. They were killed 24 h after the FST. An additional group of animals received daily injections of saline, but was not subjected to the FST. Brains used for autoradiographic analysis were rapidly dissected and frozen by immersion in cold heptane in a dry-ice bath and stored at −70°C until sectioned. Consecutive coronal sections (12-μm) were cut at −20°C using Jung CM 3000 cryostat (Leica, Wetzlar, Germany). The identification and nomenclature of the brain structures were based on the Paxinos and Watson Rat Brain Atlas (1998).

The slices were sectioned and thaw-mounted on gelatin-coated glass microscope slides. They were stored at −70°C. Just before using, the slide-mounted sections were dried at room temperature. For β-AR binding, the incubations were conducted at 25°C for 60 min in 50 mmol/l Tris-HCl buffer (pH 7.4) containing 120 mmol/l NaCl and 5 mmol/l KCl with 4 nmol/l of the β-adrenergic ligand [^{3}H]CGP12177 (specific activity: 42.5 Ci/mmol; Du Pont, Boston, Massachusetts, USA). Propranolol (5 μmol/l; Sigma, St Louis, Missouri, USA) was used to determine nonspecific labeling. Dried tissue sections were exposed for 5 days to tritium-sensitive screens (FujiFilm, Düsseldorf, Germany) along with [^{3}H]microscales (Amersham, Illinois, USA) as a standard. The images were obtained by means of a FujiFilm BAS 5000 Phosphorimager. They were analyzed using FujiFilm software (Image Gauge, Version 4.0, FujiFilm, Tokyo, Japan). The relative optical densities of structures from sections showing nonspecific binding were subtracted from the same regions of adjacent sections with total binding. After calibration by computer-generated curves derived from the standards, the results are expressed as fmol of bound radioligand per milligram of wet tissue.

Drugs

DMI was from Sigma, and IMI from Pliva (Kraków, Poland). CIT was provided by Lundbeck (Copenhagen, Denmark). The doses of DMI and IMI were chosen on the basis of previous reports (Rogóż *et al.*, 2002; Mague *et al.*, 2003); the dose of CIT was justified by our own dose–response studies (data not shown).

Statistics

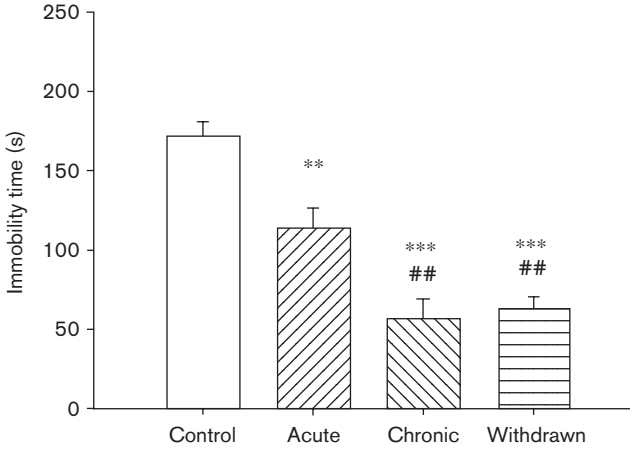
Data obtained in multiple FST experiments were analyzed by two-way analysis of variance (ANOVA) with repeated measures for behavior on different days. To compare groups, least significant difference post-hoc tests were performed. In cases in which the two-way ANOVA showed a significant interaction, data were separated into time points and analyzed with one-way ANOVA followed by Tukey's test for comparison of all pairs of groups when appropriate. Biochemical data were analyzed by one-way ANOVA, followed by Tukey's test. Effects were considered significant when $P < 0.05$.

Results

Forced swim test: experiment 1

The effects of DMI in the FST are shown in Fig. 1. Significant differences between groups were found [$F(3,25) = 18.45$; $P < 0.001$]. As expected, DMI administered in conventional manner, that is, three times (24, 5 and 1 h before the test) at a dose of 10 mg/kg, i.p. ('acute'), shortened the immobility time by ca. 30% in comparison with the control group, receiving saline ($P < 0.01$).

Fig. 1



Effect of desipramine (DMI) administered acutely (three doses), chronically or once at the beginning of the experiment (then followed by 12 saline injections on consecutive days, designated 'withdrawn'), according to the procedure described for experiment 1, on immobility time in the FST in rats. The results are the means ± SEM, $n = 7–8$ rats per group. The statistical significance was calculated using one-way analysis of variance followed by Tukey's test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\#\#}P < 0.01$ vs. acutely treated group.

