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Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7

John F. Cryan, Peter H. Kelly, Hans C. Neijt, Gilles Sansig, Peter J. Flor and Herman van Der Putten
Nervous System Research, Novartis Institutes for BioMedical Sciences, Novartis Pharma AG, Basel, Switzerland

Abstract

Glutamatergic neurotransmission has been strongly implicated in the pathophysiology of affective disorders, such as major depression and anxiety. Of all glutamate receptors, the role of group III metabotropic glutamate receptors (mGluR4, mGluR6, mGluR7, mGluR8) in such disorders is the least investigated because of the lack of specific pharmacological tools. To this end, we examined the behavioural profiles of mice with a targeted deletion of the gene for mGluR7 (mGluR7$^{-/-}$) in animal models of depression and anxiety. mGluR7$^{-/-}$ mice were compared with wild-type (mGluR7$^{+/+}$) littermates and showed substantially less behavioural immobility in both the forced swim test and the tail suspension test. Both behavioural paradigms are widely used to predict antidepressant-like activity. Further, mGluR7$^{-/-}$ mice displayed anxiolytic activity in four different behavioural tests, i.e. the light–dark box, the elevated plus maze, the staircase test, and the stress-induced hyperthermia test, while their cognitive performance was normal in the passive avoidance paradigm. Analysis of locomotor activity in a novel environment demonstrated that mGluR7$^{-/-}$ mice were slightly more active in the initial minutes following placement in the chamber only. Together, these data suggest that mGluR7 may play a pivotal role in mechanisms that regulate behavioural responses to aversive states. Therefore, drugs acting at mGluR7 may provide novel treatments for psychiatric disorders such as depression and anxiety.

Introduction

Glutamate (L-Glu) is the major excitatory neurotransmitter in the adult central nervous system. Its fast actions are mediated by ionotropic receptors. In addition, three groups comprised of eight G-protein-coupled metabotropic glutamate receptors (mGluR) mediate slower modulatory actions of L-Glu on neurotransmitter release and cell excitability (Nakanishi, 1994; Conn & Pin, 1997; Schoepp, 2001). In recent years there has been a resurgence of interest in the role of L-Glu in affective disorders such as depression and anxiety (Skolnick *et al.*, 2002; Stewart & Reid, 2002; Krystal *et al.*, 2002; Walker & Davis, 2002). It is becoming clear that manipulation of the glutamatergic system by selective activation/antagonism of various mGluRs can lead to anxiolytic- and/or antidepressant-like effects (Spooren *et al.*, 2000; Chojnacka-Wojcik *et al.*, 2001; Tatarczynska *et al.*, 2001a; Tatarczynska *et al.*, 2001b).

The availability of selective systemically active ligands and several gene knockouts for group I (mGluR1 and mGluR5) and group II receptors (mGluR2 and mGluR3) has substantially aided the elucidation of the role of these mGluR subtypes in brain function (Schoepp, 2001; Spooren *et al.*, 2001). The development of systemically active group III mGluR subtype-selective agents is lagging behind and hampers elucidating the roles of these subtypes in brain function and disease. Nonetheless, studies with group III mGluR-selective but subtype-nonselective compounds and group III mGluR subtype-selective knockout mice have been used successfully to unmask some of their roles of in-brain function. Accordingly, mGluR4 appears to have roles in neuroprotection (Bruno *et al.*, 2000), seizure-threshold (Snead *et al.*, 2000), spatial learning and memory (Pekhletski *et al.*, 1996; Gerlai *et al.*, 1998). mGluR6$^{-/-}$ mice have dysfunction of the ON visual pathway (Masu *et al.*, 1995). mGluR8-deficient mice have increased anxiety-related behaviour (Linden *et al.*, 2002) and subtle performance deficits in some learning tasks (Gerlai *et al.*, 2002). Mice lacking mGluR7, which is the most highly conserved of mGluR subtypes across different mammalian species (Makoff *et al.*, 1996), have a decreased seizure-threshold (Sansig *et al.*, 2001), deficits in amygdala-dependent behaviours (fear response and conditioned taste aversion), but show no alterations in locomotor activity or pain sensitivity (Masugi *et al.*, 1999). Electrophysiological analysis in mGluR7$^{-/-}$ mice further suggests that this receptor is a frequency-dependent regulator of neurotransmitter release (Sansig *et al.*, 2001), and alters short-term synaptic plasticity in the hippocampus (Bushell *et al.*, 2002).

