PDE4 inhibition enhances hippocampal synaptic plasticity in vivo and rescues MK801-induced impairment of long-term potentiation and object recognition memory in an animal model of psychosis

V Wiescholleck1,2 and D Manahan-Vaughan1,2

Inhibition of phosphodiesterase type 4 (PDE4) by rolipram (4–(3–cyclopentyloxy)-4–methoxyphenyl)-pyrrolidin–2–one) has been the focus of many behavioral and molecular studies in the recent years. Rolipram exhibits memory-enhancing effects in rodents. In vitro studies have shown that long-term potentiation (LTP), which may comprise a cellular substrate for learning, is also enhanced by rolipram. However, effects have not been assessed in vivo. Rolipram has antipsychotic properties. Psychosis affects cognition and in animal models of psychosis LTP is impaired. In this study, we investigated if PDE4 inhibition improves LTP in healthy animals in vivo and if PDE4 inhibition rescues impaired LTP and prevents object recognition memory deficits in an animal model of psychosis. Recordings were made from the hippocampus of adult, freely behaving Wistar rats. Thirty minutes after treatment with rolipram or vehicle, a tetanus was applied to the medial perforant path to elicit short-term potentiation (STP) in the dentate gyrus. At this time-point, radioimmunoassay revealed that rolipram significantly elevated cyclic adenosine monophosphate levels in the dorsal hippocampus, in line with reports by others that rolipram mediates decreased PDE4 activity. In healthy animals, both intracerebroventricular and subcutaneous treatment with rolipram facilitated STP into LTP, suggesting that PDE4 inhibition may have a permissive role in plasticity mechanisms that are relevant for learning and memory. One week after a single systemic treatment with the irreversible N–methyl–D–aspartate antagonist, MK801, LTP and object recognition memory were significantly impaired, but could be rescued by PDE4 inhibition. These data suggest that the relief of cognitive disturbances in psychosis models by rolipram may be mediated in part by a rescue of hippocampal LTP.

Translational Psychiatry (2012) 2, e89; doi:10.1038/tp.2012.17; published online 13 March 2012

Introduction

Inhibition of the phosphodiesterase type 4 (PDE4) by rolipram (4–(3–cyclopentyloxy)-4–methoxyphenyl)-pyrrolidin–2–one) enhances cognition in animals. Several studies have demonstrated that rolipram improves animals’ performance in memory-dependent behavioral tests, such as the object recognition task (ORT) and freezing-to-context task. It selectively inhibits PDE4, which comprises a family of four enzymes (PDE4A–D) that control the hydrolysis of cyclic adenosine monophosphate (cAMP). Consequently, PDE4 inhibition reduces degradation of cAMP, and therefore leads to elevated cAMP levels. cAMP is a second messenger that has a key role in several intracellular cascades, including the cAMP/ protein kinase A (PKA)/ cAMP response element–binding protein (CREB) pathway. The cAMP/PKA/CREB pathway has been shown to be critically involved in learning and memory.5

In vitro studies in CA1 region have further demonstrated that rolipram facilitates long-term potentiation (LTP) in hippocampal slice preparations. LTP comprises an activity-dependent long-lasting strengthening of synaptic transmission. Hippocampal LTP has been proposed to be a cellular mechanism underlying learning and memory. Interestingly, besides being involved in learning and memory, the cAMP/ PKA/CREB pathway is also crucial for LTP and transcription of LTP-related genes. Hence, cognition improvement via PDE4 inhibition might be due to the modulation of this pathway and subsequent enhancement in the ability to express synaptic plasticity. However, the question as to whether rolipram also facilitates LTP in intact brains of healthy freely moving animals has not yet been answered.

Cognition is impaired under many pathological conditions, one of which is psychosis. Although acute psychotic events occur extremely frequently—3 out of 100 people will experience a psychotic episode in their lifetimes—its pathophysiology and...
long-term consequences are not well understood. Acute psychotic episodes can be caused under various conditions, such as for example, schizophrenia, bipolar disorder, schizophreiniform disorder, schizoaffective disorder, drug-induced psychosis, brief reactive psychosis, organic psychoses and delusional disorder. Single psychotic events must be appropriately treated in order to prevent long-term consequences. Recent psychiatric research indicates that there is a critical period after first-episode psychosis, where early intervention is crucial. If treated successfully, there is a higher chance of preventing secondary morbidity, relapse or persistent disability associated with, for example, schizophrenia-related disorders.

To study the cellular and molecular mechanisms underlying single psychotic events, an animal model of acute psychosis was developed in which a single injection of the irreversible uncompetitive N-methyl-d-aspartate receptor (NMDAR)-antagonist, MK801, is applied systemically in rats. Uncompetitive NMDAR-antagonists have been shown to induce psychotic symptoms in healthy humans and to exacerbate symptoms of schizophrenic patients. After a single injection with MK801, rats display short-lasting transient behavioral aspects of psychosis-related behavior, such as disturbed pre-pulse inhibition of the acoustic startle response, disturbed stereotypy and increased ataxia. This is followed by long-term impairments in both spatial memory and in LTP at the perforant path-dentate gyrus synapse of the hippocampus. These findings suggest that long-term disruptions in cognitive ability in psychosis are associated with the loss of LTP.

Recent evidence indicates that alterations in the activity of PDE4 may contribute to the cellular mechanisms underlying psychosis. PDE4 has been shown to bind with a protein called DISC1 (disrupted in schizophrenia 1), which has disrupted chromosome aberrations in schizophrenic brains. DISC1 shows reduced expression in psychotic patients, thereby probably mediating less binding of the dephosphorylated inactive PDE4B and a subsequent pathological elevation of the amount of phosphorylated active PDE4B. Thus, PDE4B activity seems to be directly affecting DISC1 and therefore may have an important role in psychosis-related diseases.

In this study, we asked the question as to whether PDE4 inhibition may enable a rescue of LTP and memory performance in MK801-treated animals. This is not so unreasonable, given reports that rolipram has attenuating effects on MK801-induced impairments in psychosis-related behavior of rodents, indicating that PDE4 might act as a possible new receptor-independent therapeutic target in psychotic disorders by directly affecting PDE4-specific cAMP hydrolysis.