When DMI (15 mg/kg, i.p.) was administered repeatedly for 13 days ('chronic') followed by three doses of the drug necessary to evoke the behavioral reaction in the FST, the shortening in immobility time was significantly greater ($P < 0.01$), and the reduction reached ca. 60% of the control ($P < 0.001$). A very similar reduction in immobility time was observed in animals treated repeatedly with DMI for 14 days, followed by the FST performed 5 h after the last dose of the drug, that is, without additional dosing as in conventional FST (data not shown).

Rats treated acutely with DMI (15 mg/kg, i.p.) on day 1, receiving daily saline injections afterwards ('withdrawn') and then treated with three doses of DMI (10 mg/kg, i.p.) at 24, 5 and 1 h before the FST behaved almost identical to the group treated repeatedly with DMI ($P < 0.001$ vs. control, $P < 0.01$ vs. 'acute').

Forced swim test: experiment 2

Animals were treated either acutely (on day 1, then 'withdrawn') or repeatedly for 21 days with one of three ADs. All of them were subjected to six swimming sessions throughout the 21 days of experiment, always 5 h after the administration of the drug or saline: on days 1, 2, 4, 7, 14 and 21.

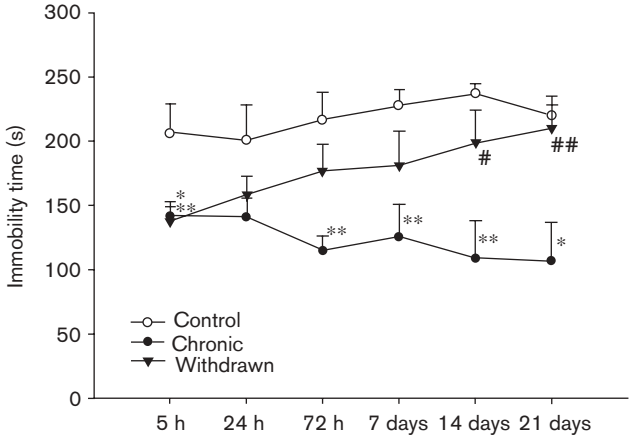
In animals receiving DMI, two-way ANOVA indicated a significant treatment × time interaction [$F(10,85) = 2.06$; $P < 0.05$]. Further analysis using one-way ANOVA for each time point showed that there were significant differences between groups at all time points [$F(2,21) = 6.02–10.02$; $P < 0.01–0.001$], except for 24 h [$F(2,21) = 3.045$; $P = 0.069$]. DMI administered chronically, reduced the immobility time of the rats during the entire duration of the experiment (Fig. 2).

Rats treated with a single dose of DMI on day 1 (then 'withdrawn') showed immobility times intermediate between control and chronically treated groups during the successive days of the experiment. On the first day, the immobility time was the same in both groups (both groups received the same treatment on day 1). The values began to differ statistically after the 14th day of the experiment.

The effect of a single dose of DMI observed on day 14 in this experiment was weaker than in experiment 1. Two differences between these experiments, however, must be underlined; in experiment 2, the same rats were subjected repeatedly to the swimming sessions, and in the experiment they did not receive additional injections of the drug to strengthen the behavioral reaction in the FST.

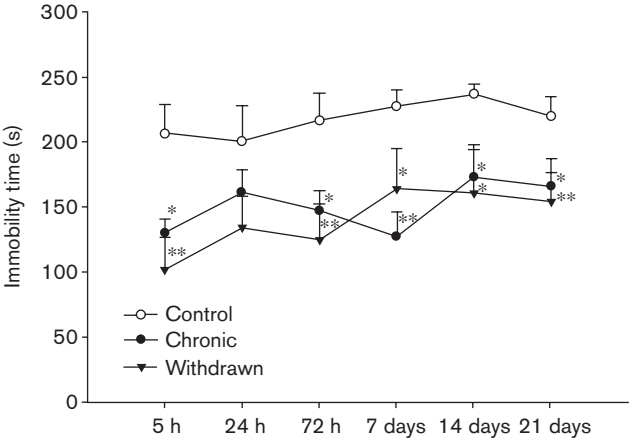
For IMI, two-way ANOVA showed significant effects of treatment [$F(2,75) = 5.37$; $P < 0.02$] and time [$F(5,75) =$

Fig. 2



Effect of desipramine (DMI, 15 mg/kg, intraperitoneal) administered daily for 21 days (chronic) or once at the beginning of experiment (then followed by 20 saline injections on consecutive days, designated 'withdrawn'), on immobility time at the indicated time of treatment. Results represent the means ± SEM, $n = 7–10$ rats per group. As two-way analysis of variance (ANOVA) for repeated measurements indicated time by treatment interaction, the statistical significance was calculated using one-way ANOVA for each time point, followed by Tukey's test. $^{*}P<0.05$, $^{**}P<0.01$ vs. control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ between DMI-treated groups.

