# Altered Brain Activation Pattern Associated With Drug-Induced Attenuation of Enhanced Depression-Like Behavior in Rats Bred for High Anxiety

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**Background:** The enhanced depression-like behavior in the forced swim test displayed by rats selectively bred for high anxiety-related behavior (HAB) as compared with their low anxiety counterparts (LAB) is abolished by chronic paroxetine treatment. The aim of the present study was to identify neuronal substrates underlying this treatment response in HABs.

**Methods:** The HAB rats received paroxetine (10 mg/kg/day) for 24 days via drinking water, and drug-induced modulation of neuronal activation patterns in response to forced swimming was mapped with the expression of the immediate early gene c-Fos as marker.

**Results:** Chronic paroxetine treatment reduced the immobility scores during forced swimming, confirming the previously observed antidepressant-like effect in these animals, and attenuated the forced swim-induced c-Fos response in a restricted set (11 of 70) of brain areas. These included limbic areas such as the prelimbic cortex, parts of the amygdala, the bed nucleus of the stria terminalis, dorsal hippocampus, dorsal lateral septum as well as hypothalamic and hindbrain areas (dorsolateral periaqueductal gray [PAG], locus coeruleus). Untreated LAB rats, which displayed low depression-like behavior comparable to that of treated HABs, also showed low swim stress-induced c-Fos response in most of these same areas, further supporting an association of attenuated neuronal excitability in the identified areas with attenuated depression-like behavior.

**Conclusions:** These findings indicate that modulation of neuronal activation in a restricted set of defined, mainly limbic as well as selected hypothalamic and hindbrain areas by paroxetine treatment is associated with the reduction of enhanced depression-like behavior in a psychopathological animal model.

**Key Words:** Antidepressant, c-Fos mapping, forced swim, HAB, HPA axis, paroxetine, stress coping

Antidepressant effects of therapeutic drugs are observed in depressed patients but not in healthy volunteers, suggesting that the neurochemical effects of antidepressant drugs might be different in disturbed versus intact systems (Blardi et al 2005; Hokfelt et al 2000). It, therefore, seems plausible to use psychopathologically relevant animal models displaying enhanced depression-like behavior to gain further insight into the neurobiology of depression-related behavior as well as treatment responses, with the ultimate goal of improving and optimizing therapeutic approaches (Adell et al 2005). Indeed, various such animal models were developed with different approaches, including selective breeding. Examples of such models used to reveal behavioral and neurochemical features of enhanced depression-like behavior are the Flinders sensitive rat line (Overstreet 1986), high 8-OH-DPAT sensitive rats (Overstreet et al 1996), rats bred for low swim test activity responses (Weiss et al 1998), the depressed fawn-hooded rats (Rezvani et al 2002), Wistar-Kyoto most immobile rats (Will et al 2003), and rat lines bred for learned helplessness (Henn et al 1993). Unfortunately, functional imaging studies looking at altered neuronal activation patterns in these animals by using positron emission tomography, functional magnetic resonance imaging, or functional stains (e.g., c-Fos-expression, $^{14}$C-2-deoxyglucose uptake) as markers of neuronal activation, likely to be related to depression-like behavior are scarce. For the present study, we, therefore, chose high anxiety-related behavior (HAB) rats and used c-Fos expression as a marker of neuronal activation. High anxiety-related behavior rats and their low anxiety-related behavior (LAB) counterparts are selectively bred depending on their behavior on the elevated plus-maze (Landgraf and Wigger 2002, 2003). Interestingly, apart from differing in anxiety-related behavior in a variety of anxiety tests and, therefore, representing a robust and consistent psychopathological animal model, these animals clearly differ in their depression-related behavior in the forced swim test, the only paradigm investigated so far (Keck et al 2003; Landgraf and Wigger 2003; Liebsch et al 1998; Salome et al 2002). This paradigm is accepted as reflecting differences in depression-like behavior across the commonly used rat strains (Cryan and Holmes 2005) and has predictive value for the efficacy of antidepressant treatment in humans (Cryan et al 2002, 2005; Lucki 1997; Porsolt et al 1977). Specifically, HAB rats float earlier and spend more time immobile/floating than their LAB counterparts, indicating that HABs adopt a passive coping strategy indicative of depression-like behavior, while LABs prefer an active coping style in this (Keck et al 2003; Liebsch et al 1998; Salome et al 2002) and other (Frank et al 2006) tests. Furthermore, chronic paroxetine treatment markedly reduced the passive stress coping in HAB rats only, whereas the behavior of LAB rats remained virtually unchanged (Keck et al 2003), resembling the preferential efficacy of antidepressant drugs in depressed patients versus healthy control subjects.

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Received February 9, 2006; revised June 29, 2006; accepted August 8, 2006.

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2006.08.035

The aims of the present study were: 1) to confirm the behavioral finding of enhanced depression-like behavior in HABs that is sensitive to chronic paroxetine (Keck et al 2003), an antidepressant drug commonly and effectively used to treat clinical depression; and 2) to identify neuronal substrates underlying this behavioral effect of paroxetine by using c-Fos expression as a marker.