We first addressed the question as to whether rolipram treatment of adult freely behaving rats results in an enhancement of LTP in healthy animals, as this has not yet been explored in vivo. We then examined if PDE4 inhibition rescues LTP that is impaired in the MK801 animal model of psychosis. Next, to validate a peripheral route of application, we investigated if the plasticity-enhancing effects of an intracerebroventricular rolipram injection can be replicated by systemic treatment. And finally, in order to examine functional aspects of our electrophysiological findings, we assessed the effect of rolipram on object recognition memory performance in control and MK801-treated animals.

Materials and methods

The present study was carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for care of laboratory animals and after approval of the local ethic committee (senate of Berlin or Bezirksamt Arnsberg). All efforts were made to reduce the number of animals used.

Animals. Six to seven-week-old male Wistar rats (Charles River, Sulzfeld, Germany) were housed on a 12-h light/dark cycle (lights on at 07:00 AM) for at least 1 week after their arrival in the animal facilities before treatment and surgery.

Surgical implantations. Animals (7–8 weeks old) were anesthetized (52 mg kg⁻¹ pentobarbital via intraperitoneal i.p.) injection and underwent chronic implantation of a monopolar recording electrode in the granule cell layer of dentate gyrus (hole diameter: ca. 1 mm; hole coordinates: 3.1 mm posterior to bregma, 1.9 mm lateral to the midline; electrode depth: ca. 4.2 mm from bone) and a bipolar stimulating electrode in the perforant pathway (hole diameter: 1 mm; hole coordinates: 3.7 mm posterior to bregma, 4.1 mm lateral to the midline; electrode depth: ca. 4.0 mm from bone), as described previously. The perforant pathway-dentate gyrus synapse was selected in line with previous studies of LTP in this animal model of psychosis conducted by our group. In addition, in terms of certain schizophrenia-relevant GABAergic markers, the dentate gyrus has been shown to react first to systemically applied MK801 as compared with all other hippocampal subfields and brain regions. Both electrodes were made of polyurethane-coated stainless steel wire. A cannula was implanted in the lateral cerebral ventricle (0.5 mm posterior to bregma, 1.6 mm lateral to the midline, 5.6 mm length, 0.8 mm diameter, 4 mm depth) to enable injections. The animals were allowed to recover from surgery for 7–10 days before experiments were conducted. Animals which then showed evoked potentials that are not characteristic of the medial perforant path-dentate gyrus synapse were excluded from the study.

Electrophysiological recordings. Throughout all experiments, the animals could move freely within the recording chamber (40 x 40 x 40 cm) and had free access to food and water. For acclimatization the animals were transferred to the experiment room 1 day in advance. The implanted electrodes were connected through a head stage by a flexible cable and a swivel connector to the stimulation unit and amplifier. Recordings were stored on a personal computer.

Responses were evoked by stimulating at low frequency (0.025 Hz, 0.2 ms stimulus duration, 10 000 Hz sample rate). For each time-point, five evoked responses were averaged. Dentate gyrus population spike (PS) amplitude, as well as field excitatory postsynaptic potential (fEPSP) slope were monitored: PS amplitude reflects summed action potentials from the somatic layer of granule cells in the dentate gyrus,
whereas alterations of fEPSP indicate dendritic changes. Each experiment started with an input-output curve (100–900 μA in steps of 100 μA) to determine the stimulus intensity required to elicit a PS that was 40% of the maximum obtained. To ensure stability of recordings and to assess basal synaptic transmission, all animals were tested in a baseline experiment first, where only test-pulse stimulation was applied. LTP was induced by high-frequency stimulation (HFS) (10 bursts of 15 pulses at 200 Hz with a 10 s inter-burst interval). Short-term potentiation (STP) was induced by a weak HFS (wHFS, 3 bursts of 15 pulses at 200 Hz and a 10 s inter-burst interval).

At the beginning of each experiment, baseline PS amplitude and fEPSP slope were obtained by averaging the response to stimulation (five sweeps at 40 s intervals), every 5 min over a period of 30 min. Drug or vehicle injections were then applied either via an intracerebroventricular cannula at the site ipsilateral to electrophysiological recordings, or subcutaneously (s.c.). Injections were followed by additional six recordings in a 30-min or 60-min period. At this point, HFS or wHFS was given (no HFS/wHFS stimulation in baseline experiments), and three additional measurements at 5-min intervals were taken, followed by recordings at 15-min intervals for 24 h.

**Histology.** At the end of the electrophysiological study, brains were removed and histological verification of electrodes and cannula localization was carried out. Brain sections (16 μm) were stained according to the Nissl method using 1% toluidine blue and then examined using a light microscope. Data from animals in which an incorrect electrode or cannula localization was found, or where hippocampal misformations were evident were excluded from the study.

cAMP radioimmunoassay. Right dorsal hippocampi were dissected 30 min after an intracerebroventricular (i.c.v.) injection of 5 μl of either vehicle or rolipram (2.75 μg), and were then shock-frozen in liquid nitrogen before being stored at −80 °C. The tissue was subsequently homogenized in 1 ml ice-cold 70% ethanol and centrifuged for 15 min at 4 °C and 15300 r.p.m. The supernatants were used for the cAMP radioimmunoassay and the pellets were kept for quantification of protein amount via the bicinchoninic acid (BCA) method, performed according to kit instructions (BCA protein assay kit, Pierce, Thermo Scientific, Rockford, IL, USA). The cAMP-radioimmunoassay (IBL International GMBH, Hamburg, Germany), based on the principle of radioimmunoassays for cyclic nucleotides described by Steiner et al. and modified by Harper and Brooker, was performed according to kit instructions. In brief, the supernatant of each sample was dried via vaporization and subsequently diluted in 100 μl radioimmunoassay buffer (100 mM sodium acetate, pH 6.0) and acetylated with triethylamine and acetate hydride (2:1). Samples were incubated for 3 h with 125I-labeled cAMP and the pre-precipitated antiserum. As radioactive and non-radioactive antigens compete for a fixed number of antibody-binding sites, the amount of 125I-labeled antigen bound to the antibody is inversely proportional to the analyzed concentration of the sample. The reaction was stopped by the addition of a co-precipitating solution and a centrifugation step. The precipitate was counted in a gamma counter (Cobra II Auto-Gamma Counter, Packard GMI, USA). cAMP levels were normalized to the amount of protein in the pellet.