Fig. 3



Effect of imipramine (IMI, 15 mg/kg, intraperitoneal) administered daily for 21 days (chronic) or once at the beginning of experiment (then followed by 20 saline injections on consecutive days, designated 'withdrawn'), on immobility time at the indicated time of treatment. Results represent the means ± SEM, $n = 7–8$ rats per group. The statistical significance was calculated using two-way analysis of variance followed by least significance difference test. $^{*}P<0.05$, $^{**}P<0.01$ vs. control.

2.76; $P < 0.025$], with no significant interaction. On day 1, both groups of experimental animals received the first dose of IMI, which induced a significant reduction in immobility time. This effect did not change significantly throughout the 21 days of the experiment, no matter whether the animals received additional doses of IMI or only saline. Therefore, repeated administration of IMI did not produce a stronger effect in the FST than the acute treatment. It must be pointed out that even the single dose of the drug, however, was sufficient for shortening the immobility time and this effect was stable for the next drug-free days (Fig. 3).

The effect of CIT was measured using the modified FST; that is, the depth of water was greater, which produced lower baseline values of immobility (Fig. 4a). Additionally, two other parameters of activity, that is, climbing and swimming were measured. As two-way ANOVA indicated a significant treatment × time interaction [$F(10,145) = 2.64$; $P < 0.01$], each time point was examined by one-way ANOVA with post-hoc Tukey's test when appropriate.

ANOVA concerning the immobility time indicated significant differences on day 4 [$F(2,45) = 4.7$; $P < 0.02$], day 7 [$F(2,45) = 4.01$; $P < 0.025$] and day 21 [$F(2,45) = 9.46$; $P < 0.001$]. The decrease in immobility time was the highest in the group that received the drug on the first day and then received saline for the remainder of the experiment ('withdrawn'). Repeated treatment with CIT decreased immobility time depending on the number of the doses, with the effect starting to be significant on day 21.

ANOVA for data concerning the swimming parameter also showed differences between groups on day 4 [$F(2,45) = 8.67$; $P < 0.001$], day 7 [$F(2,45) = 5.06$; $P < 0.02$ and day 21 [$F(2,45) = 6.78$; $P < 0.01$]. Similar to the immobility time, the effect was observed earlier in the 'withdrawn' group (on day 4) (Fig. 4c).

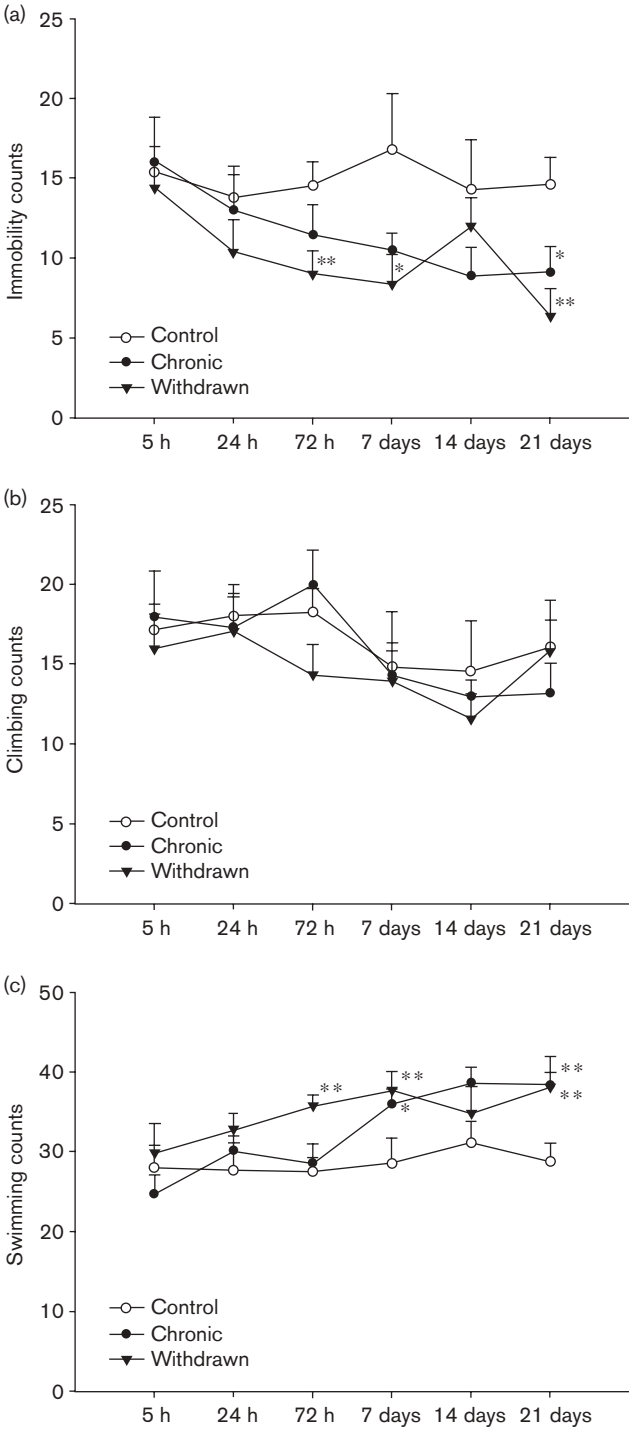
Although, for climbing, two-way ANOVA indicated a significant effect of time [$F(5,145) = 3.97$; $P < 0.005$], no significant effect of treatment and no significant interaction, post-hoc tests did not show particular differences (Fig. 4b).