mGluR7 is abundant in brain regions that are known to be critical for the manifestation of anxiolysis and antidepressant action, such as the amygdala, hippocampus and the locus coeruleus (Kinoshita *et al.*, 1998). Group III mGluRs modulate excitatory neurotransmission in the nucleus locus coeruleus (Dube & Marshall, 2000), and regulate a variety of other nonglutamatergic neurotransmitters directly or indirectly (reviewed in Cartmell & Schoepp, 2000). The latter is not surprising as mGluR7 is present in presynaptic terminals of glutamatergic, γ-aminobutyric acid (GABA)ergic and other nonglutamatergic neurons (Bradley *et al.*, 1996; Shigemoto *et al.*, 1996; Shigemoto *et al.*, 1997; Kinoshita *et al.*, 1998; Dalezios *et al.*, 2002). While these findings suggest that mGluR7 could be involved in regulating circuitries that are key in psychiatric conditions, only a limited number of studies have investigated such a proposition in animal models of psychopathology. Further, all such studies have used nonselective, group III mGluR ligands. Chojnacka-Wojcik *et al.* (1997) have shown that after intrahippocampal administration, an antagonist of group III mGlu receptors (R,S)-alpha-methylserine-

Correspondence: Dr J. F. Cryan, as above.
E-mail: john_f.cryan@pharma.novartis.com

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O-phosphate (MSOP) induced anxiolytic effects in a modified Vogel test of anxiety. However, this same research group has shown that a selective group III mGluR agonist, L-serine-O-phosphate (L-SOP), had a similar anticonflict effect (Tatarczynska *et al.*, 2001a). On the other hand, another recent study has demonstrated that the intrahippocampal injection of a different group III-preferring agonist, s-2-amino-4-phosphonobutyrate (LAP-4), failed to alter elevated plus maze behaviour in the rat (Szapiro *et al.*, 2001). Therefore, in the absence of selective mGluR7 ligands, we used mGluR7$^{-/-}$ mice to better test the hypothesis that mGluR7 could be involved in regulating circuitries that are involved in psychiatric disorders using a number of paradigms that are widely used to predict anxiolytic and antidepressant action.

Materials and methods

Animals

mGluR7$^{-/-}$ mice were generated as described previously from E14 (129/Ola) embryonic stem cells (Sansig *et al.*, 2001). Larger age-matched groups of mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice were generated using a specific pathogen-free (SPF) breeding colony of 30 F14,B6-mGluR7$^{+/-}$ males mating with 60–90 F14,B6-mGluR7$^{+/-}$ females. All the mice in the studies reported here carried wild-type or mutant mGluR7 alleles on a 14th generation C57BL/6 genetic background. Mice were weaned at the age of 3 weeks, when tail biopsies were also taken for genotyping. One–three gender-matched littermates were housed per cage. Subsequently, genotyped mice were moved to a nonSPF conventional facility in which their behaviour was assessed at the ages of 10–14 weeks. Housing was at room temperature, in a 12 h light : dark cycle, with lights on at 06.00 h. Food pellets and tap water were available *ad libitum* unless stated otherwise. All behavioural experiments were conducted during the light cycle. Experimentally naïve animals were used in all experiments, with the following exceptions: (i) those tested in the tail suspension test had previously undergone the forced swim test 1 week earlier; (ii) those tested for passive avoidance had previously been used in the staircase test; (iii) those used in the elevated plus maze were used earlier for the primary observation test (POT). All animal experiments were subject to institutional review and conducted in accordance with the Veterinary Authority of Basel-Stadt, Switzerland.

POT

A battery of behavioural and physiological observations were made to investigate if mGluR7$^{-/-}$ mice had any gross differences compared with mGluR7$^{+/+}$ mice. This was important to investigate, as these mice have been shown previously to develop an enhanced susceptibility to seizures between the ages of 8–12 weeks. This increase in spontaneous seizure episodes was primarily detected upon cage change. Also, it was specific to certain bedding material, and observed mainly in animals that were housed permanently under SPF conditions (Sansig *et al.*, 2001). The observations quantified were the presence of twitches, tremor, convulsions, piloerection, sterotyped behaviour, lacrimation, salivation, ptosis, catelepsy, passivity, falling convulsion and ataxia. In addition, the frequency and quality of breathing was observed. Alterations in skin colour, tail position, pelvic position, limb tonus, abdominal tonus and pupil width were observed. The nature of locomotion, motility in cage, rearing and grouping of animals was quantified, as was their overall flight and fear reaction. In addition, novelty behaviour was observed and a series of reflexes checked, including pinna reflex, toepinch, tailpinch and provoked biting. Body temperature was also quantified. This battery of tests has been validated in our laboratories to detect stimulant and sedative effects in mice in addition to other effects of pharmacological agents.

