Neuropharmacology 55 (2008) 908–918

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents☆

F. Josef van der Staay [a,*], Kris Rutten [c], Lars Bärfacker [b], Jean DeVry [a], Christina Erb [a], Heike Heckroth [b], Dagmar Karthaus [b], Adrian Tersteegen [b], Marja van Kampen [a], Arjan Blokland [d], Jos Prickaerts [c], Klaus G. Reymann [e], Ulrich H. Schröder [e], Martin Hendrix [b]

[a] BAYER HealthCare AG, Global Drug Discovery, Department of CNS Research, D-42096 Wuppertal-Elberfeld, Germany
[b] BAYER HealthCare AG, Global Drug Discovery, Department of Chemistry Research, D-42096 Wuppertal-Elberfeld, Germany
[c] Maastricht University, Department of Psychiatry and Neuropsychology, PO Box 616, 6200 MD Maastricht, The Netherlands
[d] Maastricht University, Department of Psychology, PO Box 616, 6200 MD Maastricht, The Netherlands
[e] Leibniz Institute for Neurobiology, Brenneckestrasse 6, 39118 Magdeburg, Germany

ARTICLE INFO

Article history:
Received 21 November 2007
Received in revised form 1 July 2008
Accepted 4 July 2008

Keywords:
Phosphodiesterase 9 inhibitor
Learning
Memory
LTP
Rat
Mouse
Social recognition
Object recognition
Passive avoidance
T-maze alternation
MK-801
Scopolamine

ABSTRACT

The present study investigated the putative pro-cognitive effects of the novel selective PDE9 inhibitor BAY 73-6691. The effects on basal synaptic transmission and long-term potentiation (LTP) were investigated in rat hippocampal slices. Pro-cognitive effects were assessed in a series of learning and memory tasks using rodents as subjects.

BAY 73-6691 had no effect on basal synaptic transmission in hippocampal slices prepared from young adult (7- to 8-week-old) Wistar rats. A dose of 10 μM, but not 30 μM, BAY 73-6691 enhanced early LTP after weak tetanic stimulation. The dose effective in young adult Wistar rats did not affect LTP in hippocampal slices prepared from young (7- to 8-week-old) Fischer 344 X Brown Norway (FBNF1) rats, probably reflecting strain differences. However, it increased basal synaptic transmission and enhanced early LTP after weak tetanic stimulation in hippocampal slices prepared from very old (31- to 35-month-old) FBNF1 rats.

BAY 73-6691 enhanced acquisition, consolidation, and retention of long-term memory (LTM) in a social recognition task and tended to enhance LTM in an object recognition task. Bay 73-6691 attenuated the scoplamine-induced retention deficit in a passive avoidance task, and the MK-801-induced short-term memory deficits in a T-maze alternation task. The mechanism of action, possibly through modulation of the NO/cGMP-PKG/CREB pathway, is discussed. Our findings support the notion that PDE9 inhibition may be a novel target for treating memory deficits that are associated with aging and neurodegenerative disorders such as Alzheimer's disease.

1. Introduction

Phosphodiesterases (PDEs) are enzymes that breakdown cyclic nucleotides, i.e. cAMP or cGMP. Thus far, 11 classes of PDEs have been identified, based on their substrate (Beavo, 1995). PDEs are selective for the second messengers cAMP, cGMP, or both. These nucleotides play an important role in intracellular signaling and in processes of neuroplasticity, such as long-term potentiation (LTP) (Frey et al., 1993; Son et al., 1998). PDE inhibitors elevate concentrations of cAMP, cGMP or both, which in turn trigger various intracellular mechanisms.

Over the years, convincing experimental evidence has accumulated supporting the cognition-enhancing properties of several classes of PDE inhibitors (for review see Blokland et al., 2006). Cognition-enhancing effects have been documented for different PDE inhibitors in rats (PDE2: Boess et al., 2004; Rutten et al., 2007; PDE4: Rutten et al., 2006; Zhang and O'Donnell, 2000; PDE5: Prickaerts et al., 2004; Rutten et al., 2007) and mice (e.g. Barad et al., 1998; Baratti and Boccia, 1999; Rutten et al., 2005), and recently in macaques (Rutten et al., 2008).

PDE inhibitors mediate cellular signaling processes by elevating the level of cAMP and/or cGMP, which ultimately can lead to gene transcription through activation of the CREB signaling pathways (Impey et al., 1996; Lu et al., 1999). Recently, the cGMP/PKG/CREB pathway and the cAMP/PKA/CREB pathway have been discussed as

☆ Further development of this compound has been stopped.

* Corresponding author. Present address: Program Emotion and Cognition, Department of Farm Animal Health, Veterinary Faculty, Utrecht University, Marburglaan 2, 3584 CN Utrecht, The Netherlands. Tel.: +31 (0) 30 253 4205; fax: +31 (0) 30 252 1887.
E-mail address: f.j.vanderstaay@uu.nl (F.J. van der Staay).

0028-3908/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2008.07.005

possible mechanisms underlying the observed cognition-enhancing effects of PDE inhibitors (Blokland et al., 2006; Prickaerts et al., 2004; Rutten et al., 2007). Furthermore, these pathways are strongly involved in synaptic transmission and long-term potentiation (LTP) (Frey et al., 1993; Son et al., 1998). Activation of these pathways is potentially beneficial in pathological conditions such as Rubinstein Taybi syndrome (Bourtchouladze et al., 2003), stroke (Dragunow, 2004), neurofibromatosis type 1 mental retardation (Weeber and Sweatt, 2002), attention and mood disorders (Einat et al., 2003), and age-associated memory impairments (Barad, 2003). Moreover, it has been proposed that inhibition of PDE4 activity in particular might effectively counteract the learning and memory deficits of patients suffering from Alzheimer's disease (AD) (Gong et al., 2004). More specifically, the PDE4 inhibitor rolipram significantly ameliorated long-term potentiation in area CA1 of hippocampal slices (Barad et al., 1998; Gong et al., 2004) and improved 24-h memory performance in a hippocampus-dependent contextual fear conditioning task in a genetic mouse model for AD (Gong et al., 2004) and in adult (12- to 16-week-old) and aging (18-month-old) C57BL mice (Barad et al., 1998).

The recently described novel potent and selective PDE9 inhibitor 1-(2-Chlorophenyl)-6-[(2*R*)-3,3,3-trifluoro-2-methylpropyl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4-one (BAY 73-6691, see Fig. 1) preferentially inhibits the breakdown of cGMP in Sf9 insect cells recombinantly expressing human PDE9 [PDE class, and ICE_{50} values (nM) ± standard error of the mean, where appropriate: PDE9A, 55 ± 9; PDE1C, 1400 ± 350; PDE2A, >4000; PDE3B, >4000; PDE4B, >4000; PDE5A, >4000; PDE7B, >4000; PDE8A; > 4000; PDE10A, >4000; PDE11A, 2600 ± 400; Wunder et al. (2005)]. Cloning of the mouse and human PDE9A enzyme revealed that it has the highest affinity for cGMP of any of the PDEs identified to date (van Staveren et al., 2002). Furthermore, PDE9A mRNA is widely distributed throughout the rodent brain. It is mainly expressed in neurons, but occasionally also in astrocytes (van Staveren et al., 2002). In addition, recent human *in vitro* studies have demonstrated that whereas PDE5 mRNA could not be detected in the brains of normal elderly people and patients suffering from AD, PDE2 mRNA and PDE9 mRNA showed a widespread distribution (Reyes-Irisarri et al., 2007). The selectivity and localization of PDE9 imply its involvement in the regulation of neuronal cGMP levels and cGMP-mediated signal transduction.

Inhibitors of soluble guanylyl cyclase or of protein kinase G prevent the induction of LTP in CA1, and cGMP analogs or activators of PKG lower the threshold for the induction of LTP (Zhuo et al., 1994). We therefore expected that inhibition of PDE9 would improve synaptic transmission and would enhance cognitive performance. Thus, the aim of the present study was to assess the effects of the novel and selective PDE9 inhibitor BAY 73-6691 on neuronal plasticity and cognitive performance. The effects of BAY 73-6691 were investigated on LTP induced by a weak tetanic stimulus in hippocampal slices prepared from young Wistar rats in addition to young and aged Fisher Brown Norway rats. Unlike most other studies investigating the role of cyclic nucleotides in LTP, we used a weak tetanic stimulus based on a theta burst, which induces an early LTP that returns to baseline within 3 h (Wilsch et al., 1998). It has recently been confirmed that increasing the number of theta-burst trains induces LTP of increasing magnitude and persistence (Raymond and Redman, 2002), i.e. a single weak stimulus does not induce the maximum achievable, saturated, type of LTP but instead induces an unsaturated LTP. Both the magnitude (e.g. Behnisch and Reymann, 1998) and the persistence (e.g. Balschun and Reymann, 1994) of this LTP can be improved or diminished by compounds that affect protein kinase activity. The early LTP induced in this study is also unsaturated and thus allows the detection of both facilitation and impairments of early LTP by drugs, as well as its prolongation to generate a late LTP.

To assess effects on memory, we used social and object recognition tasks, a passive avoidance task in combination with scopolamine, and a continuous alternation task in the T-maze in combination with MK-801, with mice or rats as subjects.

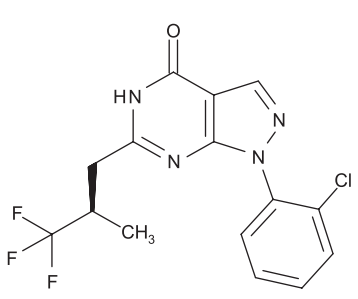

Fig. 1. The selective PDE9 inhibitor BAY 73-6691 ($C_{15}H_{12}ClF_3N_4O$, MW: 356.73).

2. Material and methods

A series of experiments was performed at the Leibniz Institute for Neurobiology, Magdeburg, Germany, the Department of Psychiatry and Neuropsychology, the Department of Psychology, Maastricht University, Maastricht, The Netherlands, and Global Drug Discovery, BayerHealthcare AG, Wuppertal, Germany, to assess the effects of BAY 73-6991 on long-term potentiation in rats and on learning and memory in rats and mice.