Binding of [^{3}H]CGP12177 to cortical beta-adrenergic receptors

The brains for autoradiographic analysis were taken from the rats examined in the FST in experiment 1. Additionally, there was a control group of animals treated repeatedly with saline but not tested in the FST.

Cortical β-ARs labeled with [^{3}H]CGP12177 were rather homogeneously distributed in different areas of the cortex; however, average binding was higher in the superficial layer (layers I–II) than in the deep layers of the cortex (layers III–V) (Fig. 5a).

Fig. 4



Effect of citalopram (CIT, 15 mg/kg, intraperitoneal) administered daily for 21 days (chronic) or once at the beginning of experiment (then followed by 20 saline injections on consecutive days, designated 'withdrawn'), on immobility (a), climbing (b) and swimming (c) in the modified forced swim test, conducted six times (as indicated) during 21 days of the experiment. Counts refer to appropriate activity qualified by behavioral sampling procedure in 5-s intervals. Results represent the means ± SEM, $n = 8–16$ rats per group. Two-way analysis of variance for repeated measurements indicated interaction. Therefore, the statistical significance was calculated using one-way ANOVA for each time point, followed by Tukey's test. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

ANOVA indicated differences between groups concerning either the superficial or the deep layers of the cortex [$F(4,30) = 9.84$; $P < 0.001$; $F(4,31) = 7.82$; $P < 0.001$, respectively]. The procedure of the FST itself did not change the binding of [^{3}H]CGP12177 in the cortex, as shown in Fig. 5b and c. In addition, there was no change in the binding of [^{3}H]CGP12177 in the brain cortex of rats treated with three doses of DMI (i.e. doses necessary to evoke the reaction in the FST).

Therefore, significant downregulation of β-ARs observed in the brain cortex of rats treated repeatedly with DMI and subjected to the FST (Fig. 5) can be regarded as an effect of the drug itself rather than the FST.