The forced swim test

The mouse forced swim test is the most widely used test to assess alterations in depression-like behaviour in genetically modified animals (Cryan *et al.*, 2002). It was conducted as previously described (Cryan *et al.*, 2001). Briefly, mice were placed individually into Plexiglas cylinders (24 cm tall $\times$ 21 cm in internal diameter) filled with water (23–25 °C) to a depth of 15 cm. All test sessions were recorded by a video camera positioned directly above the cylinders. Videotapes were subsequently scored blind by a trained observer. The behavioural measure scored from videotape was the duration of immobility during the last 4 min of the test period. A mouse was judged to be immobile when making only those movements necessary to keep its head above water.

The tail suspension test

The tail suspension test is another well-characterized test for assessing depression-like and antidepressant-like activity (Porsolt, 2000; Cryan *et al.*, 2002; Cryan *et al.*, 2003). The test was carried out essentially as described previously (Steru *et al.*, 1985). Mice were individually suspended by the tail to a horizontal ring-stand bar (distance from floor = 30 cm) using adhesive tape (distance from tip of tail = 2 cm). Typically, mice demonstrated several escape-orientated behaviours interspersed with temporally increasing bouts of immobility. A 6-min test session was employed, which was videotaped. Videotapes were subsequently scored by a highly trained observer who was unaware of the treatment. The parameter recorded was the number of seconds spent immobile. In anticipation of tail climbing behaviour, which confounds interpretation of data and has been previously described in C57/Bl6 animals (Mayorga & Lucki, 2001), the background strain which the mGluR7 allele was backcrossed on, larger than usual numbers of animals ($n = 23$) were tested in the tail suspension test.

Light–dark box

The light–dark box test was carried out essentially as described by Holmes *et al.* (2002). The apparatus consisted of a clear Plexiglas cage (44 $\times$ 21 $\times$ 21 cm) separated into two compartments by a partition, which had a small opening (12 $\times$ 5 cm) at floor level. The open compartment was open topped, transparent and brightly illuminated by a 60 Watt desk lamp overhead. The smaller compartment was 14 cm long and made from black Plexiglas. It was covered on top also by black Plexiglas. Mice were individually placed in the centre of the brightly lit compartment, facing away from the partition and allowed to freely explore the apparatus for 10 min. The apparatus was cleaned thoroughly between subjects. The number of light–dark transitions made was recorded by a trained observer.

Elevated plus maze

The elevated plus maze was carried out as described previously (Rodgers *et al.*, 1997). It comprised two open arms (30 $\times$ 5 cm) and two enclosed arms (30 $\times$ 5 $\times$ 15 cm), which extended from a common central platform (5 $\times$ 5 cm). The configuration formed the shape of a plus sign, with like arms arranged opposite one another, and the apparatus was elevated 60 cm above floor level on a central pedestal. The maze floor was made of black Plexiglas, while the side- and end-walls of the enclosed arms were made from clear Plexiglas. Grip on the open arms was facilitated by inclusion of a small raised edge (0.25 cm) around their perimeter. Animals were transported from the holding room to the laboratory at least 1 h before testing. Mice were placed

onto the central platform facing an enclosed arm. A 6-min trial was performed and, between subjects, the maze was thoroughly cleaned. Direct registrations were made by an observer sitting close to the maze using the following conventional parameters: number of open and closed arm entries (arm entry defined as all four paws entering an arm), time spent on open arms (excluding the central platform).

Staircase test

The test was carried out essentially as described by Simiand *et al.* (1984) and consisted of placing an experimentally naïve mouse in an enclosed staircase with five steps made of grey plastic. Each step was 2.5 cm in height, 7.5 cm in length and 11 cm in width. The apparatus was 45 cm in length, with one end 12 cm and the other 25 cm in height. The number of steps climbed and rearings made in a 3-min period were observed. The step-climbing count was increased every time the animal moved from one step to another in the ascending direction. The apparatus was briefly wiped with a wet paper towel and dried between animals. Animals were singly housed and moved to the testing room at least 1 h prior to testing commenced.