**Object recognition task.** The ORT was performed as described elsewhere. The apparatus consisted of a grey square polystyrene cylinder arena (80 × 80 × 80 cm). The test was performed with a constant light intensity of 18 lux. Two different kinds of objects were used: a black polystyrene rectangle tower (9 × 11 × 23 cm) and a transparent glass cylinder (10 cm diameter, 30 cm height). The objects could not be displaced by the animals, as they were weighted with sand or stones.

Three habituation sessions preceded the test, in which the animals were allowed to explore the empty arena for 5 min each. The testing was carried out directly after the last habituation session. The testing comprised two trials of 3-min duration each. A rat was always placed into arena facing the middle of the front wall. During the first trial (T1) an animal was allowed to explore two identical objects, which were placed symmetrically 20 cm away from the walls. After T1 the rat was put back into its home cage. After 4 h the second trial (T2) took place, in which the rat was placed back into the arena and exposed to a familiar and a novel object (Figure 5a). The testing session was videotaped. The times spent exploring each object during T1 and T2 were scored manually. Exploration was defined as directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered as exploratory behavior. In order to avoid the presence of olfactory cues the objects and the arena were always thoroughly cleaned with 70% ethanol, washed with water and dried before each trial. Furthermore, as the objects were available in triplicate, neither of the two identical objects from T1 had to be reused in T2. All combinations and locations of objects were used in a balanced manner to reduce potential biases, such as preferences for particular locations or objects. In addition, total exploration times have been compared between groups in T1 and T2 in order to exclude potential exploration bias. The testing order was determined randomly. Rats that did not explore any of the objects in any trial were removed from analysis.

**Compounds and drug treatment.** The PDE4 inhibitor rolipram (4–3–Cyclopenta[loxy]-4–methylphenyl)-pyrrolidin-2–one) (Biozol, Eching, Germany) was dissolved in 1.5% dimethylsulfoxide (DMSO) or in 3% DMSO and 0.9% physiological saline for i.c.v injection or for systemic injection, respectively. Dilutions of 1.5 or 3% DMSO had no effect on control recordings. For i.c.v injections, rolipram (2.75 μg or 5.5 μg) or vehicle was injected in a 5 μl volume over a 5-min period via a Hamilton syringe 30 min before stimulation to enable diffusion from the lateral cerebral ventricle to the hippocampus to occur. For systemic application, comparable concentrations were calculated, based on known pharmacokinetics of rolipram in the rat (Krause and Kühne, 1988). Rolipram (0.6 mg kg−1 or 1.2 mg kg−1) or vehicle (2 ml kg−1) were injected s.c. 30 or
60 min before stimulation. Systemic injection of the higher dose (1.2 mg kg\(^{-1}\)) caused transient dizziness and catalepsy in the animals, which disappeared approximately 20 min after treatment. In behavioral experiments, rolipram (1.2 mg kg\(^{-1}\)) was injected s.c. 24 h before T2 (Figure 5a), as with this concentration LTP was significantly enhanced for at least 24 h. Furthermore, acute stress effects arising from the injection could thus be excluded.

The NMDAR antagonist \([\mathbf{[+]}\]-5–methyl-10,11–dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine hydrogen maleate (MK801, Tocris, Bristol, UK) was dissolved in 0.9% physiological saline. MK801 (5 mg kg\(^{-1}\)) or vehicle (10 ml kg\(^{-1}\)) were injected i.p. 7 days before commencement of experiments. The concentration of MK801 was chosen in accordance with previous studies conducted by our group.\(^{15-17}\) Directly after injection, acute transient psychosis-like behaviors (locomotion, ataxia and stereotypy) were scored as described elsewhere\(^{16}\) in order to evaluate the effectiveness of the treatment. Animals that did not display this psychosis-like behavioral profile were excluded from further experiments.

**Data analysis.** In all electrophysiological experiments, data were expressed as mean % pre-injection values ± standard error of the mean (s.e.m.). Analysis of variance (ANOVA) was used to evaluate differences between groups in the both control animals studies (i.c.v. and s.c. control studies). The Bonferroni post-hoc test was applied, if more than two groups were compared. ANOVA with repeated measures was used to evaluate differences between pretreatment control experiments and experiments in which test compound or vehicle was applied intracranially in the MK801 study. Therefore, all values after stimulation (weak HFS or HFS) or corresponding values in baseline experiments were compared. In the cAMP assay, quantitative cAMP values for the vehicle and the rolipram groups have been analyzed via a one-way ANOVA. In the behavioral study percentage of time spent in exploring the novel as compared with the familiar object during T2 for each of the four groups. Differences in total exploration times in T1 and T2 have been compared. ANOVA with repeated measures was used to evaluate differences between pretreatment control animals, i.c.v. and s.c. control studies). The Bonferroni post-hoc test revealed a significant increase in potentiation of the PS amplitude after application of both, 2.75 \(\mu\)g \((P<0.01, n=4)\) and 5.5 \(\mu\)g \((P<0.0001, n=6)\) of rolipram, as compared with the control group. The same post-hoc analysis of the fEPSP revealed a significantly enhanced potentiation if the higher \((P<0.0001)\), but not the lower dose of rolipram was used. If only the values recorded in the last hour of the experiment (24–25 h after stimulation) were included in the analysis, there was still a significant effect of treatment \((PS: F (2,59)=6.657, P<0.01); fEPSP: F (2,60)=14.327, P<0.0001\). In this case, the post-hoc test showed a significantly increased PS amplitude \((P<0.01)\) and fEPSP \((P<0.0001)\) for the higher dose \((5.5 \mu\)g\) only, whereas neither PS amplitude nor fEPSP were different from control after lower dose \((2.75 \mu\)g\) treatment. Thus, although both doses were effective, the synaptic potentiation enabled by the higher dose lasted longer. In conclusion, PDE4 inhibition facilitates STP in persistent LTP in vivo, but does not affect basal synaptic transmission.

**Intracerebroventricular (i.c.v.) injection of the PDE4 inhibitor rolipram elevates cAMP levels in the hippocampus 30 min after application.** In our plasticity experiments, the afferent fibers to the hippocampus were stimulated 30 min after application of rolipram. Rolipram has been shown to elevate cAMP levels when given to the hippocampal slice preparation. This occurs as a direct consequence of rolipram-mediated decreased PDE4 activity.\(^{36}\) To check that this is also the case in vivo and within the time-frame of our plasticity experiment, we assessed if cAMP levels were altered after treatment with rolipram. We observed that the cAMP concentration in the dorsal hippocampus, 30 min after i.c.v. treatment with rolipram \((2.75 \mu\)g\) \((n=5)\), was significantly higher \((F (1,8)=11.646, P<0.01; Figure 1f)\) as compared with vehicle-treated animals \((n=5)\). Thus, at the time-point of stimulation rolipram had diffused adequately to the hippocampus and had efficiently decreased PDE4 activity.