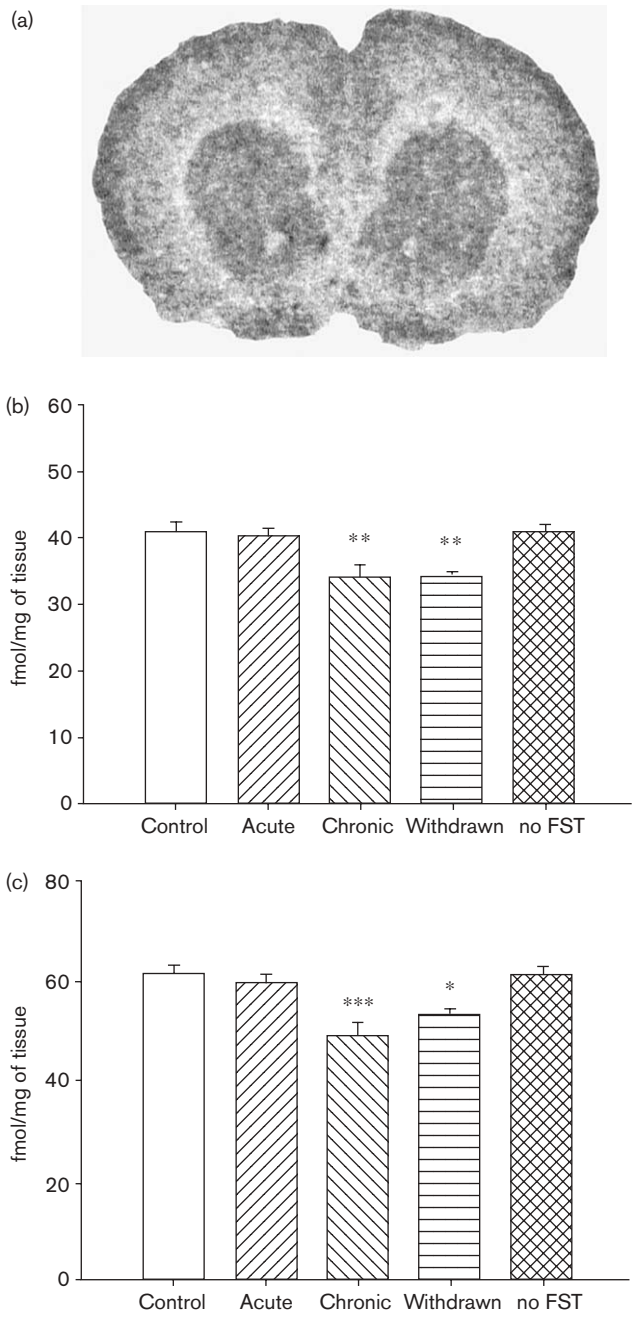
Surprisingly, similar downregulation of β-ARs was observed in the group of rats treated with the single dose of DMI on day 1, followed by daily injections of saline ('withdrawn'), and treated with three doses of DMI before the FST at the end of the experiment (Fig. 5).

Discussion

Inspired by the work of Antelman *et al.*, which concerned the delayed effects of ADs (Antelman *et al.*, 1997, 2000; Antelman and Gershon, 1998), we decided to address the issue of what is more important for the adaptive changes observed in the organism treated with these drugs: a daily dosing of the drug or the time lag following a single drug administration. To study this phenomenon, the FST was chosen as the procedure, as it is most frequently used in preclinical depression research. When the behavioral experiment was conducted in a conventional way; that is (after pretreatment), when the animals were subjected to the FST once at the end of the experiment, the results obtained clearly showed that the single administration of the drug (DMI) was able to induce behavioral changes identical to the changes observed after chronic administration. To induce the typical shortening of immobility time in the FST, all animals were treated with three doses of the drug immediately before testing; however, the shortening of immobility time in the group which was treated with a single dose of DMI on day 1 and then subjected to 'post-withdrawal DMI challenge', it was significantly greater than that observed in the group not subjected to such 'priming'. This result indicates that a single dose of DMI followed by 12 drug-free days was sufficient to trigger adaptive changes in the experimental animals similar to that with repeated daily treatment with this drug.

The same pattern of alterations was observed in these animals at the biochemical level, that is, in the brain β-AR density. A significant decrease in [^{3}H]CGP12177 binding to β-AR was observed in both cortical areas in the group of animals treated chronically with DMI, and also in the group treated acutely with that drug, but only when this

Fig. 5



Effect of desipramine (DMI) administered acutely (three doses), chronically or once at the beginning of the experiment and then following 12 saline injections on consecutive days (withdrawn), according to the procedure described for experiment 1, on [^{3}H]CGP12177 binding in the deep (b) and the superficial (c) layers of the rat cortex. The additional control group was not subjected to the forced swim test, although was treated for the whole experimental period with saline. Results represent the means ± SEM, $n = 7–8$ rats per group. The statistical significance was calculated using one-way analysis of variance followed by Tukey's test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control. Representative autoradiogram of [^{3}H]CGP12177 binding has been shown on panel (a).

treatment was followed by several drug-free days. Although there are reports showing β-AR downregulation induced by a combination of acute treatment with DMI and FST (Duncan *et al.*, 1985; Wędzony *et al.*, 1995), we did not observe such an effect in the present study. Methodological differences might underlie this discrepancy. Similarly, in our earlier studies (Dziedzicka-Wasylewska *et al.*, 1999) with conventional saturation analysis with [^{3}H]CGP12177, we observed statistically significant changes in the density and affinity of the β-AR in the rat brain cortex only in a group of animals treated repeatedly with an antidepressant (IMI).