Stress-induced hyperthermia (SIH)

The test procedure for the modified SIH was adapted from Van der Heyden *et al.* (1997). Mice were singly housed overnight in the testing room with free access to water and food. Rectal temperature was measured in each mouse twice, i.e. at $t = 0$ min (T_1) and $t = +15$ min (T_2). The first measurement of temperature serves as the stressor and results in a rapid hyperthermic response. The difference in temperature ($T_2 - T_1$) was considered to reflect the SIH. Time-points were based on previous experiments which showed that a $T_2 - T_1$ interval of 15 min was optimal in terms of SIH (Spooren *et al.*, 2002). Rectal temperature was measured to the nearest 0.1 °C by an ELLAB instruments (Copenhagen, Denmark) thermometer Model DM 852 by inserting a lubricated thermistor probe model PRA-22002-A (ELLAB) 2.2 mm diameter 20 mm into the rectum; the mouse was hand-held at the base of the tail during this determination and the thermistor probe was left in place for 15 s.

Locomotor activity

Locomotor activity was analysed in a novel environment. The activity monitor consisted of a black and white video camera, mounted in the top-centre of an enclosure ($60 \times 40 \times 50$ cm), whereby a cage ($55 \times 33 \times 19$ cm) was positioned in the enclosure. Each second, a single video frame was acquired with a highly accurate, programmable, monochrome frame grabber board (Data Translation, Marlboro, MA, USA; type DT3155). Using in-house developed software, digitized pixels of two successive frames were compared, and the total number of pixels with altered intensity was counted (independently for pixels with increased and decreased intensity). This allowed the detection of the animal's position within the cage (the centre of pixels with decreased intensity, because animals were dark compared with background). Distance travelled (distance in cm between centres of activity when movement was $> 10\%$ body size) was analysed and stored every 5 min.

Passive avoidance test

As deficits in conditioned taste aversion were previously ascribed for these mice, we sought to investigate whether they had any cognitive deficits in the passive avoidance test. Before passive avoidance training, mice were housed singly. One-trial step-through passive avoidance training was performed as previously described in detail (Venable & Kelly, 1990). In brief, on the training trial, each mouse was placed singly into the light side of a two-compartment trough-shaped apparatus. The door to the dark compartment was opened and, simultaneously, timing by a computer was initiated. When the mouse broke a photocell beam located 10.5 cm into the dark compartment, the latency from opening the door to the animal breaking the beam (step-through latency) was automatically recorded and a Campden Instruments 521 C Shock Source was automatically activated. This resulted in the application of a footshock (0.5 mA rectangular current waves) between the stainless steel plates, which comprised the dark compartment. The footshock lasted a maximum of 5 s or until the animal escaped back to the light compartment. In the present experiments, all animals escaped back to the light compartment within 5 s. Animals which did not enter the dark compartment within 150 s on the training trial were given a training latency of 150 s, received no footshock, and were excluded from the memory retention test. The memory retention test was performed on the day following the training trial and was identical to it except that no footshock was administered. Maximum latency in the retention test was 300 s.

Statistics

Forced swim test, tail suspension test, elevated plus maze, staircase test, SIH and passive avoidance data were analysed using one-way analysis of variance (ANOVA), with genotype as factor. Light–dark box and locomotor activity were analysed using a repeated-measure ANOVA, with genotype as the between factor and time as the within subject variable. Statistically significant effects were followed where appropriate with Fisher's post-hoc tests. The level of significance was set at $P < 0.05$.

Results

POT

Unlike mGluR7$^{-/-}$ mice kept under SPF conditions where spontaneous seizures were observed as reported earlier (Sansig *et al.*, 2001), this phenotype was not apparent in mGluR7$^{-/-}$ mice kept in our nonSPF facilities in which all behavioural assessments were carried out. To assess whether ablation of mGluR7 receptors had any other effects on gross behaviour and physiology, mGluR7$^{-/-}$ mice were subjected to an extensive POT battery. Other than a mild reduction in provoked biting observed in mGluR7$^{-/-}$ mice, these tests revealed no significant differences between mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice in any of the other 29 parameters assessed (see Materials and methods), including locomotion in and out of home-cage.