2.1. Long-term potentiation in hippocampal slices from young Wistar rats and young and aged Fischer 344 X Brown Norway rats

2.1.1. Hippocampal slice preparation

In a first set of experiments, hippocampal slices from 7- to 8-week-old male Wistar rats (HsdCpb:Wu; Harlan Winkelmann, Borchen, Germany) were used. These experiments were repeated using slices from young (7- to 8-week-old) and very old (31- to 35-month-old) Fischer 344 X Brown Norway hybrid rats (hereafter called FBNF1) supplied by Harlan Sprague Dawley (Indianapolis, Indiana, USA). The slices were prepared as described previously (Schröder et al., 2004). Briefly, after decapitation the brain was quickly removed and placed into ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM: NaCl 124, KCl 4.9, $MgSO_4$ 1.3, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.6, D-glucose 10, saturated with 95% O_2, 5% CO_2, pH 7.4). Both hippocampi were isolated and transverse hippocampal slices (400 mm thickness) were prepared using a tissue chopper with a cooled stage. The slices were transferred into a submerged recording chamber, where they were allowed to recover for at least 1 h before the experiment started. The chamber was constantly perfused with ACSF at a rate of 2.5 ml min^{-1} at 33 ± 1 °C.

2.1.2. LTP measurements

Synaptic responses were elicited by stimulation of the Schaffer collateral–commissural fibers in the *stratum radiatum* of the CA1 region, using lacquer-coated stainless steel stimulating electrodes. Glass electrodes (filled with ACSF, 1–4 MΩ) were placed in the apical dendritic layer, to record field excitatory postsynaptic potentials (fEPSPs). The initial slope of the fEPSP was measured, and the stimulus strength of the test pulses was adjusted to 30% of the fEPSP maximum. When the baseline was recorded, three single stimuli (10 s interval) were averaged every 5 min, but after high-frequency stimulation recordings were taken as indicated in the figures. Once a stable baseline had been established, LTP was induced using a weak high-frequency stimulus paradigm (Schröder et al., 2004) consisting of four paired pulses with an interpulse interval of 10 ms applied with a pulse width of 0.2 ms at the theta frequency of 5 Hz (i.e. every 200 ms). BAY 73-6691 was dissolved in DMSO, diluted in experimental solution to a final concentration of either 10 or 30 μM, and applied 30 min before until 15 min after tetanic stimulation.

2.1.3. Statistical analysis

Recording, analysis, and supervision of the experiments were performed by means of a software program (PWIN), which was developed at the Leibniz Institute for Neurobiology (Department of Neurophysiology) in Magdeburg, Germany. EXCEL and a customized program were used to average fEPSP slope values and to draw diagrams. All values are given as mean ± SEM. ANOVA with repeated measures was used to compare the field potentials between two groups of slices (i.e. control vs. drug treatment).

2.2. Effects of BAY 73-6991 on social recognition performance in rats and mice

2.2.1. General description of material and methods

2.2.1.1. Animals. Four- to five-month-old male Wistar rats and 4- to 5-week-old juveniles were supplied by Charles River (Sulzfeld, Germany). Adult C57BL/6J mice

and juvenile mice aged 4 weeks were obtained from Harlan Sprague Dawley (The Netherlands). Adult rats were housed in groups of three in type IV Makrolon™ cages. Juvenile rats were housed in groups of three in type III Makrolon™ cages, and adult and juvenile mice were housed in groups of 10 in type III Makrolon™ cages, under a 12-h light-dark schedule: white lights were on from 06:00 to 18:00 hours. In the night phase from 18:00 to 06:00 hours, the laboratory was illuminated by red strip lighting. The laboratory was temperature ($22 \pm 1\,^{\circ}C$) and humidity ($55 \pm 5\%$) controlled. Food (pelleted standard lab chow for rats and mice: ssniff, Soest, Germany) and water were available ad libitum, except during testing. The animals were randomly assigned to their respective treatment groups.

2.2.1.2. Procedure. The social recognition task was performed as described previously (Dantzer et al., 1987). All animals were adapted to the laboratory for 1 week prior to behavioral testing. Then, one habituation session was performed under essentially similar conditions as the test session (see below).

Testing consisted of pairs of trials, trial 1 (T1) and trial 2 (T2).

T1 Adult animals were individually housed 30 min before testing. An enclosure (63 cm × 41 cm × 40 cm; two aluminum sidewalls, an aluminum back wall, and a Plexiglas front) was put over the type IV Makrolon™ observation cage 4 min prior to testing. The lid of the cage was removed. Then, a juvenile was placed into the cage and social investigation by the adult animal was registered continuously for 2 min by a trained observer. Sniffing and grooming of body parts, anogenital sniffing, and close following were scored. Contacts between adult and juvenile rats were not scored as social investigation when the behavior of the adult was not directed toward the juvenile. Animals were returned to their home cages during the 24-h retention interval.

T2 *Retesting* was performed after a retention interval under essentially the same experimental conditions as during the first trial. In the tests conducted with a familiar juvenile (exp. 1–5), each adult was exposed to the same juvenile already used during T1. In the fifth social recognition experiment, one group of adult rats was exposed to a new juvenile, to verify the behavioral specificity of drug effects.

2.2.1.3. Statistical analysis. The percent reduction in social investigation time (%RSIT) between T1 and T2 was used as an index of social memory performance. This measure was analyzed by analysis of variance (ANOVA) with the factor Treatment (vehicle vs. different doses of the test compound), supplemented by Fisher's least significant difference (LSD) post hoc test, where all treatment groups were compared pair-wise. Differences with an associated probability <0.05 were considered as statistically reliable.

2.3. Effect of BAY 73-6691, administered 60 min before T1, on the social recognition performance of rats

2.3.1. Animals

Thirty-two 15- to 16-week-old adult male Wistar [Crl:(WI)WU BR] rats and 32 4-week-old male juvenile Wistar rats were used.

2.3.2. Procedure

Each adult animal was randomly assigned to one of four treatment groups. The adult rats were treated p.o. 60 min before T1 with either vehicle (5% EtOH + 10% solutol + 85% H_2O; $n = 8$), or 0.03 ($n = 8$), 0.3 ($n = 8$) or 3 mg kg^{-1} BAY 73-6691 ($n = 8$), with an application volume of 1 ml kg^{-1} body weight. Recognition performance was tested after a retention interval of 24 h with the same juvenile that was used in T1.

2.4. Effect of BAY 73-6691, administered 30 min before T1, on the social recognition performance of mice

2.4.1. Animals

Forty 8-week-old adult male C57BL (C57BL/6JOlaHsd) mice and forty 3- to 4-week-old juvenile C57BL mice were used.

2.4.2. Procedure

The adult mice were assigned to one of four treatment conditions. The adult male mice were treated p.o. 30 min before T1 with either vehicle (5% EtOH + 10% solutol + 85% H_2O; $n = 9$), 0.03 ($n = 9$), 0.3 ($n = 8$) or 3 mg kg^{-1} BAY 73-6691 ($n = 10$), in an application volume of 10 ml kg^{-1}. Recognition performance was tested after a retention interval of 24 h with the same juvenile that was used in T1.

2.5. Effect of BAY 73-6691, administered immediately after T1, on the social recognition performance of rats

2.5.1. Animals

Thirty-two 15- to 16-week-old adult male Wistar [Crl:(WI)WU BR] rats and thirty-two 4-week-old male juvenile Wistar rats were used.

2.5.2. Procedure

The adult male rats were treated p.o. immediately after T1 with either vehicle (5% EtOH + 10% solutol + 85% H_2O) or 0.03, 0.3 or 3 mg kg^{-1} BAY 73-6691, in an application volume of 1 ml kg^{-1} body weight ($n = 8$ per treatment group). Recognition performance was tested after a retention interval of 24 h with the same juvenile that was used in T1.

2.6. Effect of BAY 73-6691, administered 60 min before T2, on the social recognition performance of rats

2.6.1. Animals

Thirty-two 15- to 16-week-old adult male Wistar [Crl:(WI)WU BR] rats and thirty-two 4-week-old male juvenile Wistar rats were used.

2.6.2. Procedure

The adult rats were assigned to one of four treatment conditions. The adult rats were treated p.o. 60 min before T2 with either vehicle (5% EtOH + 10% solutol + 85% H_2O) or 0.03, 0.3, or 3 mg kg^{-1} BAY 73-6691, in an application volume of 1 ml kg^{-1} body weight ($n = 8$ per treatment group). Recognition performance was tested after a retention interval of 24 h with the same juvenile that was used in T1.

2.7. Effect of BAY 73-6691 on the social recognition performance of rats confronted with a familiar or a novel juvenile

2.7.1. Animals

Twenty-four 15- to 16-week-old adult male Wistar [Crl:(WI)WU BR] rats and thirty-two 4-week-old male juvenile Wistar rats were used.

2.7.2. Procedure

The rats were assigned to one of three treatment conditions: (1) vehicle control group, confronted with the *familiar* juvenile at T2, 24 h after T1 ($n = 8$), (2) a group treated p.o. with 1 mg kg^{-1} BAY 73-6691 1 h before T1 and confronted with a *familiar* juvenile at T2 ($n = 8$), and (3) a group treated p.o. with 1 mg kg^{-1} BAY 73-6691, 1 h before T1 and confronted with a *novel* juvenile rat at T2 ($n = 8$). Vehicle was 5% EtOH + 10% solutol + 85% H_2O. The application volume was 1 ml kg^{-1} body weight. Recognition performance was tested after a retention period of 24 h with either a familiar of a novel juvenile.

2.8. Effect of BAY 73-6691, administered 30 min before T1, on the object recognition performance of rats

2.8.1. Animals

Twenty-four 5-month-old male Wistar rats (Charles River, The Netherlands) were used. The animals were housed individually in type III Makrolon™ cages on sawdust bedding in an air-conditioned room (about 20 °C) and were kept under a 12/12-h light/dark cycle (lights on from 18.00 to 6.00 hours) with free access to food and water. Rats were not housed in the room where they were tested. A radio, which was playing softly, provided background noise in all rooms. All testing was performed between 9.00 and 16.00 hours.

