# Enhanced Long-Term Synaptic Depression in an Animal Model of Depression

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**Background:** A growing body of evidence suggests a disturbance of brain plasticity in major depression. In contrast to hippocampal neurogenesis, much less is known about the role of synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) regulate the strength of synaptic transmission and the formation of new synapses in many neural networks. Therefore, we examined the modulation of synaptic plasticity in the chronic mild stress animal model of depression.

**Methods:** Adult rats were exposed to mild and unpredictable stressors for 3 weeks. Thereafter, long-term synaptic plasticity was examined in the hippocampal CA1 region by whole-cell patch clamp measurements in brain slices. Neurogenesis was assessed by doublecortin immunostaining.

**Results:** Exposure to chronic mild stress facilitated LTD and had no effect on LTP. Chronic application of the antidepressant fluvoxamine during the stress protocol prevented the facilitation of LTD and increased the extent of LTP induction. Neurogenesis in the dentate gyrus was impaired after chronic stress.

**Conclusions:** In addition to neurogenesis, long-term synaptic plasticity is an important and ubiquitous form of brain plasticity that is disturbed in an animal model of depression. Facilitated depression of synaptic transmission might impair function and structure of brain circuits involved in the pathophysiology of major depression. Antidepressants might counteract these alterations.

**Key Words:** Animal model, chronic mild stress, depression, fluvoxamine, hippocampus, long-term synaptic plasticity, neurogenesis

Depression is a devastating disorder with high prevalence and mortality, resulting in massive socioeconomic burden (World Health Organization 2001). Despite its importance, the pathophysiology of affective disorders and the mode of action of antidepressants have been poorly understood. In the last few years, evidence from preclinical and clinical research has accumulated that brain plasticity might be disturbed in depressed patients.

One prominent form of brain plasticity is adult neurogenesis (Duman 2004; Lledo *et al.* 2006). Neural progenitor cells differentiate to granule cells in the olfactory bulb and in the dentate gyrus of the adult hippocampus (Altman and Das 1965; Gage 2000). Stress decreases adult neurogenesis (Gould *et al.* 1998), whereas antidepressant treatment increases the formation of new neurons and blocks the effects of stress (Czeh *et al.* 2001; Malberg *et al.* 2000; Malberg and Duman 2003). It has been postulated that functional neurogenesis is necessary for the action of antidepressants in an animal model of depression and anxiety (Santarelli *et al.* 2003). These preclinical findings might reflect clinical evidence for hippocampal atrophy in mood disorders (Bremner *et al.* 2000).

Despite these important findings, it has been questioned if disturbed neurogenesis could account for all aspects of the clinical course and the etiology of depression (Henn and Vollmayr 2004; Vollmayr *et al.* 2003). Reduced hippocampal volume correlates with the total lifetime number of depressive episodes but not with mood state (Videbech and Ravnkilde 2004). A recent study found a decreased neural stem cell proliferation in the postmortem brains of patients with schizophrenia but not with depression (Reif *et al.* 2006).

This has stimulated research on the role of other more ubiquitous forms of brain plasticity in the etiology of depression. Synapses underlie profound functional and morphological plastic changes. Long-term synaptic plasticity regulates the strength of synaptic transmission. Long-term potentiation (LTP) increases, whereas long-term depression (LTD) decreases, synaptic transmission (Linden 1999; Normann *et al.* 2000). Long-term potentiation and LTD have been demonstrated in most brain regions of rodents and also in slices from human brain (Chen *et al.* 1996). In brain slices, long-term synaptic plasticity can be observed for hours and in living animals it can be observed even for weeks or months. Long-term synaptic plasticity is believed to be the molecular basis of learning and memory (Bliss and Collingridge 1993; Kandel 2001). When efficiency changes of synaptic transmission are consolidated, late phases of long-term synaptic plasticity involve gene transcription, protein synthesis, and ultimately synaptogenesis (Engert and Bonhoeffer 1999; Toni *et al.* 1999), finally leading to permanent morphological changes in the synaptic structure of neuronal networks.

It has been proposed that long-term synaptic plasticity or its modulation might be disturbed in depressed patients (Castrén 2005; Garcia 2002; Popoli *et al.* 2002; Spedding *et al.* 2003; Stewart and Reid 2002). Different antidepressants and electroconvulsive therapy have been shown to effectively modulate synaptic plasticity in the dentate gyrus and the CA1 subfield of the hippocampus and in the neostriatum (De Murtas *et al.* 2004; Levkovitz *et al.* 2001; Shakesby *et al.* 2002; Stewart and Reid 2000; Von Frijtag *et al.* 2001).

We examined hippocampal long-term synaptic plasticity and neurogenesis in the chronic mild stress (CMS) animal model of depression (Willner *et al.* 1987; Willner 2005). The repeated exposure of rodents to mild and unpredictable stressors has been shown to produce behavioral changes that resemble certain core features of human major depression. After CMS, animals show a reduced sensitivity to reward. This anhedonic state can be