**PDE4 inhibition rescues impaired LTP 1 week after an acute MK801 injection.** In controls, we first showed that systemic (i.p.) treatment with vehicle does not have any effect on the expression of LTP. HFS (10 bursts of 15 pulses at 200 Hz, 10 s interburst interval) was used to elicit STP that persisted for approximately 2 h. Intracerebral treatment with rolipram significantly facilitated STP into LTP \((PS: F (2,300)=14.322, P<0.0001, Figure 1c); fEPSP: F (2,304)=19.230, P<0.0001, Figure 1d)\). The subsequent Bonferroni post-hoc test revealed a significant increase in potentiation of the PS amplitude after application of both, 2.75 \(\mu\)g \((P<0.01, n=4)\) and 5.5 \(\mu\)g \((P<0.0001, n=6)\) of rolipram, as compared with the control group. The same post-hoc analysis of the fEPSP revealed a significantly enhanced potentiation if the higher \((P<0.0001)\), but not the lower dose of rolipram was used. If only the values recorded in the last hour of the experiment (24–25 h after stimulation) were included in the analysis, there was still a significant effect of treatment \((PS: F (2,59)=6.657, P<0.01); fEPSP: F (2,60)=14.327, P<0.0001\). In this case, the post-hoc test showed a significantly increased PS amplitude \((P<0.01)\) and fEPSP \((P<0.0001)\) for the higher dose \((5.5 \mu\)g\) only, whereas neither PS amplitude nor fEPSP were different from control after lower dose \((2.75 \mu\)g\) treatment. Thus, although both doses were effective, the synaptic potentiation enabled by the higher dose lasted longer. In conclusion, PDE4 inhibition facilitates STP in persistent LTP in vivo, but does not affect basal synaptic transmission.

**Results**

**PDE4 inhibition facilitates STP into LTP in vivo, but does not affect basal synaptic transmission.** To date, effects of PDE4 inhibitors on hippocampal synaptic plasticity have only been studied in vitro.\(^2,6,35\) We investigated whether an i.c.v. injection with the PDE4 inhibitor, rolipram, leads to alterations in excitability or in ability to express synaptic potentiation in the dentate gyrus of adult freely behaving rats. Basal synaptic transmission was unaffected by rolipram, as no significant changes were found between vehicle-treated control animals \((n=6)\) and animals treated with rolipram \((5.5 \mu\)g, \(n=6)\) in a baseline experiment (PS: \(F (1,228)=0.780, P<0.006, NS\); Figure 2a; fEPSP: \(F (1,229)=1.311, NS, Figure 1b)\). In control animals \((n=6)\), weak HFS (wHFS: 3 bursts of 15 pulses at 200 Hz, 10 s interburst interval) elicited STP that persisted for approximately 2 h. Intracerebral treatment with rolipram significantly facilitated STP into LTP \((PS: F (2,300)=14.322, P<0.0001, Figure 1c); fEPSP: F (2,304)=19.230, P<0.0001, Figure 1d)\). The subsequent Bonferroni post-hoc test revealed a significant increase in potentiation of the PS amplitude after application of both, 2.75 \(\mu\)g \((P<0.01, n=4)\) and 5.5 \(\mu\)g \((P<0.0001, n=6)\) of rolipram, as compared with the control group. The same post-hoc analysis of the fEPSP revealed a significantly enhanced potentiation if the higher \((P<0.0001)\), but not the lower dose of rolipram was used. If only the values recorded in the last hour of the experiment (24–25 h after stimulation) were included in the analysis, there was still a significant effect of treatment \((PS: F (2,59)=6.657, P<0.01); fEPSP: F (2,60)=14.327, P<0.0001\). In this case, the post-hoc test showed a significantly increased PS amplitude \((P<0.01)\) and fEPSP \((P<0.0001)\) for the higher dose \((5.5 \mu\)g\) only, whereas neither PS amplitude nor fEPSP were different from control after lower dose \((2.75 \mu\)g\) treatment. Thus, although both doses were effective, the synaptic potentiation enabled by the higher dose lasted longer. In conclusion, PDE4 inhibition facilitates STP in persistent LTP in vivo, but does not affect basal synaptic transmission.

**Intracerebroventricular (i.c.v.) injection of the PDE4 inhibitor rolipram elevates cAMP levels in the hippocampus 30 min after application.** In our plasticity experiments, the afferent fibers to the hippocampus were stimulated 30 min after application of rolipram. Rolipram has been shown to elevate cAMP levels when given to the hippocampal slice preparation. This occurs as a direct consequence of rolipram-mediated decreased PDE4 activity.\(^{36}\) To check that this is also the case in vivo and within the time-frame of our plasticity experiment, we assessed if cAMP levels were altered after treatment with rolipram. We observed that the cAMP concentration in the dorsal hippocampus, 30 min after i.c.v. treatment with rolipram \((2.75 \mu\)g\) \((n=5)\), was significantly higher \((F (1,8)=11.646, P<0.01; Figure 1f)\) as compared with vehicle-treated animals \((n=5)\). Thus, at the time-point of stimulation rolipram had diffused adequately to the hippocampus and had efficiently decreased PDE4 activity.
In order to replicate the long-term effects of MK801 on synaptic plasticity, that have been reported in previous studies, a second group of animals was injected with MK801 and one week later their ability to express hippocampal LTP was assessed. In line with previous findings, systemic treatment with MK801 resulted in a significant impairment of LTP in the dentate gyrus compared with control LTP. Here, a lower induction magnitude and reduced duration of LTP (2.5 h) were evident compared to pretreatment control LTP.

A third group of animals was treated with MK801 and 1 week later the PDE4 inhibitor rolipram was applied intracerebrally 30 min before an attempt to induce LTP. Under control conditions, vehicle injection was applied i.c.v and here, HFS elicited LTP that lasted for over 24 h (Figure 2c). PDE4 inhibition attenuated the impairment in the induction phase and rescued fully the maintenance phase of LTP. Thus, the MK801-induced impairment in the ability to express LTP was rescued by PDE4 inhibition.