2.8.2. Apparatus

The object recognition test was performed as described elsewhere (Prickaerts et al., 2002). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40-cm-high wall was made of gray polyvinyl chloride (PVC) and the other half of transparent PVC. The light intensity was equal in the different parts of the apparatus. Two objects were placed in a symmetrical position about 10 cm from the gray wall. Each object was available in triplicate. We used four different sets of objects: (1) a cone consisting of a gray polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), (2) a standard 1-l transparent glass bottle (diameter 10 cm, height 22 cm) filled with sand, (3) a solid metal cube (10.0 × 5.0 × 7.5 cm) with two holes (diameter 1.9 cm), and (4) a solid aluminum cube with a tapered top (13.0 × 8.0 × 8.0 cm). The rats could not move the objects.

2.8.3. Procedure

In the first week, the animals were handled daily and were adapted to the procedure on 3 days, i.e. they were allowed to explore the test arena (without any objects) twice for 5 min each day. Fluorescent red tubes and a light bulb provided a constant illumination of about 20 lux on the floor of the apparatus. During the two following weeks, the rats were tested until they showed a good discrimination performance. A saline injection (0.5 ml) was always given after T1. Testing of the drugs began the next week.

A testing session consisted of two trials. The duration of each trial was 3 min. During T1, the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the middle of the transparent front wall. After T1 the rat was put back in its home cage. Subsequently, after a predetermined interval, the rat was put back in the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent exploring each object during T1 and T2 were recorded manually using a personal computer.

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 trails the objects were always thoroughly cleaned. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

Since we expected the drug treatments to improve memory performance, the interval between tests needed to be long enough to prevent discrimination between the objects. For this reason, we used a delay interval of 24 h, since there is no (or minimal) discrimination between the two objects after this interval (Ennaceur et al., 1989). Each week, two testing sessions were given, one session on Monday and Tuesday and the other on Thursday and Friday. Doses were tested in a random order.

2.8.4. Treatment

BAY 73-6691 was prepared daily and dissolved in solutol:ethanol:water (10:5:85). The drug was administered p.o. at doses of 0.1, 0.3, 1 and 3 mg kg^{-1} ($n = 24$ per dose; within subjects design) 30 min before T1. The administration volume was 1 ml kg^{-1} body weight. The rats were treated once with vehicle and each of the doses of BAY 73-6691.

2.8.5. Statistical analysis

The basic measure in the object recognition task is the time rats spent exploring each object during T2. The measure *d*2 is considered as index of discrimination between the new and the familiar object in T2. It is calculated as: d2 = (time spent exploring the novel object in T2 – time exploring the familiar object in T2)/(time spent exploring the novel object in T2 + time spent exploring the familiar object in T2) In fact, *d*2 is a relative measure of discrimination that is corrected for differences between individual rats with respect to exploratory activity. We consider *d*2 as a better discrimination index than *d*1, the absolute difference in exploration times because this is dependent on the level of exploration.

2.9. Effects of BAY 73-6691 on scopolamine-induced retention deficits in the passive avoidance task

2.9.1. Animals

Forty male Wistar (HsdCpb:Wu) rats weighing between 267 and 299 g were supplied by Harlan Winkelmann (Borchen, Germany). They were randomly assigned to one of five treatment conditions: (1) vehicle–vehicle; (2) 0.03 mg kg^{-1} scopolamine–vehicle; (3) 0.03 mg kg^{-1} scopolamine–0.3 mg kg^{-1} BAY 73-6691; (4) 0.03 mg kg^{-1} scopolamine–1 mg kg^{-1} BAY 73-6691; and (5) 0.03 mg kg^{-1} scopolamine–3 mg kg^{-1} BAY 73-6691. Each treatment group consisted of eight animals.

2.9.2. Apparatus

The inhibitory avoidance apparatus consisted of a two-compartment box with a light compartment and a dark compartment, each measuring 36 cm (height) × 27 cm (depth) × 37 cm (width). The apparatus was made of black plastic, except for the sidewalls of the light compartment, which were made of white plastic. The floor consisted of a metal grid (diameter of stainless steel bars: 6.3 mm, free space between bars: 11.3 mm; shock floor manufactured by Coulbourn) connected to a shock scrambler. The two compartments were separated by a guillotine door that could be raised 9 cm. A threshold of 2 cm separated the two compartments when the guillotine door was raised. When the door was open, the illumination in the dark compartment was about 2 lux. The light intensity was about 500 lux at the center of the floor of the light compartment. The equipment was situated in a quiet laboratory illuminated by red fluorescent strip lights.

2.9.3. Procedure

One habituation session, one shock session, and a retention session were given, separated by intervals of 24 h. In the habituation and retention sessions, the rat was allowed to explore the apparatus for 300 s. The rat was placed in the light compartment, facing the wall opposite the guillotine door. After 15 s, the guillotine door was opened so that all parts of the apparatus could be visited freely. In the shock session the guillotine door between the compartments was lowered as soon as the rat had entered the dark compartment with its four paws, and a scrambled 1 mA footshock was administered for 2 s. The rat was removed from the apparatus 10 s after shock termination and put back into its home cage.

2.9.4. Treatment

(−)-Scopolamine HCl was supplied by Sigma–Aldrich (Schnelldorf, Germany). It was dissolved in 0.9% NaCl solution, and BAY 73-6691 was dissolved in EtOH:solutol:0.9%NaCl 5:10:85. Scopolamine or its vehicle was administered s.c. 30 min before the shock session, whereas BAY 73-6691 or its vehicle was administered p.o. 60 min before the shock session. The injection volume was 2 ml kg^{-1} body weight.

2.9.5 . Statistical analysis

The step-through latency, that is the latency to enter the dark compartment (in s), was recorded. Data were analyzed by ANOVA with the factor treatments, supplemented with Fisher's LSD post hoc pair-wise comparisons between treatment groups.

2.10. Effects of BAY 73-6691 on the spontaneous alternation behavior of mice in the T-maze

2.10.1. Animals

One hundred male C57BL (C57BL/6JIco) mice, weighing 22–26 g, were supplied by IFFA Credo (l'Arbresle, France), in two separate shipments of 50 animals each. The mice were housed in groups of 10 in standard type III Makrolon™ cages in a temperature- (22 ± 1 °C)- and humidity (55 ± 5%)-controlled laboratory. The animals were allowed to habituate to the laboratory for 1 week before drug testing started. Before testing, all mice were singly housed in standard type II Makrolon™ cages.

2.10.2. Apparatus

The T-maze was constructed by Sembach (Ratingen, FRG; for details see Spowart-Manning and van der Staay, 2004) and was made of transparent Plexiglas. The guillotine doors and the floor of the apparatus were black. The guillotine doors could be operated by the experimenter through a system of pulley strings.

2.10.3. Forced-choice trial

In the first trial, the 'forced-choice trial', either the left or right goal arm was blocked by lowering the guillotine door. After the mouse had been released from the start arm, it negotiated the T-maze eventually entered the open goal arm, and returned to the start position. There, the animal was confined for 5 s by lowering the guillotine door of the start arm.

2.10.4. Free-choice trials

During 14 'free-choice' trials, the mouse could choose freely between the left and right goal arms. After the guillotine door of the start arm was opened, the mouse was free to choose between both goal arms (all guillotine doors open). As soon as the mouse had entered one goal arm, the other goal arm was closed. The mouse eventually returned to the start arm, and the next free-choice trial started after a 5-s confinement in the start arm. A session was terminated and the animal was removed from the T-maze as soon as 14 free-choice trials had been performed or 30 min had elapsed, whatever event occurred first. During the session, the animals were not handled by the experimenter.

2.10.5. Procedure

The mice were assigned at random to one of five treatment combinations: (1) vehicle of MK-801 (vehicle 1) or vehicle of BAY 73-6691 (vehicle 2); (2) 0.06 mg kg^{-1} MK-801 and vehicle 2; (3) 0.06 mg kg^{-1} MK-801 and 1 mg kg^{-1} BAY 73-6691; (4) 0.06 mg kg^{-1} MK-801 and 3 mg kg^{-1} BAY 73-6691; and (5) 0.06 mg kg^{-1} MK-801 and 10 mg kg^{-1} BAY 73-6691. Each group consisted of 20 mice, half of them from each of the two shipments.

Mice were trained in a single session, which started with one forced-choice trial, followed by 14 free-choice trials. The study was performed in two series, each with 10 animals per treatment condition. BAY 73-6691 or its vehicle (5% EtOH + 40% solutol + 55% 0.9% NaCl solution) was administered p.o. 60 min before the start of testing, whereas MK-801 or its vehicle (0.9% NaCl solution) was injected s.c. 30 min before the T-maze experiment started. The application volume for both compounds was 5 ml kg^{-1} body weight.

2.10.6. Statistical analysis

The data from both series were pooled for statistical analysis. The data of animals that completed fewer than eight free-choice trials during 30 min were excluded from further analyses. The percent alteration in arm choice during the 14 trials (or the total number of free-choice trials within a session, if less than 14 free-choice trials were completed) was calculated. This percentage and the total time needed to complete a session (in s) were evaluated statistically by ANOVA, with the factor Treatment, supplemented with Fisher's least significant difference (LSD) post hoc comparisons. One-sample *t*-statistics were used to test whether particular treatment groups alternated above chance level.