The results discussed above justify the conclusion that acute treatment with ADs might be sufficient to trigger the cascade of events leading to the altered biosynthesis as well as the functioning of brain receptors.

Investigations of the long-term effects of antidepressant treatment showed changes in the density and function of monoaminergic receptors, in both experimental animals and depressed patients. Therefore, it has been suggested that the acute increase in the synaptic levels of monoamines may only be an early step in a potentially complex cascade of events that ultimately results in antidepressant efficacy (Pineyro and Blier, 1999; Blier, 2001, 2003; Elhwnegi, 2004; Millan, 2004).

From some studies conducted so far, it may be concluded that such an acute increase in the synaptic concentration of amines may be sufficient to begin the biochemical changes without the need of successive daily drug treatment. For example, some of our previous behavioral and biochemical studies have shown that the effects of acute treatment with ADs on α_2-adrenoceptors as well as presynaptic dopamine D_2 and D_3 receptors (Dziedzicka-Wasylewska, 1997; Dziedzicka-Wasylewska and Rogóż, 1997; Dziedzicka-Wasylewska *et al.*, 1999), measured 14 days after drug administration, were similar to (and in some cases stronger than) the effects induced by repeated administration of these drugs. There are also other published data showing the delayed effects of acute ADs administration (Antelman *et al.*, 1983; Lace and Antelman, 1983; Stewart and Rajabi, 1996).

Although difficult to comprehend, these results might be compared with the results of studies concerning long-lasting changes in the hypothalamus–pituitary–adrenal axis after a single session of stress, which also appear to mature with time (Van Dijken *et al.*, 1993; Schmidt *et al.*, 1995, 1996), and, in some aspects, are similar to those observed after chronic repeated stress (De Goeij *et al.*, 1991, 1992a, b; Aubry *et al.*, 1999). On the other hand, the same mechanism might be postulated for sensitization to a stressor and episode sensitization, which may leave

residual traces and produce vulnerability to recurrence of an affective illness (Duman *et al.*, 1994; Kendler *et al.*, 2001), as it is well established that affective disorders develop gradually (Post, 1992). They are triggered by stressful stimuli, which stimulate various neurotransmitter receptors, activating second and third messengers, leading to long-lasting changes in gene expression.

Still, the question of when ADs begin to alleviate depressive symptomatology is very difficult to answer. Using the procedure of the repeated FST, we attempted to address this issue. It should be, however, underlined that, apart from serving as a procedure for testing the effects of ADs, forced swimming is a stressful situation of a mixed psychological (novelty, water) and physiological (exercise, temperature) nature. Dal-Zotto *et al.* (2000) reported that repeated experience with swimming reduced struggling and increased immobility. We did not observe any statistically significant alterations in the behavior of control animals upon repeated exposure to the FST, but rats in our experiments were forced to swim six times over a period of 21 days, and in experiments described by Dal-Zotto *et al.* (2000), the rats were forced to swim every day for consecutive 14 days. Therefore, the experimental schedule – apart from different rat strains used in the two studies (Wistar vs. Sprague–Dawley) – might be a reason for the observed differences.

The effect of a single dose of antidepressant drugs was similar to the effect of repeated dosing of the drug. This effect of IMI persisted throughout the whole experimental period. As for CIT, its influence specifically on immobility and climbing was similar after acute treatment and delayed testing to the effects seen after repeated drug exposure. Acute treatment with DMI did not induce an effect similar to that of repeated treatment throughout the whole period of experiment 2. In experiment 1, however, when an additional dosing of the drug was introduced before the swimming session, the shortening of immobility time was almost identical in both chronically treated and 'withdrawn' groups of animals.

The FST is by no means a model of depressive illness, nor does the repeated FST model the process of recovery, but it is used only to detect the antidepressant-like effects of drugs. Our results obtained with this test indicate that even a single dose of an antidepressant is sufficient to trigger changes similar to those observed upon repeated treatment. If at all these changes are present, they start to be significant relatively early. These results might be regarded as an analogy to the recent clinical data reported by Katz *et al.* (2004), who have shown early drug-specific behavioral changes in depressive patients, which were highly predictive of ultimate clinical response.

On the other hand, the results obtained in the present study may question some conclusions that are usually drawn from the behavioral and especially biochemical studies concerning the need for repeated treatment with antidepressant drugs to induce various adaptive changes in the brain, thought responsible for therapeutic efficacy of these drugs. If ADs efficacy does not require repeated treatment, but if the same effects are produced by just a single dose of the drug, followed by a certain drug-free period of time, then we should correct our understanding of the mechanism of action of ADs.