Depression-like behaviour in mGluR7$^{-/-}$ mice

In light of the pronounced localization of mGluR7 in regions known to be implicated in depression and the mediation of the antidepressant response, we investigated whether mGluR7$^{-/-}$ mice had an altered behaviour in the forced swim test and the tail suspension test. These tests are the two most widely used paradigms for assessing alterations in depression-related behaviour in genetically altered animals (Porsolt, 2000; Cryan *et al.*, 2002; Seong *et al.*, 2002).

The forced swim test

As shown in Fig. 1A, mGluR7$^{-/-}$ mice had significantly lower immobility scores than their mGluR7$^{+/+}$ counterparts ($F_{1,46} = 13.53$, $P = 0.001$), which is indicative of an antidepressant-like phenotype in mGluR7$^{-/-}$ mice.

The tail suspension test

The test revealed (Fig. 1B) that mGluR7$^{-/-}$ mice had significantly lower immobility scores than age- and sex-matched mGluR7$^{+/+}$ mice from the same set of litters ($F_{1,30} = 68.74$, $P < 0.001$). The outcomes in

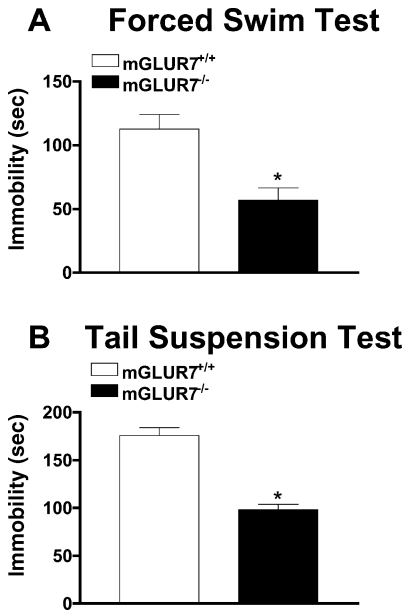

FIG. 1. Antidepressant-like activity in mGluR7$^{-/-}$ mice. (A) mGluR7$^{-/-}$ mice ($n = 24$) had a much lower immobility score than mGluR7$^{+/+}$ ($n = 24$) in the mouse forced swim test, which indicates an antidepressant-like effect. (B) Similarly, mGluR7$^{-/-}$ mice ($n = 19$) had a much lower immobility score than mGluR7$^{+/+}$ ($n = 13$) in the mouse tail suspension test, which is also indicative of an antidepressant-like effect. All bars represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from mGluR7$^{+/+}$ mice.

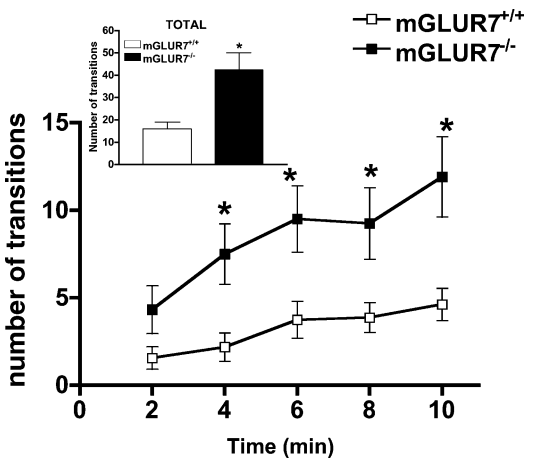

FIG. 2. Anxiolytic-like effects of mGluR7$^{-/-}$ mice in the light–dark box test. (A) mGluR7$^{-/-}$ mice ($n = 12$) had a markedly higher number of transitions between the dark and light compartments throughout the 10 min of the test when compared with mGluR7$^{+/+}$ animals ($n = 16$). (Inset) Analysis of the total number of light–dark transitions further demonstrates that mGluR7$^{-/-}$ mice are less anxious than their wild-type counterparts. All data points or bars represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from mGluR7$^{+/+}$ mice.