3. Results

3.1. Long-term potentiation in hippocampal slices from young Wistar rats and young and aged FBNF1 rats

To investigate the effects of PDE9 inhibition on synaptic plasticity in rat hippocampal slices, we measured the effect of BAY 73-6691 on sub-maximal LTP in the CA1 region induced by weak tetanic stimulation of the Schaffer collateral pathway. In young Wistar rats, basal synaptic transmission was not affected by either 10 or 30 μM BAY 73-6691 (see Fig. 2A and B, respectively). Treatment with 10 μM BAY 73-6691 slightly increased the slope of the fEPSPs when present from 30 min before until 15 min after a weak

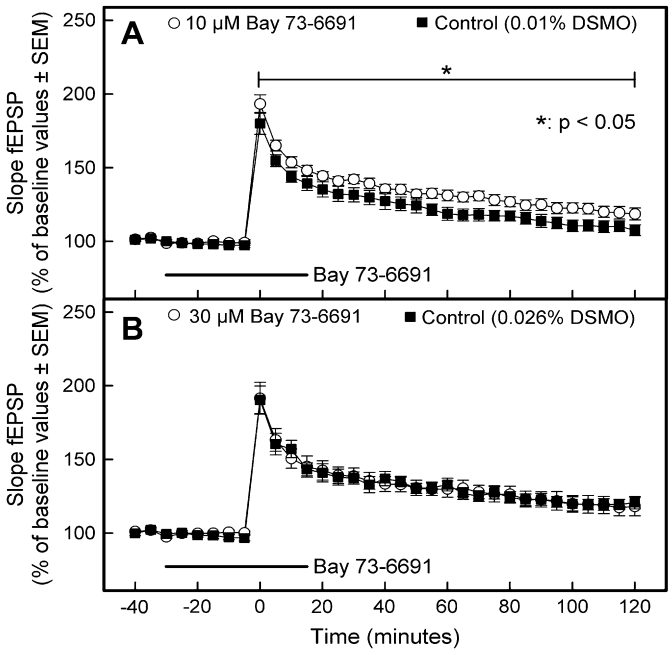

Fig. 2. Effect of BAY 73-6691 on the potentiation of fEPSPs elicited by weak tetanic stimulation of hippocampal slices from young Wistar rats. Panel A depicts the results from 10 control slices and from 11 slices treated with 10 μM BAY 73-6691. Panel B depicts the results from nine control slices and from eight slices treated with 30 μM BAY 73-66911.

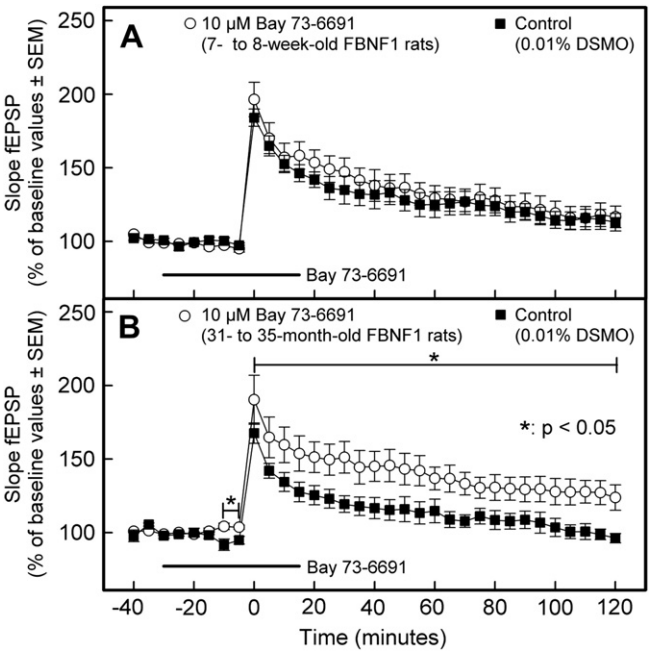

Fig. 3. Effect of 10 μM BAY 73-6691 on the potentiation of the fEPSPs elicited by weak tetanic stimulation of slices from young (7- to 8-week-old; eight control slices; eight slices treated with BAY 73-6691; panel A) and aged (31- to 35-month-old; eight control slices and of nine slices treated with 10 μM BAY 73-6691; panel B) Fischer 344 X Brown Norway hybrid rats. The compound was present from 30 min before to 15 min after weak tetanic stimulation.

tetanic stimulation [repeated measures ANOVA: $F_{1,19} = 4.41$, $p < 0.05$, Fig. 2, panel A, post-tetanic potentiation and potentiation 10 min, 60 min, and 120 min after stimulation; control ($n = 10$) vs. drug ($n = 11$), percent of baseline values: $180.3 \pm 7.4.0\%$ vs. $193.2 \pm 6.2\%$, $143.9 \pm 3.8\%$ vs. $153.6 \pm 3.7\%$, $118.7 \pm 4.4.0\%$ vs. $131.2 \pm 3.6\%$, and $107.5 \pm 3.7\%$ vs. $118.6 \pm 4.0\%$]. In contrast, 30 μM BAY 73-6691 did not affect the potentiation of the slope of the fEPSPs when present from 30 min before to until 15 min after weak tetanic stimulation [Fig. 2, panel B, post-tetanic potentiation, and potentiation 10 min, 60 min, and 120 min after stimulus, control ($n = 9$) vs. drug ($n = 8$), percent of baseline values: $190.4 \pm 9.4.0\%$ vs. $191.4 \pm 10.8\%$, $157.1 \pm 5.9\%$ vs. $150.6 \pm 6.6\%$, $129.8 \pm 5.5\%$ vs. $132.9 \pm 4.4\%$, and $120.6 \pm 4.1\%$ vs. $118.0 \pm 6.3\%$].

In contrast, BAY 73-6691 (10 μM) did not affect LTP in age-matched FBNF1 rats [see Fig. 3, panel A, post-tetanic potentiation and potentiation 10 min, 60 min and 120 min. after stimulus; control ($n = 8$) vs. drug ($n = 8$), percent of baseline values: $196.4 \pm 11.7.0\%$ vs. $184.0 \pm 5.9\%$, $157.0 \pm 9.6\%$ vs. $152.5 \pm 5.7\%$, $129.3 \pm 8.5\%$ vs. $124.8 \pm 8.1\%$, and $116.6 \pm 6.0\%$ vs. $113.0 \pm 7.3\%$]. However, the compound slightly improved basal synaptic transmission (Mann–Whitney *U*-test, $U = 11.00$; $p < 0.05$) and augmented the potentiation of the slope of the fEPSPs (repeated measures ANOVA, $F_{1,15} = 5.62$; $p < 0.05$) when present from 30 min before to until 15 min after weak tetanic stimulation in slices from aged FBNF1 rats [see Fig. 3, panel B, post-tetanic potentiation and potentiation 10 min, 60 min and 120 min. after stimulus; control ($n = 8$) vs. drug ($n = 9$), percent of baseline values: $167.6 \pm 6.9.0\%$ vs. $190.3 \pm 10.7\%$, $134.4 \pm 6.4\%$ vs. $159.6 \pm 12.1\%$, $114.7 \pm 8.0\%$ vs. $136.8 \pm 7.7\%$, and $100.7 \pm 5.4\%$ vs. $123.8 \pm 8.6\%$].

3.2. Effects of BAY 73-6991 on social recognition performance in rats and mice

Effects of putative cognition enhancers on the retention performance in the social recognition task are usually expressed as percent reduction in social investigation times from T1 to T2. In addition, we calculated and presented the effects of the compound on the social investigation times in seconds during T1 and T2 for the first three experiments.

3.2.1. BAY 73-6691, administered 60 min before T1, on the social recognition performance of rats

3.2.1.1. Percent reduction social investigation times. Treatment with BAY 73-6691 improved the social recognition performance at T2 ($F_{3,28} = 12.95$, $p < 0.0001$; see Fig. 4, panel A). Post hoc LSD comparisons confirmed that the doses of 0.3 and 3 mg kg^{-1} reduced the social investigation time at T2, i.e. improved memory performance, whereas the performance of the group treated with 0.03 mg kg^{-1} was not different from that of the control group. The performance levels of the groups treated with 0.3 and 3 mg kg^{-1} did not differ from one another.

3.2.1.2. Social investigation times (s). When considering the social investigation times as T1 and T2, the following picture emerged: investigation times did not differ between doses at T1 ($F_{3,28} = 1.18$, NS; see Fig. 4, panel D), i.e. the compound had not effect on the social investigation per se. At T2, the social investigation times were affected by the treatment with BAY 73-6691 ($F_{3,28} = 9.76$, $p < 0.0001$). Post hoc LSD comparisons confirmed that the social investigation times were lower in the groups treated with 0.3 and 3 mg kg^{-1} BAY 73-6691 than in the vehicle-treated group and in the group treated with 0.03 mg kg^{-1} BAY 73-6691.

3.2.2. BAY 73-6691, administered 30 min before T1, on the social recognition performance of mice

3.2.2.1. Percent reduction social investigation times. Treatment with BAY 73-6691 30 min before T1 improved the retention performance of adult mice 24 h later, at T2 ($F_{3,32} = 17.009$, $p < 0.0001$, Fig. 4, panel B). Post hoc LSD comparisons revealed that the groups treated with 0.3 or 3.0 mg kg^{-1} BAY 73-6691 had a better retention performance than the vehicle-treated group, whereas the group treated with 0.03 mg kg^{-1} had a similar retention performance as