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assessed by various behavioral paradigms such as reduced preference for sucrose solutions (Willner *et al.* 1987), attenuated place-preference conditioning (Papp *et al.* 1991), and reduced intracranial self-stimulation behavior (Moreau *et al.* 1992). Stressed animals showed a decrease in sexual activity (D'Aquila *et al.* 1994) and grooming behavior resulting in a degradation of the physical state of the fur (Griebel *et al.* 2002). Sleep architecture was disturbed with rapid eye movement (REM) disinhibition and fragmented sleep patterns (Moreau *et al.* 1995). Plasma corticosterone levels were increased (Grippo *et al.* 2005). The behavioral effects of CMS could be reversed by different classes of antidepressants (Grippo *et al.* 2006; Moreau *et al.* 1992, 1994; Muscat *et al.* 1992) and electroconvulsive therapy (ECT) (Moreau *et al.* 1995) but not by antipsychotics (Moreau 1998; Papp *et al.* 1996). Recent work has demonstrated a suppression of neurogenesis in the hippocampal dentate gyrus of CMS-treated mice and rats and a correlation of behavioral recovery and restored neurogenesis after application of an antidepressant (Alonso *et al.* 2004; Jayatissa *et al.* 2006; Joels *et al.* 2004). The model has been criticized, as the replication in some laboratories has turned out to be difficult (Reid *et al.* 1997). Taken together, however, CMS is an animal model of depression with high face validity (phenomenological reproduction of signs and symptoms of the disorder) and predictive validity (differentiation between effective and ineffective treatments).

By using the chronic mild stress protocol, we tested if long-term synaptic plasticity is altered in experimentally depressed animals and if this could be prevented by an antidepressant.

## Methods and Materials

### Animals and Stress Protocol

Wistar Rats were used for all experiments. Adult rats were 2- to 3-months-old (weight 350–450 g) and were housed in individual cages with free access to food and water in a temperature- (21°C) and light-controlled (12:12 hour light:dark cycle) environment. Juvenile rats (11–14 days postnatal) were used for some LTD experiments.

The CMS protocol was applied at the Hoffmann-La Roche research facility in Basel, Switzerland. Two-month-old rats were subjected to a stress regimen for 3 weeks as described in Table 1 (adapted from Moreau *et al.* 1994). Restraint (R) consisted of confinement to small (24 cm x 10 cm x 9 cm) cages for 1 hour. One night of food and water deprivation (F/Wd) was immediately followed by exposure to restricted food for 2 hours (Fr, scattering of 30 pellets of 20 mg in the cage). Another night of water deprivation (Wd) was followed by exposure to an empty bottle (Eb) for 1 hour. Other elements of the stress protocol were overnight group housing in a soiled cage (GsC) and overnight illumination (Oi). Moreover, the rats were maintained on a reversed light/dark cycle (rLDC) from Friday evening to Monday morning. The animals did not lose weight during the stress protocol. When post-stress behavior was assessed by ventral tegmentum self-stimulation (VTSS) in previous experiments from the same laboratory, CMS proved to be very reliable in inducing an increase in VTSS threshold in >90% of all rats. The VTSS threshold gradually returned to baseline values 10 to 20 days after the termination of the stress regimen (Moreau *et al.* 1992, 1994).

Immediately after the end of the CMS protocol, five groups of eight stressed and eight nonstressed animals each, which were kept at identical conditions except the CMS treatment, were consecutively transferred to the Department of Psychiatry, University of Freiburg, where the electrophysiological recordings were performed within 8 days after the end of the stress protocol. Electrophysiological experiments were done and analyzed in a blinded manner: site staff was not aware if the rats had been previously stressed or not. All protocols were approved by local animal care committees in accordance with institutional guidelines.

### Pharmacological Treatment and Plasma Levels

A group of 12 rats was treated with 20 mg/kg body weight fluvoxamine (dissolved in Tween 80 .3% sodium chloride [NaCl]) (Sigma-Aldrich, Munich, Germany) administered by intraperitoneal injection once daily for 21 days. In rats subjected to CMS ($n$ = 8), fluvoxamine was administered during the complete course of the stress protocol. Control experiments with four stressed rats injected with Tween 80 .3% NaCl once daily for 21 days revealed no difference in the modulation of plasticity. Immediately after decapitation, blood was taken from all rats treated with fluvoxamine and plasma levels were measured by high-pressure liquid chromatography. Plasma adrenocorticotropic hormone (ACTH) and corticosterone levels were determined in a sample of six stressed and six nonstressed rats by radioimmunoassay.

### Slice Preparation

The animals were exposed to a pure oxygen atmosphere for 10 min and immediately afterward anesthetized by isoflurane and killed by decapitation. The brain was removed and transverse 350-μm hippocampal slices were cut using a vibratome (DTK-1000; Dosaka, Kyoto, Japan). Slices were incubated at 34°C for 20 min and subsequently held at room temperature. For dissection, slicing, and storage, a solution containing 125 mmol/L NaCl, 25 mmol/L sodium bicarbonate ($NaHCO_3$), 32.5 mmol/L glucose, 2.5 mmol/L potassium chloride (KCl), 1.25 mmol/L sodium dihydrogen phosphate ($NaH_2PO_4$), 2 mmol/L calcium dichloride ($CaCl_2$), and 1 mmol/L magnesium dichloride ($MgCl_2$) (equilibrated with 95% $O_2$/5% $CO_2$) was used.

### Electrophysiology

Slices were transferred to the recording chamber and superfused with saline solution containing 20 μm picrotoxin to isolate

**Table 1.** The Chronic Mild Stress Protocol

| | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday | Sunday |
|---|---|---|---|---|---|---|---|
| 9:00 | R | R | Fr 2 hours | Eb 1 hour | R | rLDC | rLDC |
| 12:00 | | | | R | | | |
| 16:00 | R | | | R | | | |
| 20:00 | Oi | F/Wd | Wd | GsC | rLDC | | |

R, restraint; Oi, overnight illumination; F/Wd, food and water deprivation; Fr, food restriction; Wd, water deprivation; Eb, exposure to empty bottle; GsC, group housing in soiled cage; rLDC, reversed light dark cycle. For details see Methods and Materials.