The PDE4 inhibitor, rolipram, enhances synaptic plasticity in vivo, even if applied via a systemic route. On the basis of these results, PDE4 could be considered as a therapeutic target to address, for example, cellular mechanisms of cognition in psychosis-related diseases. However, in this case, peripheral administration would be essential. In order to investigate if rolipram can also enhance plasticity if it is applied systemically, we injected either 0.6 mg kg⁻¹ or 1.2 mg kg⁻¹ amount of rolipram s.c. 30 min before wHFS stimulation. In control animals, wHFS elicited STP in the presence of vehicle (Figure 3a). Subcutaneous treatment with rolipram significantly facilitated STP into LTP (PS: F (2,391) = 10.999, P < 0.0001, Figure 3a; fEPSP: F (2,391) = 20.454, P < 0.0001).
The subsequent Bonferroni post-hoc test revealed that both the lower (PS: $P < 0.05$; fEPSP: $P < 0.0001$) and the higher doses (PS: $P < 0.0001$; fEPSP: $P < 0.0001$, $n = 8$) of rolipram potentiated control STP. Thus, if applied systemically, both doses were sufficient to enhance synaptic plasticity. Analysis of the values recorded in the last hour of the experiment (24–25 h after stimulation) revealed that the higher dose is more effective (PS: $F (2,72) = 5.026$, $P < 0.01$; fEPSP: $F (2,60) = 4.492$, $P < 0.05$).

Systemic application of the PDE4 inhibitor rolipram at the higher dose (1.2 mg kg$^{-1}$) induces transient chemical potentiation, which lasts for 1 h after injection. Subcutaneous rolipram injection at the dose of 1.2 mg kg$^{-1}$ significantly potentiated the PS amplitude and the fEPSP immediately after injection (Figure 3a) (PS: $F (2,117) = 12.594$, $P < 0.0001$, Figure 3a; fEPSP: $F(2,117) = 14.185$, $P < 0.0001$, not shown). In order to study this phenomenon of chemical potentiation in more detail, a baseline experiment was performed in which either vehicle ($n = 9$) or rolipram was applied s.c. at the dose of 1.2 mg kg$^{-1}$ ($n = 11$). This baseline experiment revealed that PS amplitude and fEPSP remained significantly enhanced for 1 h (PS: $F (1,131) = 66.084$, $P < 0.0001$, Figure 3b; fEPSP: $F (1,131) = 38.137$, $P < 0.0001$, not shown). Therefore, PS amplitude and fEPSP values returned to baseline levels and remained stable for the remainder of the 25 h recording period. Following their return to pre-injection baseline levels, the evoked potentials were no longer different from controls (PS: $F (1,18) = 0.347$, NS; fEPSP: $F (1,18) = 0.025$, NS). Thus, systemic injection of the higher dose of rolipram (1.2 mg kg$^{-1}$) induces a transient chemical potentiation of synaptic strength.

Systemically administered rolipram facilitates STP into LTP, if wHFS is applied after synaptic transmission returns to basal levels. In this experiment (Figure 4), we waited for more than 1 h after the systemic application of the higher dose of rolipram (1.2 mg kg$^{-1}$) before tetanic stimulation was applied. In this way, we wanted to make sure that basal synaptic transmission was comparable between controls and treated animals, when wHFS was applied. The last time-point before wHFS did not differ between controls ($n = 8$) and rolipram-treated animals ($n = 8$) (PS: $F (1,14) = 1.042$, NS; Figure 4a; fEPSP: $F
In this case, wHFS in the presence of rolipram still resulted in a significantly facilitated STP as compared with controls (PS: F (1,352) = 6.449, P < 0.05, Figure 4a; fEPSP: F (1,352) = 6.145, P < 0.05; not shown). Thus, systemic application of rolipram facilitates STP into LTP independent of changes in basal synaptic transmission.

One week after i.p. treatment with MK801 (n = 10), object recognition memory performance was significantly impaired, as no difference between time spent exploring the familiar and the novel objects could be observed (F (1,18) = 0.213, NS; Figure 5e). In contrast, the control group (n = 10), treated with saline 1 week before behavioral testing, clearly distinguished between the familiar and the novel objects 4 h after T1 (F (1,18) = 13.813, P < 0.01; Figure 5d).

If rolipram was injected s.c. 24 h before T2, vehicle- (n = 10) and MK801-treated animals (n = 8), both significantly remembered the familiar object after 4 h (F (1,18) = 60.691, P < 0.0001 and F (1,14) = 8.078, P < 0.01, respectively; Figures 5f and g, respectively). Thus, treatment with rolipram prevented MK801-induced deficits in object recognition memory.
Discussion

The present study demonstrates that PDE4 inhibition facilitates hippocampal LTP in freely behaving healthy animals after intracerebroventricular (i.c.v.) as well as after systemic (s.c.) administration, and that it rescues long-term impairments in LTP and in object recognition memory that occur in an animal model of psychosis.

Phosphodiesterases (PDEs) are enzymes that break down cyclic nucleotides, and have an important role in intracellular signaling. Hence, PDE inhibitors elevate concentrations of second messengers, such as cAMPs and cyclic guanosine monophosphates (cGMPs), thereby affecting major intracellular cascades involved in synaptic plasticity. The activity of PKA is determined by cAMP, and PKA mediates factors such as CREB that triggers gene transcription, which is in turn essential for long-lasting LTP and memory. Rolipram is known to selectively inhibit cAMP-specific PDE4 and to increase cAMP levels by preventing its hydrolysis. Our results support that this is also the case when rolipram is injected in vivo; i.e., injection of rolipram increases the hippocampal cAMP concentration. Rolipram activates the
mitogen-activated protein kinase (MAPK) pathway via Rap1 but not Ras signaling. Treatment with rolipram also increases hippocampal expression and phosphorylation of CREB. Therefore, the cAMP/PKA/MAPK/CREB pathway appears to be specifically involved in the rolipram-mediated effects on synaptic plasticity and memory.

Behavioral studies have demonstrated an improving effect of rolipram in memory-related tasks. Rolipram also enhances synaptic plasticity in the hippocampal slice preparation. Our study is the first one to complete this picture of evidence, in vivo, by demonstrating that PDE4 inhibition also facilitates hippocampal LTP in the intact brains of freely behaving rats.