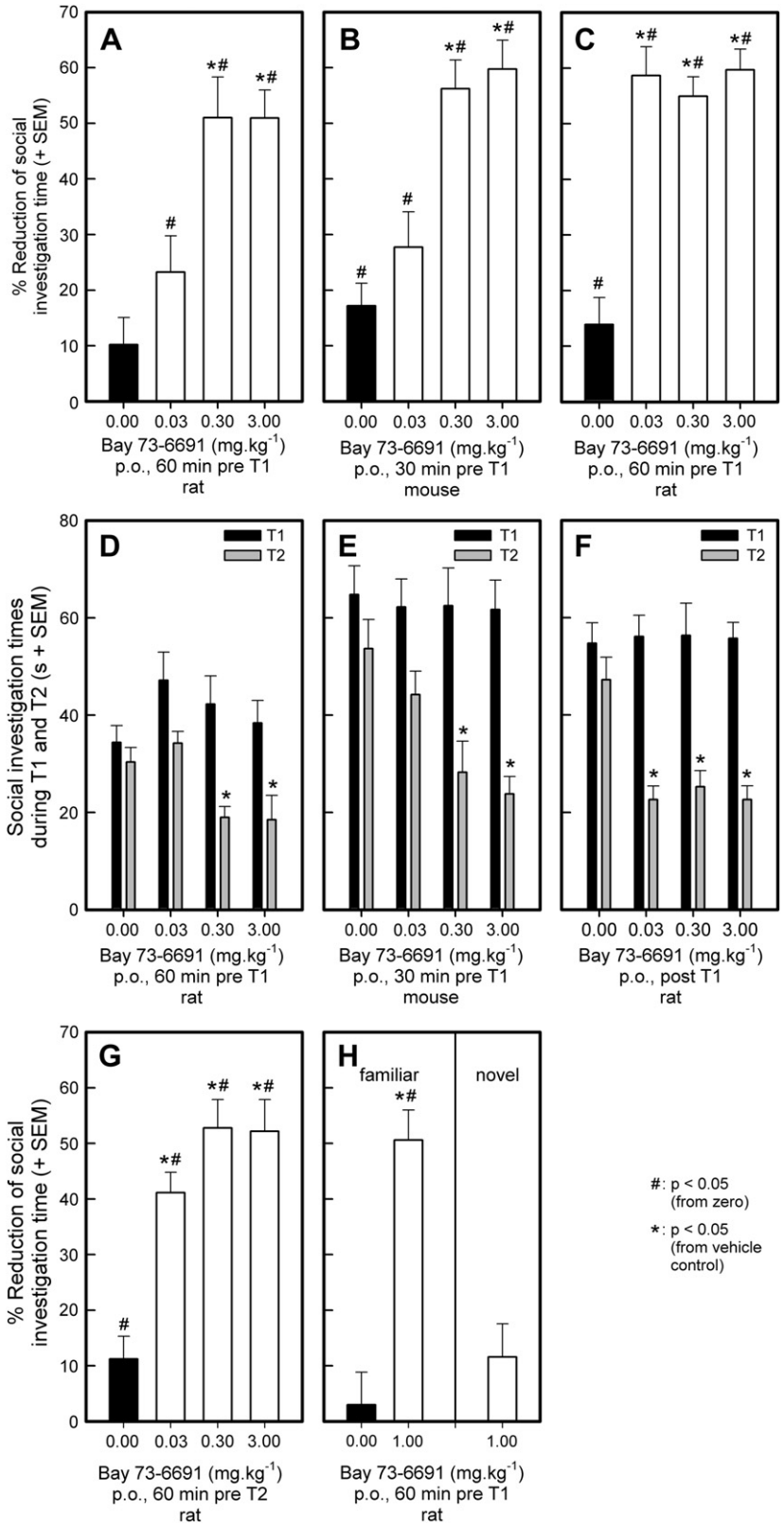

Fig. 4. Effects of BAY 73-6691 on the social recognition performance of adult Wistar rats. The percent reduction of social investigation time at T2 relative to the investigation time at T1 [and for the first three social recognition experiments the times (s) investigating the juvenile] during T1 and T2 is presented as mean and SEM per treatment group. Treatment groups consisted of 8–10 animals. Panels A and D: rats were treated p.o. with vehicle or BAY 73-6691, 60 min before T1. Panels B and E: mice were treated p.o. with vehicle or BAY 73-6691, 30 min before T1. Panels C and F: rats were treated p.o. with vehicle or BAY 73-6691, immediately after T1. Panel G: rats were treated p.o. with vehicle or BAY 73-6691, 60 min before T2. Panel H: rats were treated p.o. with vehicle or 1 mg kg^{-1} BAY 73-6691, 60 min before T1. The drug-treated rats were confronted with the same (familiar) or an unknown (novel) juvenile at T2, 24 h after T1. $^{*}p < 0.05$, i.e. significantly different from the vehicle control group. $\#p < 0.05$ that the percent change in social investigation time at T2 deviates from zero (assessed by one-sample *t*-statistics per treatment group).

the vehicle-treated group. There were no differences in the retention of the groups treated with 0.3 or 3.0 mg kg^{-1} BAY 73-6691.

3.2.2.2. Social investigation times (s). The social investigation times during T1 were not affected by BAY 73-6691 that had been administered 30 min before the start of the trial ($F_{3,32} = 0.05$, NS; see Fig. 4, panel E). However, treatment affected the social investigation times during T2 ($F_{3,32} = 7.34$, $p < 0.0007$). Post hoc LSD comparisons confirmed that the social investigation times were reduced in the groups treated with 0.3 and 3 mg kg^{-1} BAY 73-6691, compared with the groups treated with vehicle or 0.03 mg kg^{-1} BAY 73-6691.

3.2.3. BAY 73-6691, administered immediately after T1, on the social recognition performance of rats

3.2.3.1. Percent reduction social investigation times. Treatment with 0.03, 0.3, or 3.0 mg kg^{-1} BAY 73-6691 immediately after T1 improved the social recognition performance of rats ($F_{3,28} = 25.49$, $p < 0.0001$; Fig. 4, panel C). LSD post hoc comparisons confirmed that all drug-treated groups remembered the juvenile better than the vehicle-treated control group did, and that the performance of the drug-treated groups did not differ from one another.

3.2.3.2. Social investigation times (s). No differences between treatment groups were found at T1 ($F_{3,28} = 0.02$, NS; see Fig. 4, panel F). Drug treatment immediately after T1 affected the social investigation times during T2 ($F_{3,28} = 11.90$, $p < 0.0001$). Post hoc LSD comparisons revealed that all groups treated with BAY 73-6691 spent less time investigating the juvenile rat during T2 than the group treated with vehicle. There was no difference between the three doses (0.03, 0.3, and 3 mg kg^{-1}) tested.

3.2.4. BAY 73-6691, administered 60 min before T2, on the social recognition performance of rats

Treatment with 0.03, 0.3, or 3 mg kg^{-1} BAY 73-6691 60 min before T2 improved the social recognition performance of rats ($F_{3,28} = 17.14$, $p < 0.0001$; Fig. 4, panel G). LSD post hoc comparisons confirmed that all drug-treated groups remembered the juvenile better than the vehicle-treated control group did, and that the performance of the drug-treated groups did not differ from one another.

3.2.5. BAY 73-6691 on the social recognition performance of rats confronted with a familiar or a novel juvenile

The treatments affected the social investigation performance, expressed as percent reduction in social investigation time ($F_{2,21} = 19.47$, $p < 0.0001$; Fig. 4, panel H). Post hoc comparisons between groups revealed that the investigation time of vehicle-treated rats confronted with a familiar juvenile was not shorter at T2 than at T1, whereas it was shorter in rats treated with 1 mg kg^{-1} BAY 73-6691. This indicates that the vehicle-treated controls did not recognize the juvenile after a 24-h retention interval whereas the group treated with 1 mg kg^{-1} did. The rats treated with 1 mg kg^{-1} BAY 73-6691 and confronted with a novel juvenile at T2 did not show a shorter social investigation time during T2, as expected. Thus, the improved recognition performance at T2 of treated rats was not due to a non-mnemonic effect of the test compound *per se*.

3.3. Effects of BAY 73-6691 administered 30 min before T1 on the object recognition performance of rats

The effects of BAY 73-6691, injected 30 min before T1, on the relative discrimination index *d*2 are presented in Fig. 5. Comparison of the effects of the different doses revealed only a trend for a larger *d*2 value ($F_{4,118} = 2.10$, $0.05 < p < 0.1$, Fig. 5, left panel), which is possibly due to a rather large variation. However, one-sample *t*-test showed a within group effect on *d*2 values, suggesting that discrimination performance was different from zero after administration of 0.1 and 0.3 mg kg^{-1} doses.

In addition, we analyzed the exploration times at the novel and familiar objects, using repeated measures ANOVA with the two within subjects factors Dose (vehicle vs. 0.1, 0.3, 1 and 3 mg kg^{-1} Bay 73-6691) and Object (novel vs. familiar) (Fig. 5, right panel). This analysis was complemented with contrasts of the total exploration times (familiar + novel) and the exploration times for the familiar and the novel objects in which the successive doses

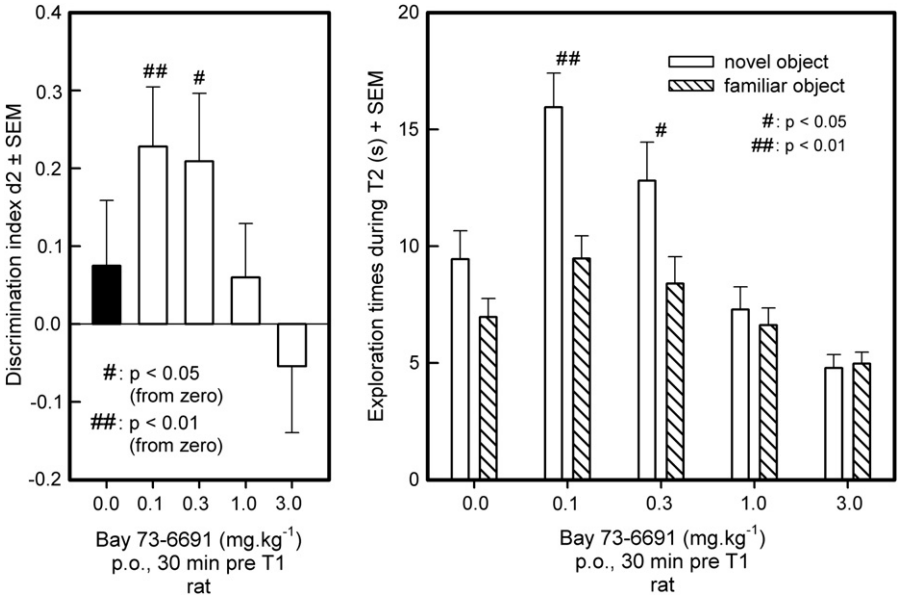

Fig. 5. Effects of BAY 73-6691 on discrimination performance of 5-month-old male rats in an object recognition task. The effects on the discrimination index *d*2 (left panel) and on the exploration times with familiar and novel objects are depicted as means and standard errors of the means (SEM). In the vehicle session, rats were treated with solutol:ethanol:water (10:5:85). In the drug session, rats were treated with doses of 0.1, 0.3, 1 and 3 mg kg^{-1} BAY 73-6691. *$p < 0.01$ [one-sample *t*-statistics, within session effects (different from zero)].

were compared (vehicle – 0.01, 0.01–0.3, 0.3–1.0, 1.0–3 mg kg^{-1}; SAS GLM procedure, PROFILE contrasts). Bay 73-6691 affected the exploration times (Dose: $F_{4,92} = 17.56$, $p < 0.0001$). The rats discriminated between the familiar object and the novel object (Object: $F_{1,23} = 32.10$, $p < 0.0001$), and this discrimination was affected by the dose administered (Dose by Object interaction: $F_{4,92} = 3.84$, $p < 0.0063$).