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References

Antelman SM, Gershon S (1998). Clinical application of time-dependent sensitization to antidepressant therapy. *Prog Neuro-Psychopharmacol Biol Psychiatry* **22**:65–78.

Antelman SM, DeGiovanni LA, Kocan D, Perel JM, Chiodo LA (1983). Amitriptyline sensitization of a serotonin-mediated behavior depends on the passage of time and not repeated treatment. *Life Sci* **33**:1727–1730.

Antelman SM, Soares JC, Gershon S (1997). Time-dependent sensitization: possible implications for clinical psychopharmacology. *Behav Pharmacol* **6–7**:505–514.

Antelman SM, Levine J, Gershon S (2000). Time-dependent sensitization: the odyssey of a scientific heresy from laboratory to the door of the clinic. *Mol Psychiatry* **5**:350–356.

Aubry JM, Bartanusz V, Jezova D, Belin D, Kiss JZ (1999). Single stress induces long-lasting elevations in vasopressin mRNA levels in CRF hypophysiotrophic neurons, but repeated stress is required to modify AVP immunoreactivity. *J Neuroendocrinol* **11**:377–384.

Blier P (2001). Possible neurobiological mechanisms underlying faster onset of antidepressant action. *J Clin Psychiatry* **62** (**Suppl 4**): S7–S11.

Blier P (2003). The pharmacology of putative early-onset antidepressant strategies. *Eur Neuropsychopharmacol* **13**:57–66.

Borsini F, Meli A (1988). Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology* **94**:147–160.

Chiodo LA, Antelman SM (1980). Repeated tricyclics induce a progressive dopamine autoreceptor subsensitivity independent of daily drug treatment. *Nature* **287**:451–454.

Cryan JF, Markou A, Lucki I (2002). Assessing antidepressant activity In rodents: recent developments and future Leeds. *Trends Pharmacol Sci* **23**:238–245.

Cryan JF, Page ME, Lucki I (2004). Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacol* **182**:335–344.

Cryan JF, Valentino RJ, Lucki I (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev* **29**:547–569.

Dal-Zotto S, Marti O, Armario A (2000). Influence of single or repeated experience of rats with forced swimming on behavioral and psysiological responses to the stressor. *Behav Brain Res* **114**:175–181.

De Goeij DCE, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJH (1991). Repeated stress-induced activation of corticotrophin-releasing factor neurons enhances vasopressin stores and colocalization with corticotrophin-releasing factor in the median eminence of rats. *Neuroendocrinology* **53**:150–159.

De Goeij DCE, Binnekade R, Tilders FJH (1992a). Chronic stress enhances vasopressin, but not corticotrophic-releasing factor recreation during hypoglycemia. *Am J Physiol* **263**:E394–E399.

De Goeij DCE, Dijkstra H, Tilders FJH (1992b). Chronic psychosocial stress enhances vasopressin, but not corticotrophin-releasing factor, in the external zone of median eminence of male rats: relationship to subordinate status. *Endocrinology* **131**:847–853.

Delgado PL (2000). Depression: the case for monoamine deficiency. *J Clin Psychiatry* **61**:7–11.

Detke MJ, Lucki I (1996). Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. *Behav Brain Res* **73**:43–46.

Duman RS, Heninger GR, Nestler EJ (1994). Adaptations of receptor-coupled signal transduction pathways underlying stress- and drug-induced neuronal plasticity. *J Nerv Ment Dis* **182**:692–700.

Duman R, Heninger GR, Nestler EJ (1997). A molecular and cellular theory of depression. *Arch Gen Psychiatry* **54**:597–606.

Duncan GE, Paul IA, Harden K, Mueller RA, Stumpf WE, Breese GR (1985). Rapid down regulation of beta adrenergic receptors by combining antidepressant drugs with forced swim: a model of antidepressant-induced neural adaptation. *J Pharmacol Exp Ther* **234**:402–408.

Dziedzicka-Wasylewska M (1997). The effect of imipramine on the amount of mRNA coding for rat dopamine D_2 autoreceptors. *Eur J Pharmacol* **337**:291–296.

Dziedzicka-Wasylewska M, Rogóż Z (1997). Time-dependent effects of antidepressant drugs on the low dose of apomorphine-induced locomotor hypoactivity in rats. *Pol J Pharmacol* **49**:337–343.