both forced swim test and the tail suspension test are therefore indicative of an antidepressant-like phenotype in mGluR7$^{-/-}$ mice. One of the drawbacks of the tail suspension test is that certain strains of mice climb up their tail onto the supporting bar (or other support), making it impossible to interpret the behaviour of such animals in terms of depression-related behaviour, as they are no longer in an inescapable situation. Mayorga & Lucki (2001) recently demonstrated that C57/Bl6 mice have a very high incidence of tail climbing compared with other strains. Indeed, in our studies 14 out of 46 mice (30%) (of which four mice were mGluR7$^{-/-}$ and 10 mGluR7$^{+/+}$) climbed their tails. This climbing is to be predicted given that the mGluR7 mutant allele was backcrossed for 14 generations onto the C57/Bl6 background strain. We excluded all animals from analysis that showed tail climbing in order not to confound our behavioural analysis. Furthermore, based on the limited dataset on tail climbing in the literature, it is difficult to give any significance to the fact that slightly less mGluR7$^{-/-}$ mice climbed their tails than did wild-type controls.

Anxiety-like behaviour in mGluR7$^{-/-}$ mice

Three distinct behavioural and one physiological paradigm were used to assess anxiety-related behaviours in mGluR7$^{-/-}$ mice.

The light–dark box

Upon being placed in the lit side of the apparatus, some freezing behaviour was observed in many of the mGluR7$^{+/+}$ mice (7/16, 44%) as compared with only 2/12 (17%) mGluR7$^{-/-}$ mice failed to enter the dark compartment within 6 min. These observations are in agreement with previous findings that showed a decrease in freezing behaviour in mGluR7$^{-/-}$ mice (Masugi *et al.*, 1999). In a similar manner, ANOVA revealed that: (i) mGluR7$^{-/-}$ mice had a significantly higher number of transitions between the light and dark compartments ($F_{1,26} = 12.62$, $P = 0.001$); and (ii) their number of transitions increased during the test ($F_{4,104} = 9.27$, $P < 0.001$). Altogether, these effects are indicative of anxiolytic-like behaviour of mGluR7$^{-/-}$ mice (Fig. 2).

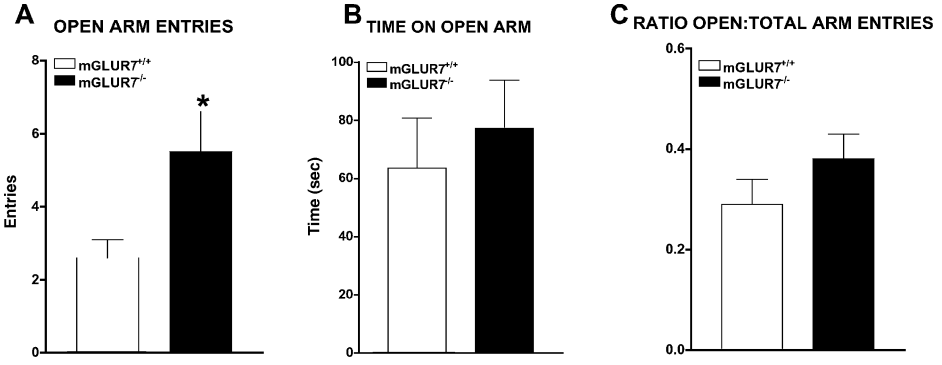

FIG. 3. Anxiolytic-like effects of mGluR7$^{-/-}$ mice in the elevated plus maze. (A) mGluR7$^{-/-}$ mice ($n = 24$) had significantly more entries into the open arms of the elevated plus maze compared with mGluR7$^{+/+}$ mice ($n = 24$). (B) mGluR7$^{-/-}$ mice did not have a significant increase in the time spent on the open arms compared with mGluR7$^{+/+}$ mice. (C) mGluR7$^{-/-}$ mice had an increased ratio of open : total arm entries compared with mGluR7$^{+/+}$ mice. However, this failed to reach the level of statistical significance. All bars represent mean values with vertical lines indicating 1 SEM. *Groups that differed significantly from mGluR7$^{+/+}$ mice.

The elevated plus maze

In the elevated plus maze (Fig. 3), mGluR7$^{-/-}$ mice had a significantly higher number of open arm entries as compared with their mGluR7$^{+/+}$ littermates ($F_{1,46} = 6.07$, $P = 0.018$). There was no difference between both genotypes on the number of closed arm entries (data not shown), supporting the proposition that the anxiolytic-like behavioural effects of mGluR7$^{-/-}$ mice are not due to nonspecific alterations on locomotor activity. The ratio of open arm : total arm entries was somewhat higher (31%) in mGluR7$^{-/-}$ mice as compared with mGluR7$^{+/+}$ mice, but this difference did not reach statistical significance. Likewise, mGluR7$^{-/-}$ mice showed a nonsignificant trend to spend more time (27% more as compared with mGluR7$^{+/+}$ mice) on the open arms. Although the magnitude of the response was not as pronounced as that commonly observed after treatment with benzodiazepine-type anxiolytics (Lister, 1987; Griebel *et al.*, 2000), the behavioural effects associated with the lack of mGluR7 in the elevated plus maze are consistent with anxiolytic-like behaviour in these mice.