Although primarily known from the neuroenhancement research area and from aborted clinical trials as an antidepressant, recent behavioral studies indicate a possible therapeutic role of PDE4 inhibition in psychosis-related diseases. On the molecular level, common antipsychotics primarily block the dopamine D2-receptors and thereby cause an increase in cAMP, implying a possible therapeutic potential for cAMP-concentration-elevating PDE inhibitors in schizophrenia spectrum disorders. Of note, currently available antipsychotics are able to treat schizophrenia-related cognitive dysfunctions. In this respect, PDE4 might be a more promising target.

In this study, we demonstrated that PDE4 inhibition prevents MK801-induced long-term deficit in the ability to express LTP in freely moving rats. Through application of rolipram 1 week after MK801 treatment, LTP was enabled that lasted for over 24 h. Specifically, the maintenance phase was fully rescued. This is in line with the finding that cAMP has a role primarily in the later phase of LTP, as well as in long-term rather than in short-term memory. In parallel, demonstrating a functional aspect of these electrophysiological findings, we found that a single MK801 treatment leads to long-term impairments in object recognition memory and that PDE4 inhibition prevents these MK801-induced memory deficits. In our behavioral study, animals were treated with rolipram 20 h before the first trial of the ORT. Thus, the ability of rolipram to prevent MK801-induced deficit in object recognition memory lasted far beyond its half-life time of 1–3 h. Others have already demonstrated persistent effects of subchronic and chronic rolipram treatment. Our behavioral and electrophysiological results indicate that an acute injection with rolipram is sufficient to trigger molecular changes related to memory and synaptic plasticity lasting for more than 20 h. Presumably, this happens via activation of CREB-dependent gene expression.

Besides suggesting a possible therapeutic target, these findings also give us insights into potential pathophysiological mechanisms underlying schizophrenia. Why are plasticity and object recognition memory still impaired 1 week after an acute MK801-induced psychotic event? Which mechanisms lead to the ability of PDE4 inhibition to restore it? Our animal model is based on the NMDAR-hypofunction hypothesis of schizophrenia, which assumes that a deficiency in NMDAR functioning has a major role in schizophrenia. If given systemically, MK801 increases spontaneous firing rates in the medial prefrontal cortex, specifically via a hippocampo-prefrontal pathway. Thus, among these two schizophrenia-related brain structures, the medial prefrontal cortex and the hippocampus, the latter seems to be activated first by MK801. However, presumably effects within both regions and their circuits lead to the overall psychotomimetic profile of MK801. After a single application of MK801, a high proportion of NMDARs are irreversibly blocked. This treatment results in a physiological long-term change, which lasts for at least 1 week, as reflected by the impairment of synaptic plasticity and object recognition memory that we have observed in our study. The mechanism underlying this effect may relate to NMDAR hypofunction resulting from a loss of receptors but also to a functional impairment of the receptor itself. This possibility is supported by findings that increasing available glycine rescued impaired LTP in the same MK801 animal model. However, our current finding that impaired LTP is also rescued by PDE4 inhibition suggests that alterations of the cAMP concentration in the brain might additionally have an important role in this phenomenon. Indeed, some evidence exists for a possible direct link between NMDARs and cAMP levels. For instance, Skeberdis et al. have shown that PKA regulates Ca²⁺ influx through NMDARs in vitro. Specifically, this study demonstrated that the Ca²⁺ permeability of neuronal NMDARs is controlled by the cAMP-PKA signaling cascade. PKA inhibition led to a decreased NMDAR-mediated Ca²⁺ -rise in activated dendritic spines, indicating that PKA might also have a role in the regulation of both the induction and maintenance of LTP.

Another interesting link in this respect is provided by the fact that the NR1 subunit of the NMDAR is phosphorylated by PKA at the S897 site and that mice deficient for this phosphorylation display a schizophrenia-relevant behavioral profile. Additionally, the phosphorylation at this site has been shown to be reduced in schizophrenic patients. Zhang et al. have shown that inhibition of PDE4 reverses memory deficits associated specifically with NMDAR antagonism. Moreover, PDE4 inhibitor rolipram has been demonstrated to enhance the ability of NMDA to increase cAMP in neurons. This effect could be antagonized by application of MK801. Finally, it has been recently reported that cAMP production is modulated by PDE4 in response to NMDAR stimulation via a pharmacological NMDAR agonist in mouse cortical neurons, thereby providing another link between NMDAR-functioning and PDE4 (source: schizophrenia research forum website).

Despite these first pieces of evidence, it remains speculative as to whether cAMP levels depend on the NMDAR, if they change as a consequence of NMDAR hypofunction, or if cAMP itself has a causative role in this respect by influencing NMDAR currents. It is furthermore unclear if PDE4 inhibition rescues MK801-induced deficits by ‘kicking-in’ at a more downstream level of NMDAR-dependent signaling cascades, leaving the dysfunctional upstream part unaffected, or if elevated cAMP levels directly interact with NMDARs, thereby restoring relevant cascades at an early phase of the process. The latter point is supported by in vitro data that suggest that PDE4 and DISC1 associate with NR1, a mandatory NMDAR-subunit (source: schizophrenia research forum website).

In order to further study the therapeutic potential of PDE4 inhibition, it is important to see if rolipram would display similar...
effects on hippocampal synaptic plasticity after a peripheral application. We show here that a systemic injection of rolipram has similar beneficial effects as an application directly into the brain. Behavioral studies have already indicated that systemic application of rolipram leads to an enhanced effect on cognitive performance, \textsuperscript{1,2,41} and our study suggests a possible mechanism underlying this effect: namely, facilitation of hippocampal synaptic plasticity. Our data support that, after peripheral administration in vivo, rolipram reaches the hippocampus, the brain structure that is most prominently involved in learning and memory processes, and facilitates its ability to express LTP on the cellular level. Interestingly, a high-dose subcutaneous injection induced a transient potentiation of PS, suggesting that a brief increase in somatic excitability occurred. This finding is interesting in its own right as it suggests that rolipram is able to enhance cellular excitability in the absence of external electrical stimulation. Higher doses of rolipram could be expected to prolong the duration of this chemical potentiation, and this may also have interesting therapeutic implications.