Dziedzicka-Wasylewska M, Rogóż Z, Margas W, Dlaboga D, Góralska M (1999). Some behavioral effects of antidepressant drugs are time-dependent. *Prog Neuro-Psychopharmacol Biol Psychiatry* **25**:373–393.

Elhwnegi AS (2004). Central monoamines and their role in major depression. *Prog Neuro-Psychopharmacol Biol Psychiatry* **28**:435–451.

Holoubek G, Nöldner M, Treiber K, Müller WE (2004). Effect of chronic antidepressant treatment on β-receptor coupled signal transduction cascade. Which effect matters most? *Pharmacopsychiatry* **37** (**Suppl 2**): S113–S119.

Katz MM, Tekell JL, Bowden CL, Brannan S, Houston JP, Berman N, Frazer A (2004). Onset of early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology* **29**:566–579.

Kempermann G, Kronenberg G (2003). Depressed new neurons: adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol Psychiatry* **54**:499–503.

Kendler KS, Thornton LM, Gardner CO (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *Am J Psychiatry* **158**:582–586.

Lace JW, Antelman SM (1983). Cortical beta-adrenergic subsensitivity after desmethylimipramine may depend on the passage of time rather than daily treatment. *Brain Res* **278**:359–361.

Lucki I (1997). The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* **8**:523–532.

Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC Jr, *et al.* (2003). Antidepressant-like effects of κ-opioid receptor antagonists in the forced swimming test in rats. *J Pharmacol Exp Ther* **305**:323–330.

Manji HK, Quiraz JA, Sporn J, Payne JL, Denicoff K, Gray NA, *et al.* (2003). Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol Psychiatry* **53**:707–742.

Millan MJ (2004). The role of monoamines in the actions of established and 'novel' antidepressant agents: a critical review. *Eur J Pharmacol* **500**: 371–384.

Pacher P, Kohogyi E, Kecskemati V, Furst S (2001). Current trends in the development of new antidepressants. *Curr Med Chem* **8**:89–100.

Paxinos G, Watson C (1998.) *The rat brain in stereotaxic coordinates*. London: Academic Press.

Pineyro G, Blier P (1999). Autoregulation of serotonic neurons: role in antidepressant drug action. *Pharmacol Rev* **51**:533–591.

Porsolt RD (2000). Animal models of depression: utility for transgenic research. *Rev Neurosci* **11**:53–58.

Porsolt RD, Le Pichon M, Jalfre M (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**:730–732.

Porsolt RD, Anton G, Blavet N, Jelfre M (1978). Behavioral despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* **47**:379–391.

Post RM (1992). Transduction of psychosocial stress into the neurobiology of recurrent affective disorders. *Am J Psychiatry* **149**:999–1110.

Rogóż Z, Skuza G, Maj J, Danysz W (2002). Synergistic effect of uncompetitive NMDA receptor antagonists and antidepressant drugs In the forced swimming test In rats. *Neuropharmacology* **42**:1024–1030.

Schmidt ED, Janszen AWJW, Wouterlood FG, Tilders FJH (1995). Interleukin-1-induced long-lasting changes in hypothalamic corticotrophin-releasing hormone (CRH)-neurons and hyperresponsiveness of the hypothalamus–pituitary–adrenal axis. *J Neurosci* **15**:7417–7426.

Schmidt ED, Binnekade R, Janszen AWJW, Tilders FJH (1996). Short stressor induced long-lasting increases of vasopressin stores in hypothalamic corticotrophin-releasing hormone (CRH) neurons in adult rats. *J Neuroendocrinol* **8**:703–712.

Stewart J, Rajabi H (1996). Initial increases in extracellular dopamine in the ventral tegmental area provide a mechanism for the development of desipramine-induced sensitization within the midbrain dopamine system. *Synapse* **23**:258–264.

Sulser F, Vetulani J, Mobley PL (1978). Mode of action of antidepressant drugs. *Biochem Pharmacol* **27**:257–261.

Van Dijken HH, De Goeij DCE, Sutanto W, Mos J, De Kloet ER (1993). Short inescapable stress produces long-lasting changes in the brain–pituitary–adrenal axis of adult male rats. *Neuroendocrinology* **58**:57–64.

Wędzony K, Klimek V, Nowak G (1995). Rapid down-regulation of β-adrenergic receptors evoked by combined forced swimming test and CGP 37849: a competitive antagonist of NMDA receptors. *Pol J Pharmacol* **47**:537–540.