The staircase test

As shown in Fig. 4, mGluR7$^{-/-}$ mice had a significantly lower number of rearings in the staircase test than mGluR7$^{+/+}$ littermates ($F_{1,29} = 12.30$, $P = 0.002$). There was no difference between both genotypes on the number of steps climbed, again (see elevated plus maze) supporting the interpretation that the effects are not attributable to nonspecific effects on locomotor activity caused by the lack of mGluR7. The ratio of steps : rears, the primary index of anxiolysis in this test (Simiand *et al.*, 1984), was significantly higher ($F_{1,29} = 12.62$, $P = 0.001$) in mGluR7$^{-/-}$ as compared with mGluR7$^{+/+}$ mice. Furthermore, in the novel environment, mGluR7$^{-/-}$ unlike mGluR7$^{+/+}$ mice showed a strong trend toward a significant reduction in urination ($F_{1,29} = 3.16$, $P = 0.086$) and production of faecal boli ($F_{1,29} = 2.39$, $P = 0.133$). Sixty-nine per cent of mGluR7$^{+/+}$ mice showed signs of physiological anxiety (defecation or urination) compared with 25% of mGluR7$^{-/-}$ mice.

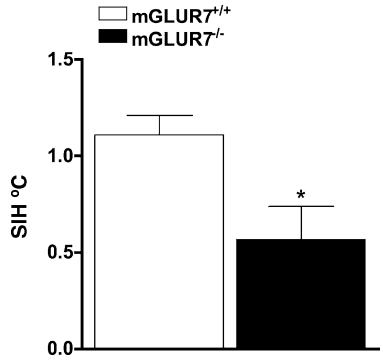

FIG. 5. Anxiolytic-like effects of mGluR7$^{-/-}$ mice in the SIH test. The effects are shown of mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice in the SIH test, a physiological test of anticipatory anxiety. mGluR7$^{-/-}$ mice ($n = 10$) had a much lower SIH than mGluR7$^{+/+}$ ($n = 10$) mice, which is indicative of a reduced anxiety. Bars represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from saline-treated animals.

The SIH test

Similar to what we had seen in our POT battery, there was no significant difference in basal temperature (T_1) between mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice (mean ± SEM = 36.1 ± 0.14 °C for mGluR7$^{+/+}$ mice; 36.3 ± 0.19 °C for mGluR7$^{-/-}$ animals). However, 15 min