Contrasts between successive doses revealed that 0.1 mg kg^{-1} increased the total exploration time compared with vehicle, that the exploration times after injection of 0.1 and 0.3 mg kg^{-1} Bay 73-6691 did not differ from one another, and that the exploration times after 1 and 3 mg kg^{-1} were shorter than that after 0.3 mg kg^{-1} Bay 73-6691. We performed these analyses separately for the familiar and novel objects. Analysis of the exploration time for the novel object after administration of increasing doses of Bay 73-6691 yielded the same results as for the total exploration time, with there being no difference in exploration time for the familiar object between the successive doses. Thus treatment with 0.1 and 0.3 mg kg^{-1} Bay 73-6691 increased the exploration time, but selectively for novel objects, suggesting that the compound improved object recognition.

3.4. Effects of BAY 73-6691 on scopolamine-induced retention deficits in the passive avoidance task

Latency to enter the dark compartment (see Fig. 6) was not different between treatment groups during the habituation session ($F_{4,35} = 1.05$, NS) and the shock session ($F_{4,35} = 1.93$, NS). Treatment with scopolamine and BAY 73-6691 affected the performance of the rats in the retention session ($F_{4,35} = 10.02$, $p < 0.0001$). Post hoc Fisher's LSD comparisons revealed that 0.03 mg kg^{-1} scopolamine disrupted the retention performance; this effect was attenuated, at least partially, by 1 and 3 mg kg^{-1} BAY 73-6691 (see Fig. 6).

3.5. Effects of BAY 73-6691 on the spontaneous alternation behavior of mice in the T-maze

Spontaneous alternation behavior was affected by the drug treatment ($F_{4,95} = 6.40$, $p < 0.0001$; Fig. 7, panel A). Post hoc LSD comparisons revealed that the groups of mice treated with MK-801 alone or in combination with 1 or 3 mg kg^{-1} BAY 73-6691 alternated less than the vehicle control group. However, 10 mg kg^{-1} BAY 73-6691 at least partially attenuated the MK-801-induced decrease in the spontaneous alternation rate. The performance of this group did not differ from that of the vehicle-treated control group and from the MK-801-only treated group. One-sample t-statistics confirmed that the vehicle-treated group and the group of mice treated with the

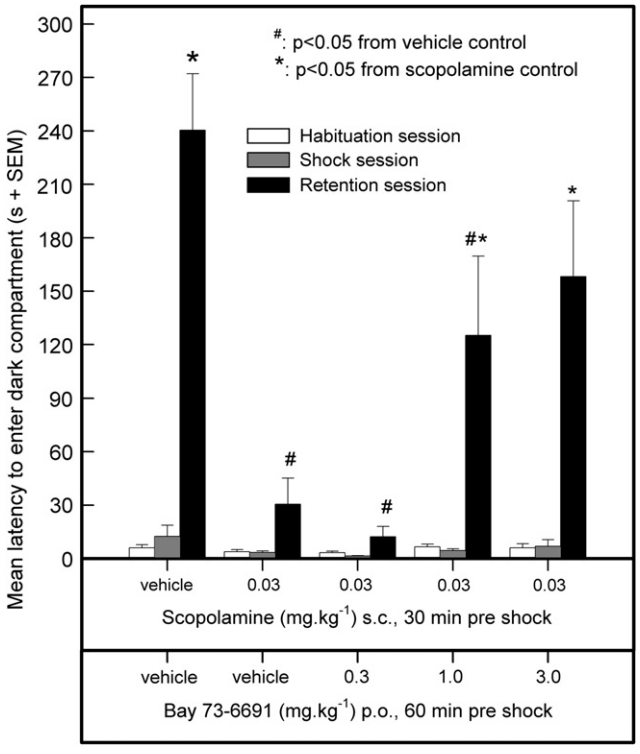

Fig. 6. Effects of scopolamine (0.03 mg kg^{-1}, s.c.) alone or in combination with 0.3, 1, or 3 mg kg^{-1} BAY 73-6691, administered p.o., on the retention performance of male Wistar rats in a passive avoidance task. Scopolamine was administered approximately 30 min before the start of the shock session. BAY 73-6691 was administered 60 min before the shock session. The shock intensity was 1 mA. The means and standard errors of the means (SEM) to enter the dark compartment during the habituation session, the shock session, and the retention session are shown.

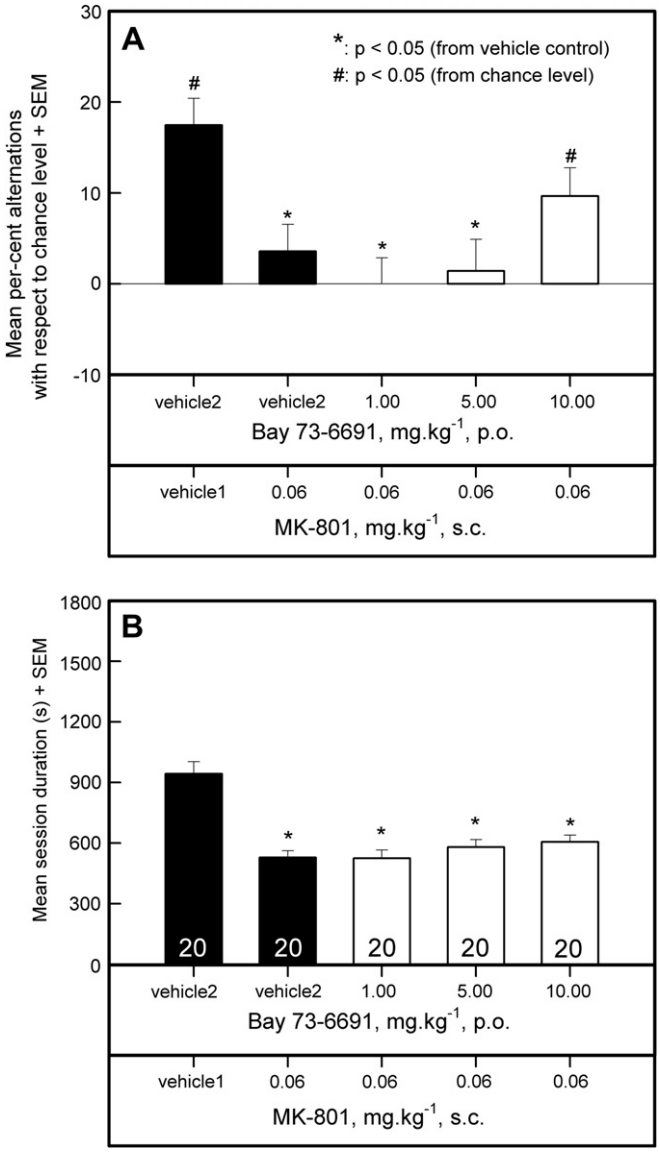

Fig. 7. The effects of treatment with MK-801 or MK-801 in combination with 1, 3 or 10 mg kg^{-1} BAY 73-6691 on the T-maze continuous alternation performance of C57BL mice. The means and standard errors of the means (SEM) of the percent alternations (panel A) and of the time to complete a session (consisting of one forced-choice trial and 14 free-choice trials, panel B) are depicted. The number of animals per treatment group is shown in the bars of the lower panel. Vehicle 1: 0.9% NaCl solution; vehicle 2: (5% EtOH + 40% solutol + 55% 0.9% NaCl solution).

combination of MK-801 and 10 mg kg^{-1} BAY 73-6691 alternated above chance level, whereas none of the other groups of mice did.

Session duration was affected by drug treatment ($F_{4,95} = 16.88$, $p < 0.0001$; see Fig. 7, panel B). All MK-801-treated groups were non-dose dependently faster than the vehicle-treated control group, and treatment with BAY 73-6691 did not affect this MK-801-induced effect.

4. Discussion

We assessed the effects of the selective PDE9 inhibitor BAY 73-6991 on LTP and on learning and memory in rodents. We found that neither 10 nor 30 μM BAY 73-6691 affected basal synaptic transmission in hippocampal slices from young Wistar rats. While 10 μM, but not 30 μM, BAY 73-6691 enhanced early LTP after weak tetanic stimulation in young Wistar rats, it did not affect LTP in hippocampal slices from young FBNF1 rats; however, it increased basal synaptic transmission and enhanced early LTP after weak tetanic stimulation in the very old FBNF1 rats.

BAY 73-6691 enhanced the acquisition, consolidation, and retention of long-term memory in the social recognition task and appeared to attenuate forgetting after a 24-h retention interval in the object recognition task. It attenuated the scopolamine-induced retention deficits in passive avoidance and the MK-801-induced short-term memory deficits in the T-maze alternation task. The results of behavioral testing are summarized in Table 1.

4.1. Synaptic transmission and LTP

Treatment with BAY 73-6691 did not affect basal synaptic transmission in hippocampal slices from young Wistar and FBNF1 rats. Thus, inhibition of PDE9 by itself does not induce synaptic changes that lead to an enhancement of synaptic efficacy in young animals. The very small but significant enhancement of basal synaptic transmission in the slices from the senescent FBNF1 rats could reflect a compound-induced compensation for the aging-related decrease in cGMP levels observed in other studies (Chalimoniuk and Strosznajder, 1998; Vallebuona and Raiteri, 1995).

PDE9 in not the only PDE present; other cGMP degrading PDEs may compensate for the inhibited PDE9. In addition, the cGMP/cAMP ratio may play a role. One may hypothesize that compensatory mechanisms are less effective in aged animals. Moreover, in slices the inhibitory potency of the compound may be masked by compensatory mechanisms and by the efficacy of the compound to penetrate the slice (e.g. due to slow diffusion, adhesion to membrane constituents) and cell membranes. The apparent potency in tissue (slices) may thus be lower than the potency seen in single cells or isolated enzyme preparations.