Expression of the immediate early gene c-Fos was chosen as an established method for labeling of neuronal activation, with high (cellular) spatial resolution in widespread regions of the brain (Hoffman and Lyo 2002; Kovacs 1998; Morgan and Curran 1991), which has proven useful to aid the therapeutic classification of psychoactive drugs, including antidepressant drugs (Sumner et al 2004). With this functional anatomical tool, it has been demonstrated that acute swim stress induces a widespread, pronounced activation of stress-relevant brain areas (Bellchambers et al 1998; Cullinan et al 1995; Duncan et al 1993, 1996). Because it is speculated that antidepressant drugs modify distinct dysfunctional neuronal excitability in depressed patients (Mayberg 2003), we hypothesized that the antidepressant-like effect of paroxetine in HAB animals would be associated with a modified activation profile in depression-related brain areas. We further predicted that the c-Fos response in paroxetine-treated HAB rats within these key areas should be similar to the c-Fos response in untreated LAB rats, provided that the depression-like behavior of LABs and treated HABs would be at a comparable low level.

## Methods and Materials

### Animals

All animals tested were bred in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany, as described previously (Landgraf and Wigger 2002). In brief, Wistar rats were selected and mated according to the results of an elevated plus maze test, to establish the lines termed HAB and LAB. Rats that spend $<$ 5% or more than 50% in the open arms are considered as HABs or LABs, respectively. All offspring (including those used for the present study) are tested routinely at an age of 10 weeks in Munich to ensure assignment to the HAB or LAB line. This testing also confirmed that the HABs and LABs used did not differ in locomotor activity, as evidenced by a similar "number of closed arm entries" (data not shown; Henniger et al 2000, Landgraf and Wigger 2002). The experiments were carried out on outbred adult male 16–18-week-old HAB and LAB rats weighing 300–400 g, approximately 6–8 weeks after their arrival in Innsbruck. They were housed in groups of 3–5/cage under standard controlled laboratory conditions (12-hour light/dark cycle with lights on at 7:00 AM, 21°C, 50% humidity, pelleted food and water ad libitum). All experimental protocols were approved by the local Ethical Committees on Animal Care and Use.

### Antidepressant Drug Treatment

Male HAB ($n$ = 15) and LAB ($n$ = 8) animals received paroxetine (GlaxoSmithKline, Harlow, United Kingdom) via drinking water over a period of 24 days. The paroxetine solutions were renewed every 2 days, and the paroxetine concentrations were calculated according to body weight and the mean water intake of the rats, which was evaluated by weighing the drinking bottles at each time of renewal. This resulted in a mean drug intake of approximately 10 mg/kg/day. Control HAB rats ($n$ = 20) and LAB rats ($n$ = 22) were supplied with normal drinking water.

### Forced Swim Test

Twenty-four hours before this experiment, animals were taken in their home cages to the experimental room (same lighting cycle) and allowed to habituate. The behavioral test was carried out between 9:00 AM and 2:00 PM, according to the standard protocol/method in our laboratory, which differs slightly from the method used by Keck et al (2003). Our procedure was validated in the past by using different antidepressant drugs, such as citaloprame and NK1 receptor antagonists, all reducing the immobility time (Georg Singewald, Peter Salchner, unpublished).

Rats were individually subjected to the forced swim procedure in a square plastic tank (40 cm × 40 cm, 41 cm high) filled with tap water (23°C) to approximately 30 cm. During the 10-min swim test (light intensity 100 lux), the behavior of the rats was videotaped. After the test session, rats were dried with an absorbent towel. A trained observer blind to the treatment scored the immobility time (floating time) from the videotape with a computer program for ethological analyses (The Observer, Noldus, Wageningen, The Netherlands). Immobility was scored when no additional activity was observed other than that required to keep the rat′s head above water. We focused on this parameter, because: 1) immobility, described as a symptom of "behavioral despair," is generally considered to be the main indicator of depressive-like behavior (Porsolt et al 1977); and 2) immobility is clearly defined and easy to distinguish from the active coping parameters (struggling/swimming), which are sometimes difficult to dissect, leading to varying less reliable results depending on the subjective observations of a certain experimenter. One of the paroxetine-treated HABs had to be excluded from the behavioral data set, because the video stopped recording after 5 min of the forced swim procedure.

### c-Fos Immunohistochemistry

Two hours after the onset of the forced swim test, animals were deeply anesthetized with an overdose of sodium pentobarbital (200 mg/kg) and transcardially perfused with 100 mL of .9% saline followed by 100 mL of 4% paraformaldehyde in .1 mol/L phosphate buffered solution (PBS, pH 7.4). Animals not exposed to the test paradigm were treated identically immediately after removal from their cages in the experimental room. Brains were then removed and postfixed at 4°C overnight in 4% paraformaldehyde in phosphate buffered saline. Coronal sections (100 μm) were cut with a vibratome (Ted-Pella, Redding, California) and collected in immunobuffer. The sections were processed for c-Fos–like immunoreactivity as described previously (Singewald et al 2003), diluted with a polyclonal primary antibody (1:20000; sc-52, Santa Cruz Biotechnology, Santa Cruz, California) and a biotinylated goat antirabbit secondary antibody (1:200; Vector Laboratories, Burlingame, California). Cells containing a nuclear brown-black reaction product were considered as c-Fos-positive cells, and were counted at different levels of the brain in 70 different structures, which (amongst others) are known to show stress-induced increase in c-Fos expression (Cullinan et al 1995; Salchner et al 2006; Salome et al 2004). Many of these regions have been implicated in the depression circuitry (Drevets 2000). The anatomical localization of c-Fos-positive cells was aided by use of adjacent Nissl stained sections and