Nonetheless, although PDE4 inhibition displays an interesting new target with neuroenhancing and therapeutic potential and although rolipram provides a valuable tool in order to study this mechanism of action, this particular substance has to be first pharmacologically tuned in order to make it relevant for clinical consideration. A known side-effect of rolipram is nausea.\textsuperscript{46} And indeed, we also observed transient dizziness in our rats right after a systemic injection of the higher dose of rolipram. This emetic effect makes it rather difficult to test rolipram in human trials. Additionally, as molecular evidence has shown specifically PDE4B to be a genetic susceptibility factor for schizophrenia,\textsuperscript{23} more subtype-selective PDE4 inhibitors would be interesting to study in the future.

In conclusion, our results support that treatment with a PDE4 inhibitor facilitates synaptic plasticity in the intact brains of healthy animals and restores impaired LTP and object recognition memory in an animal model of psychosis. These data provide further insight into possible mechanisms underlying psychosis pathology. We suggest that the antipsychotic-like profile of PDE4 inhibition is mediated, in part, by a rescue of impaired LTP that enables improved hippocampus-depending cognition.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. We thank Jens Klausnitzer for technical assistance, Nadine Gomell for animal care and Prof Dr Doris Koesling, as well as Isabel Schönle for support and advice on the RIA experiments. WV is a scholarship recipient of the International Graduate School of Neuroscience (IGSN) of the Ruhr University.

1. Rutten K, Prikkaerts J, Schaedele G, Rosenbrock H, Blokland A. Sub-chronic rolipram treatment leads to a persistent improvement in long-term object memory in rats. Neurobiol Learn Mem 2008; 90: 569–575.
2. Barad M, Bourchouladze R, Winder DG, Golan H, Kandel E. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. Proc Natl Acad Sci USA 1998; 95: 15020–15025.
3. van Staveren WC, Markenink-van Itersum M, Steinbusch HW, de Vente J. The effects of phosphodiesterase inhibition on cyclic GMP and cyclic AMP accumulation in the hippocampus of the rat. Brain Res 2001; 888: 275–286.
4. Walteret R, Weller M. Signaling from cAMP/PKA to MAPK and synaptic plasticity. Mol Neurobiol 2003; 27: 99–106.
5. Barco A, Pittenger C, Kandel ER, CREB, memory enhancement and the treatment of memory disorders: promises, pitfalls and prospects. Expert Opin Ther Targets 2003; 7: 101–114.
6. Navalkode S, Sajikumar S, Frey JU. The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. J Neurosci 2004; 24: 7740–7744.
7. Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. J Physiol 1973; 222: 331–356.
8. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993; 361: 31–39.
9. Kemp A, Manahan-Vaughan D. Hippocampal long-term depression: master or minion in declarative memory processes? Trends Neurosci 2007; 30: 111–118.
10. Douki S, Taleik MJ, Ben Zineb S, Cheour M. Therapeutic strategies in the first psychotic episode. Encephale 1999; 25(Spec No 3): 44–51.
11. McCorry PD, Killackey E, Yung A. Early intervention in psychosis: concepts, evidence and future directions. World Psychiatry 2008; 7: 148–156.
12. Goldner-Vukov M, Cupina DD, Moore LJ, Baba-Mikie N, Milovanovie S. Early intervention in first episode psychosis: hope for a better future. Sip Arh Celob Lek 2007; 132: 672–678.
13. Birchwood M, Todd P, Jackson C. Early intervention in psychosis. The critical period hypothesis. Br J Psychiatry Suppl 1998; 172: 53–59.
14. Bird V, Premkumar F, Kendall T, Whittington C, Mitchell J, Kuipers E. Early intervention services, cognitive-behavioural therapy and family intervention in early psychosis: systematic review. Br J Psychiatry 2010; 197: 350–356.
15. Wohlt R, Eisenach S, Manahan-Vaughan D, Heinemann U, von Haebler D. Acute and long-term effects of MK-801 on direct cortical input evoked homosynaptic and heterosynaptic plasticity in the CA1 region of the female rat. Eur J Neurosci 2007; 26: 2973–2983.
16. Manahan-Vaughan D, von Haebler D, Winter C, Juckel G, Heinemann U. A single application of MK801 causes symptoms of acute psychosis, deficits in spatial memory, and impairment of synaptic plasticity in rats. Hippocampus 2008; 18: 125–134.
17. Manahan-Vaughan D, Wildfoster V, Thomassen C. Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). Eur J Neurosci 2008; 28: 1342–1350.
18. Lahl AC, Weiler MA, Tamara Michaelaids BA, Parwani A, Tammnga CA. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 2001; 25: 455–467.
19. Tammnga CA, Holcomb HH, Gao XM, Lahl AC. Glutamate pharmacology and the treatment of schizophrenia: current status and future directions. Int Clin Psychopharmacol 1995; 10 Suppl 3: 29–37.
20. Davis JA, Gould TJ. Rolipram attenuates MK-801-induced deficits in latent inhibition. Behav Neurosci 2005; 119: 595–602.
21. Kanes SJ, Tokarzycyk J, Siegel SJ, Bilkcr W, Abel T, Kcly MP. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. Neuroscience 2007; 144: 239–246.
22. Millar JK, Mackie S, Clapcote SJ, Murdoch H, Pickard BS, Christie S et al. Disrupted in schizophrenia 1 and phosphodiesterase 4B: towards an understanding of psychiatric illness. J Physiol 2007; 586(Pt 2): 401–405.
23. Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate AMP signaling. Science 2005; 310: 1187–1191.
24. Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders— cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am J Hum Genet 2001; 69: 428–433.
25. Sachs NA, Sawa A, Holmes SE,Ross CA,DeLiis LE, Margolis RL. A framework mutation in Disrupted in Schizophrenia 1 is an American family with schizophrenia and schizoaffective disorder. Mol Psychiatry 2005; 10: 758–764.
26. Sawa A, Snyder SH. Genetics. Two genes link two distinct psychoses. Science 2005; 310: 1128–1129.
27. Mackie S, Millar JK, Porteous DJ. Role of DISC1 in neural development and schizophrenia. Curr Opin Neurobiol 2007; 17: 15–22.
28. Zhang HT, Crissman AM, Dorosay NR, Chandler LJ, O’Donnell JM. Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. Neuropsychopharmacology 2000; 23: 198–204.
29. Buciaik JA, Chapi OS, McCarthy SA, Martin AN. Antipsychotic profile of rolipram: efficacy in rats and reduced sensitivity in mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. Psychopharmacology 2007; 192: 415–424.
30. Manahan-Vaughan D, Braunevell KH, Reymann KG. Subtype-specific involvement of metabotropic glutamate receptors in two forms of long-term potentiation in the dentate gyrus of freely moving rats. Neuroscience 1998; 85: 709–721.
31. Romón T, Mengod G, Adell A. Expression of parvalbumin and glutamic acid decarboxylase-67 after acute administration of MK-801. Implications for the NMDA hypofunction model of schizophrenia. *Psychopharmacology* 2011; 217: 231–238.