Long-term potentiation of synaptic transmission in the hippocampus is a widely studied model of synaptic plasticity and is thought to underlie learning and memory (Bliss and Collingridge, 1993; Reymann and Frey, 2007). Among the various molecules involved in LTP and learning and memory, cAMP and cGMP play a prominent role (Bailey et al., 1996; Son et al., 1998). We found that the LTP elicited by a weak tetanic stimulation was amplified by 10 μM of BAY 73-6691 in young Wistar rats. These findings are in line with results obtained with the PDE2 inhibitor BAY 60-7550 in rats (Boess et al., 2004). The dose of 30 μM BAY 73-6691 had no effect on either synaptic transmission or LTP. As the PDE9 inhibitor BAY 73-6691 preferentially inhibits the breakdown of cGMP in brain tissue, the inefficiency of BAY 73-6691 at this dose could be due to an excessive increase in cGMP levels relative to the augmentation in the cAMP level.

In line with a study using aged (23–24 months) rats that suffered from an impaired memory (Hsu et al., 2002), the very old FBNF1 rats displayed a markedly reduced LTP compared to that of the young rats. Hsu et al. (2002) showed in addition that phosphatase inhibition enhanced synaptic responses in slices from aged, but not young adult, rats. Thus, it is possible that the increase in intracelullar cGMP levels produced by BAY 73-6691 corrects an age-related impairment of LTP as a result of alterations in the balance of protein kinase/phosphatase activities.

A possible explanation for the effects of BAY 73-6691 on synaptic plasticity is that elevated cGMP levels act pre-synaptically during early LTP as part of an NO/sGC/cGMP pathway (Arancio et al., 2001; Son et al., 1998; Zhuo et al., 1994), where nitric oxide (NO) is believed to act as a retrograde messenger. Elevation of cGMP could result in either increased glutamate release via activation of presynaptic voltage-gated cGMP-dependent ion channels, or more glutamate synthesis via cGMP-dependent protein kinase (PKG). Moreover, beside the already known cAMP/PKA/CREB pathway (Impey et al., 1996), a postsynaptic cGMP/PKG/CREB pathway has been proposed to explain the role of cGMP in longer lasting late LTP (Lu et al., 1999).. Thus, the effects of BAY 73-6691 on memory processes can be explained by an enhancement of early LTP, although the drug may also affect late LTP. This requires further investigation.

4.2. Social recognition task

Rodents are social animals that are innately interested in their conspecifics. Their social discrimination capabilities can be used to measure recognition of a conspecific. Social recognition is measured as the difference in time spent between the first and the second encounters of an adult animal with a juvenile. This test has been found to be sensitive to cognition-enhancing compounds such as α7 nicotinic acetylcholine receptor agonists (Boess et al., 2007) and PDE2 inhibitors in rats (Boess et al., 2004).

One-trial learning tasks, such as the social recognition task, make it possible to investigate different memory processes, dependent on the timing of drug administration (Abel and Lattal, 2001; Rutten et al., 2007). By injecting a drug either before or after the learning trial, or before the test trial, its effects on acquisition, consolidation, or retention processes can be investigated. In the present study, we investigated the effects of BAY 73-6691 on acquisition, consolidation, and retention in the social recognition task in rats and on acquisition of this task in mice. Surprisingly, PDE9 inhibition enhanced social recognition performance, regardless of the timing of its administration. Thus, BAY 73-6691 enhanced acquisition, consolidation, and retention of social recognition memory. However, the lowest dose (0.03 mg kg^{-1}) was not effective when administered in the acquisition phase (60 min before training), whereas it was when administered during the consolidation and retention phases. Similar effects of BAY 73-6691 on the acquisition of social recognition memory were also observed in C57/BL6 mice, suggesting that the results can be generalized to another rodent species.

Table 1
Effects of Bay 73-6691 on the performance in the social and object recognition tasks, and on scopolamine-induced deficits in the passive avoidance task, and on MK-801-induced deficits in the spontaneous T-maze alternation task

	Dose (mg kg^{-1} body mass) of BAY 73-6691						
	0.03	0.1	0.3	1	3	5	10
'Natural', retention-interval dependent forgetting							
Social recognition	●↑		●↑	●↑	●↑		
Object recognition		●↑	●↑	●	●		
Deficit models (scopolamine or MK-801-induced deficits)							
Passive avoidance			●↔	●↑	●↑		
T-maze alternation				●↔		●↔	●↑

↑: Improvement; ●: tested dose; ↔: no change.

Taking into account the half-life of BAY 73-6691, i.e. approximately 2 h, it can be concluded that BAY 73-6691 improves consolidation and retrieval processes in the social recognition task. However, whether a drug affects consolidation processes when injected during the acquisition phase depends on its pharmacokinetic properties (Prickaerts et al., 2005). Further experiments are needed before specific conclusions can be drawn about the effect of BAY 73-6691 on acquisition processes.

To control for possible non-mnemonic effects of the drug on social recognition performance, we compared the time spent inspecting a familiar juvenile with that spent inspecting a novel juvenile after BAY 73-6691 treatment (60 min before T1). If in the previous social recognition task, the reduction in investigation time was not due to remembrance of the juvenile, then the introduction of a novel juvenile in T2 would also lead to a reduction in investigation time after treatment with BAY 73-6691. This was not the case; the investigation time in T2 was not reduced when there was a novel juvenile whereas it was when the familiar juvenile was present. This implies that social recognition performance depends on successful mnemonic representation of the familiar juvenile in long-term memory.

Separate analysis of the effects of BAY 73-6691 confirmed that the compound did not affect the social investigation times during T1 when administered before T1. It reduced, however, the investigation times during the retention trial T2. This observation is compatible with the view that the treatment improves the recognition performance.

4.3. Object recognition task

The object recognition test was developed to assess the effects of experimental manipulations on the cognitive performance of rodents (rats: Ennaceur et al., 1989; mice: Dodart et al., 1997). This task is sensitive to the pharmacological modulation of memory performance by putative cognition-enhancing compounds (nootropics: Ennaceur et al., 1989; PDE2 inhibitors: Boess et al., 2004; PDE5 inhibitors: Prickaerts et al., 2004; Rutten et al., 2005) and to compounds that impair memory performance (Dodart et al., 1997). Here, we observed that BAY 73-6691 had cognition-enhancing effects on acquisition (and eventually consolidation) processes in the object recognition test when injected in doses of 0.1 and 0.3 mg kg^{-1} 30 min before acquisition (T1).

4.4. Passive avoidance task

The passive or inhibitory avoidance test is used in most drug-screening programs. Similar to social and object recognition tasks, the passive avoidance task is a one-trial learning task. Here both the anticholinergic cognition impairer scopolamine (30 min) and BAY 73-6691 (60 min) were administered before the shock trial (learning trial). In this task, scopolamine impaired memory in the retention session and BAY 73-6691 attenuated the scopolamine induced long-term memory deficit at doses of 1 and 3 mg kg^{-1}. Of note, scopolamine and BAY 73-6691 did not affect the rats' behavior in the shock session.

4.5. T-maze continuous alternation task

Gerlai (1998) developed a T-maze continuous alternation task to assess the spatial memory performance of mice. Spontaneous alternation is defined as a visit to the other of the two goal arms of a T-maze visited in the previous trial. In order to alternate or avoid a revisit, a mouse must *remember* the goal arm chosen in the previous trial. The information about the previous visit is stored in spatial working memory. Information held in the working memory is relevant for only one trial. This task appears to depend on the normal functioning of the hippocampus, is guided by extra-maze cues, and is sensitive to strain differences (Gerlai, 1998).

The task is suited to assess the effects of putative cognition-enhancing and cognition-impairing interventions (Spowart-Manning and van der Staay, 2004). Here, we observed that the cognition impairer MK-801 disrupted task performance and that BAY 73-6691 partially attenuated the deficit in working memory. The effective dose of BAY 73-6691 (10 mg kg^{-1}) in this task was considerably higher than the doses that improved memory performance in the social and object recognition tasks and the passive avoidance task. In addition, the deficit induced by MK-801 was not completely reversed, i.e. the percent alteration did not reach the level of vehicle performance. These results suggest that the cognition-enhancing effects of BAY 73-6691 are less pronounced in short-term or working memory processes and more pronounced in long-term memory processes, a notion that needs further experimental investigations.

4.6. Presumed mode of action

PDE9 inhibitors preferentially inhibit the breakdown of cGMP (Wunder et al., 2005). Intrahippocampal injection of cGMP analogs has been shown to enhance memory in an object recognition task (Prickaerts et al., 2002). Therefore it is feasible that the effects of BAY 73-6691 are caused by an increase in cGMP concentrations in brain structures associated with memory, such as the hippocampus. Of note, in a cGMP reporter cell line, BAY 73-6691 alone did not increase basal cGMP levels. However, BAY 73-6691 increased cGMP signals when administered in combination with activators of soluble guanylate cyclase (sGC), such as BAY 58-2667 or BAY 41-2272 (Wunder et al., 2005). Similar findings have been observed after addition of the PDE5 inhibitor sildenafil to hippocampal slices. When sildenafil alone was added to hippocampal slices prepared from Swiss mice, it did not increase cGMP levels. An NO donor was required for cGMP elevation, but memory enhancement in the object recognition task was nevertheless observed after sildenafil treatment only (Rutten et al., 2005).

The PDE9 enzyme has the highest affinity for cGMP of any of the PDEs identified to date (van Staveren et al., 2002). In addition, recent human *in vitro* studies have demonstrated that whereas PDE5 mRNA was not detected in the brains of normal elderly people and of AD patients, PDE9 mRNA showed a widespread distribution (Reyes-Irisarri et al., 2007). This might indicate that PDE5 enzymes are present in lower concentrations or are less active than PDE9 enzymes in the aged brain, which increases the therapeutic potential of PDE9 inhibitors as opposed to PDE5 inhibitors for age-related cognitive decline or neurodegenerative diseases that are accompanied by a decline in memory performance, such as AD.

In this context, it is of interest that BAY 73-6691 appeared to increase the fEPSP slope after mild tetanic stimulation of hippocampal slices more efficiently in slices from very old rats (Brown-Norway X Fischer 344 hybrids, aged 31–35 months) than in those from (young) adult Wistar rats and young adult FBNF1 rats. Under our experimental conditions, PDE9 inhibition facilitated early LTP. Because early LTP is assumed to be related to short-term memory and late LTP to long-term memory (Izquierdo et al., 2002), it seems plausible that late LTP is affected as well since PDE9 clearly improved long-term memory. Whether an additional prolongation of early into late LTP would take place, as is seen after PDE4 inhibition (Barad et al., 1998), remains to be demonstrated.

Taken together, this study is the first to provide experimental evidence for pro-cognitive effects of PDE9 inhibitors. Our results are in concordance with previous studies demonstrating an enhancement of memory and neuronal plasticity by PDE5, PDE2, or PDE4 inhibitors (Barad et al., 1998; Boess et al., 2004; Prickaerts et al., 2004; Rutten et al., 2007). Our data with unimpaired subjects and with subjects treated with scopolamine or MK-801 suggest that

BAY 73-6691 may act as a putative cognition enhancer. Investigations with AD relevant animal models are needed to assess whether PDE9 inhibition may serve as novel therapeutic principle for treating patients suffering from memory impairments that are symptomatic for neurodegenerative diseases such as AD.

Acknowledgments

The authors wish to thank Katrin Böhm for expert technical assistance.

References

Abel, T., Lattal, K.M., 2001. Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology 11, 180–187.

Arancio, O., Antonova, I., Gambaryan, S., Lohmann, S.M., Wood, J.S., Lawrence, D.S., Hawkins, R.D., 2001. Presynaptic role of cGMP-dependent protein kinase during long-lasting potentiation. Journal of Neuroscience 21, 143–149.

Bailey, C.H., Bartsch, D., Kandel, E.R., 1996. Toward a molecular definition of long-term memory storage. Proceedings of the National Academy of Sciences of the United States of America 93, 13445–13452.

Balschun, D., Reymann, K.G., 1994. Cholecystokinin (CCK-8S) prolongs 'unsaturated' theta-pulse induced long-term potentiation in rat hippocampal CA1 *in vitro*. Neuropeptides 26, 421–427.

Barad, M., 2003. Later developments: molecular keys to age-related memory impairment. Alzheimer's Disease and Associated Disorders 17, 168–176.

Barad, M., Bourtchouladze, R., Winder, D.G., Golan, H., Kandel, E., 1998. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. Proceedings of the National Academic of Sciences of the United States of America 95, 15020–15025.

Baratti, C.M., Boccia, M.M., 1999. Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. Behavioural Pharmacology 10, 731–737.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiology Reviews 75, 725–748.

Behnisch, T., Reymann, K.G., 1998. Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus *in vitro*. Neuroscience Letters 253, 91–94.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Blokland, A., Schreiber, R., Prickaerts, J., 2006. Improving memory: a role for phosphodiesterases. Current Pharmacological Design 12, 2511–2523.

Boess, F.G., De Vry, J., Erb, C., Flessner, T., Hendrix, M., Luithle, J., Methfessel, C., Riedl, B., Schnizler, K., van der Staay, F.J., van Kampen, M., Wiese, W.B., Koenig, G., 2007. The novel alpha7 nicotinic acetylcholine receptor agonist *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide improves working and recognition memory in rodents. Journal of Pharmacology and Experimental Therapeutics 321, 716–725.

Boess, F.G., Hendrix, M., van der Staay, F.J., Erb, C., Schreiber, R., van Staveren, W., de Vente, J., Prickaerts, J., Blokland, A., Koenig, G., 2004. Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. Neuropharmacology 47, 1081–1092.

Bourtchouladze, R., Lidge, R., Catapano, R., Stanley, J., Gossweiler, S., Romashko, D., Scott, R., Tully, T., 2003. A mouse model of Rubinstein–Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. Proceedings of the National Academy of Sciences of the United States of America 100, 10518–10522.

Chalimoniuk, M., Strosznajder, J.B., 1998. Aging modulates nitric oxide synthesis and cGMP levels in hippocampus and cerebellum. Effects of amyloid beta peptide. Molecular and Chemical Neuropathology 35, 77–95.

Dantzer, R., Bluthe, R.M., Koob, G.F., Le Moal, M., 1987. Modulation of social memory in male rats by neurohypophyseal peptides. Psychopharmacology (Berl) 91, 363–368.

Dodart, J.C., Mathis, C., Ungerer, A., 1997. Scopolamine-induced deficits in a two-trial object recognition task in mice. Neuroreport 8, 1173–1178.

Dragunow, M., 2004. CREB and neurodegeneration. Frontiers in Bioscience: A Journal and Virtual Library 9, 100–103.

Einat, H., Yuan, P., Gould, T.D., Li, J., Du, J., Zhang, L., Manji, H.K., Chen, G., 2003. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. Journal of Neuroscience 23, 7311–7316.

Ennaceur, A., Cavoy, A., Costa, J.C., Delacour, J., 1989. A new one-trial test for neurobiological studies of memory in rats. II: effects of piracetam and pramiracetam. Behavioural Brain Research 33, 197–207.

Frey, U., Huang, Y.Y., Kandel, E.R., 1993. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 260, 1661–1664.

Gerlai, R., 1998. A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study. Behavioural Brain Research 95, 91–101.

Gong, B., Vitolo, O.V., Trinchese, F., Liu, S., Shelanski, M., Arancio, O., 2004. Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. Journal of Clinical Investigations 114, 1624–1634.

Hsu, K.S., Huang, C.C., Liang, Y.C., Wu, H.M., Chen, Y.L., Lo, S.W., Ho, W.C., 2002. Alterations in the balance of protein kinase and phosphatase activities and age-related impairments of synaptic transmission and long-term potentiation. Hippocampus 12, 787–802.

Impey, S., Mark, M., Villacres, E.C., Poser, S., Chavkin, C., Storm, D.R., 1996. Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron 16, 973–982.

Izquierdo, L.A., Barros, D.M., Vianna, M.R., Coitinho, A., deDavid e Silva, T., Choi, H., Moletta, B., Medina, J.H., Izquierdo, I., 2002. Molecular pharmacological dissection of short- and long-term memory. Cellular and Molecular Neurobiology 22, 269–287.

Lu, Y.F., Kandel, E.R., Hawkins, R.D., 1999. Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. Journal of Neuroscience 19, 10250–10261.

Prickaerts, J., de Vente, J., Honig, W., Steinbusch, H.W., Blokland, A., 2002. cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. European Journal of Pharmacology 436, 83–87.

Prickaerts, J., Sik, A., van der Staay, F.J., de Vente, J., Blokland, A., 2005. Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. Psychopharmacology (Berl) 177, 381–390.

Prickaerts, J., Sik, A., van Staveren, W.C., Koopmans, G., Steinbusch, H.W., van der Staay, F.J., de Vente, J., Blokland, A., 2004. Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. Neurochemistry International 45, 915–928.

Raymond, C.R., Redman, S.J., 2002. Different calcium sources are narrowly tuned to the induction of different forms of LTP. Journal of Neurophysiology 88, 249–255.

Reyes-Irisarri, E., Markerink-Van Ittersum, M., Mengod, G., de Vente, J., 2007. Expression of the cGMP-specific phosphodiesterases 2 and 9 in normal and Alzheimer's disease human brains. European Journal of Neuroscience 25, 3332–3338.

Reymann, K.G., Frey, J.U., 2007. The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 52, 24–40.

Rutten, K., Basile, J.L., Prickaerts, J., Blokland, A., Vivian, J.A., 2008. Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. Psychopharmacology (Berl) 196, 643–648.

Rutten, K., Prickaerts, J., Blokland, A., 2006. Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. Neurobiology of Learning and Memory 85, 132–138.

Rutten, K., Prickaerts, J., Hendrix, M., van der Staay, F.J., Sik, A., Blokland, A., 2007. Time-dependent involvement of cAMP and cGMP in consolidation of object memory: studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. European Journal of Pharmacology 558, 107–112.

Rutten, K., Vente, J.D., Sik, A., Ittersum, M.M., Prickaerts, J., Blokland, A., 2005. The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. Behavioural Brain Research 164, 11–16.

Schröder, U.H., Hock, F.J., Wirth, K., Englert, H.C., Reymann, K.G., 2004. The ATP-regulated K+-channel inhibitor HMR-1372 affects synaptic plasticity in hippocampal slices. European Journal of Pharmacology 502, 99–104.

Son, H., Lu, Y.F., Zhuo, M., Arancio, O., Kandel, E.R., Hawkins, R.D., 1998. The specific role of cGMP in hippocampal LTP. Learning and Memory 5, 231–245.

Spowart-Manning, L., van der Staay, F.J., 2004. The T-maze continuous alternation task for assessing the effects of putative cognition enhancers in the mouse. Behavioural Brain Research 151, 37–46.

van Staveren, W.C., Glick, J., Markerink-van Ittersum, M., Shimizu, M., Beavo, J.A., Steinbusch, H.W., de Vente, J., 2002. Cloning and localization of the cGMP-specific phosphodiesterase type 9 in the rat brain. Journal of Neurocytology 31, 729–741.

Vallebuona, F., Raiteri, M., 1995. Age-related changes in the NMDA receptor/nitric oxide/cGMP pathway in the hippocampus and cerebellum of freely moving rats subjected to transcerebral microdialysis. European Journal of Neuroscience 7, 694–701.

Weeber, E.J., Sweatt, J.D., 2002. Molecular neurobiology of human cognition. Neuron 33, 845–848.

Wilsch, V.W., Behnisch, T., Jager, T., Reymann, K.G., Balschun, D., 1998. When are class I metabotropic glutamate receptors necessary for long-term potentiation? J Neurosci 18, 6071–6080.

Wunder, F., Tersteegen, A., Rebmann, A., Erb, C., Fahrig, T., Hendrix, M., 2005. Characterization of the first potent and selective PDE9 inhibitor using a cGMP reporter cell line. Molecular Pharmacology 68, 1775–1781.

Zhang, H.T., O'Donnell, J.M., 2000. Effects of rolipram on scopolamine-induced impairment of working and reference memory in the radial-arm maze tests in rats. Psychopharmacology (Berl) 150, 311–316.

Zhuo, M., Hu, Y., Schultz, C., Kandel, E.R., Hawkins, R.D., 1994. Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature 368, 635–639.