32. Steiner AL, Parker CW, Kipnis DM. Radioimmunoassay for cyclic nucleotides. I. Preparation of antibodies and isolated cyclic nucleotides. *J Biol Chem* 1972; 247: 1056–1113.

33. Harper JF, Brooker G. Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2’O acetylation by acetic anhydride in aqueous solution. *J Cyclic Nucleotide Res* 1975; 1: 207–218.

34. Prickaerts J, de Vente J, Honig W, Steinbusch HWM, Blokland A. cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol* 2002; 436: 83–87.

35. Navakkode S, Sakjukom S, Frey JU. Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging. *J Neurosci* 2005; 25: 10664–10670.

36. Navakkode S, Korte M. Cooperation between cholinergic and glutamatergic receptors are essential to induce BDNF-dependent long-lasting memory storage. *Hippocampus* 2011; 22: 335–346. doi: 10.1002/hipo.20992.

37. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* 2001; 76: 1–10.

38. Beavo JA. Multiple isozymes of cyclic nucleotide phosphodiesterase. *Adv. Second Messenger Phosphoprotein Res* 1988; 22: 1–38.

39. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 2006; 58: 488–520.

40. Monti B, Bertotti C, Contatlibale A. Subchronic rolipram delivery activates hippocampal CREB and arc, enhances retention and slows down extinction of conditioned fear. *Neuropsychopharmacology* 2008; 31: 278–286.

41. Li Y, Cheng Y, Huang Y, Conti M, Wilson SP, O’Donnell JM et al. Phosphodiesterase-4D knock-out and RNA interference-mediated knock-down enhance memory and increase hippocampal neurogenesis via increased cAMP signaling. *J Neurosci* 2011; 31: 172–183.

42. Rutten K, Basle JL, Prickaerts J, Blokland A, Vivian JA. Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology* 2009; 196: 643–648.

43. Rennertken OA, Rutten K, Steinbusch HW, Blokland A, Prickaerts J. Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. *Psychopharmacology* 2009; 202: 419–443.

44. Normann C, Berger M. Neuroenhancement: status quo and perspectives. *Eur Arch Psychiatry Clin Neurosci* 2006; 256(Suppl 5): 110–114.

45. Zeller E, Stief HJ, Pflug B, Sastre-y Hernández M. Results of a phase II study of the antidepressant effect of rolipram. *Pharmacopsychiatry* 1984; 17: 188–190.

46. Hebenstreit GF, Fellerer K, Fichte K, Fischer G, Geyer N, Maya U et al. Rolipram in major depressive disorder: results of a double-blind comparative study with imipramine. *Pharmacopsychiatry* 1989; 22: 156–160.

47. Halene TB, Siegel SJ. Antipsychotic-like properties of phosphodiesterase 4 inhibitors: evaluation of 4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone (RO-20-1724) with auditory event-related potentials and prepulse inhibition of startle. *J Pharmacol Exp Ther* 2008; 326: 230–239.

48. Carpenter WT, Koenig J. The evolution of drug development in schizophrenia: past issues and future opportunities. *Neuropsychopharmacology* 2008; 33: 2061–2079.

49. Hyo U, Huang YY, Kandel ER. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 1993; 260: 1661–1664.

50. Krause W, Köhne G. Pharmacokinetics of rolipram in the rhesus and cynomolgus monkeys, the rat and the rabbit. Studies on species differences. *Xenobiotica* 1988; 18: 581–571.

51. Gong B, Vitolis OV, Trinchese F, Liu S, Shelnazi M, Aranoto O. Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J Clin Invest* 2004; 114: 1624–1634.

52. Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S et al. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurol* 2008; 31: 234–242.

53. Gordon JA. Testing the glutamate hypothesis of schizophrenia. *Nat Neurosci* 2010; 13: 2–4.

54. Jodo E, Suzuki Y, Katayama T, Hoshino K, Takeuchi S, Niwa S et al. Activation of medial prefrontal cortex by phenycyclidine is mediated via a hippocampal-prefrontal pathway. *Cereb Cortex* 2005; 15: 663–669.

55. Rosenmund C, Clements JD, Westbrook GL. Nonuniform probability of glutamate release at a hippocampal synapse. *Science* 1993; 262: 754–757.

56. Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Sudicani SO et al. Protein kinase A regulates calcium permeability of NMDA receptors. *Nat Neurosci* 2006; 9: 501–510.

57. Li B, Devizne N, Barengotis D, Prostak N, Spicolas E, Apicella AJ et al. NMDA receptor phosphorylation at a site affected in schizophrenia controls synaptic and behavioral plasticity. *J Neurosci* 2009; 29: 11965–11972.

58. Emmanian ES, Karayiorgou M, Gogas JA. Decreased phosphorylation of NMDA receptor type 1 at serine 897 in brains of patients with Schizophrenia. *J Neurosci* 2004; 24: 1561–1564.

59. Zhang H, Huang Y, Suvarna NU, Deng C, Crissman AM, Hopper AT et al. Effects of the novel PDE4 inhibitors MEM1018 and MEM1091 on memory in the radial-arm maze and inhibitory avoidance tests in rats. *Psychopharmacology* 2005; 179: 613–619.

60. Suvarna NU, O’Donnell JM. Hydrolysis of N-methyl-D-aspartate receptor-stimulated cAMP and cGMP by PDE4 and PDE2 phosphodiesterases in primary neuronal cultures of rat cerebral cortex and hippocampus. *J Pharmacol Exp Ther* 2002; 302: 249–256.

61. Rampino A, DISC 2010 – protein with many talents. *Schizophrenia Research Forum* website, 5 November 2010, accessed 10 April 2011. Available from: http://www.schizophreniaforum.org/new/detail.asp?id = 1632.

---

**Translational Psychiatry** is an open-access journal published by *Nature Publishing Group*. This work is licensed under the Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0/