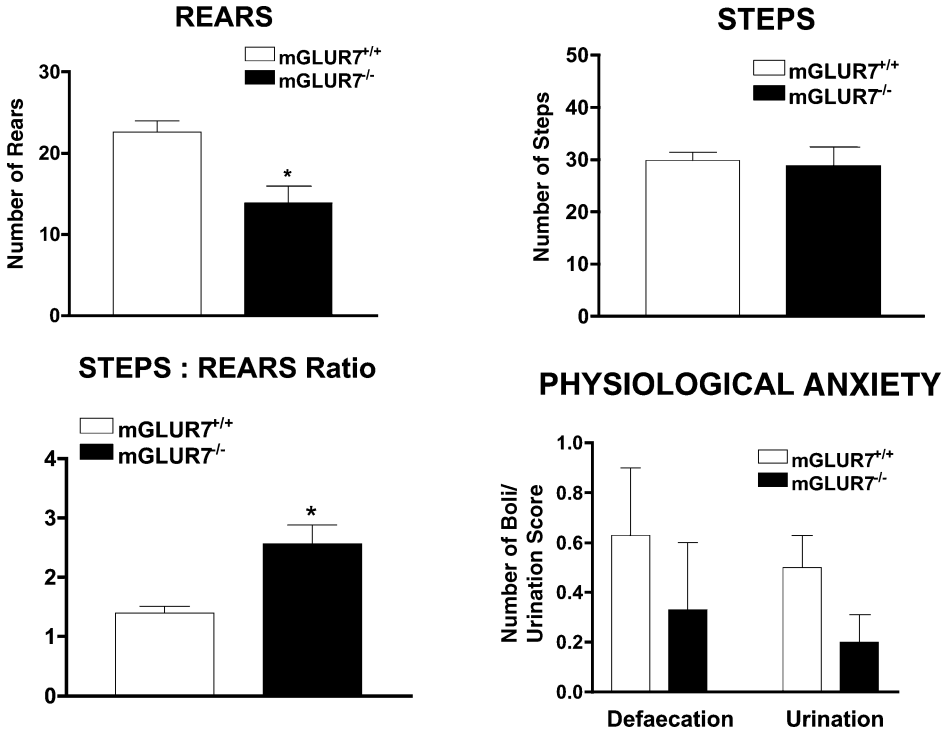

FIG. 4. Anxiolytic-like effects of mGluR7$^{-/-}$ mice in the staircase test. (A) mGluR7$^{-/-}$ mice ($n = 16$) had significantly less rearings in the staircase apparatus compared with mGluR7$^{+/+}$ mice ($n = 16$). (B) mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice did not differ in the total number of steps climbed. (C) mGluR7$^{-/-}$ mice had an increased ratio of steps : rears as compared with mGluR7$^{+/+}$ mice, which is the primary indicator of reduced anxiety in this test. (D) mGluR7$^{-/-}$ mice did not manifest the physiological aspects of anxiety to the same extent as mGluR7$^{+/+}$ mice. This is indicated by the reduced amount of urination and defecation in the mGluR7$^{-/-}$ mice compared with mGluR7$^{+/+}$ mice. All bars represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from mGluR7$^{+/+}$ mice.

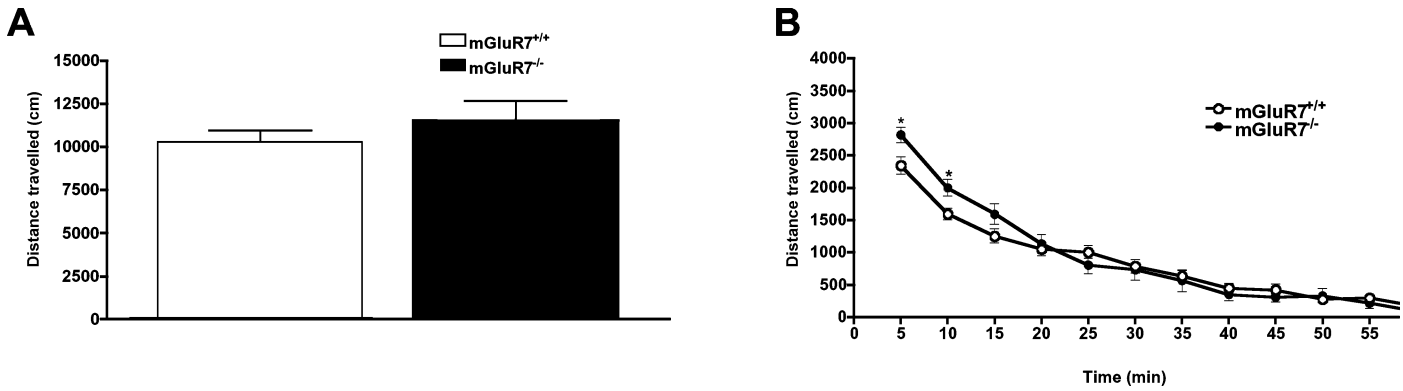

FIG. 6. The effects of mGluR7$^{-/-}$ mice on locomotor activity. (A) mGluR7$^{-/-}$ mice ($n = 11$) did not differ from their wild-type controls ($n = 16$) on locomotor activity over the entire 60 min observed. Bars represent mean values, with vertical lines indicating 1 SEM. (B) mGluR7$^{-/-}$ mice had significantly higher activity during the first 10 min of the testing period only compared with their wild-type controls. Datapoints represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from saline-treated animals.

following mild stress (T_2) [the taking of the initial temperature (at T_1) serves as the stressor], the body temperature of mGluR7$^{-/-}$ animals was significantly lower than that of mGluR7$^{+/+}$ mice ($F_{1,18} = 5.98$, $P = 0.025$, data not shown). When SIH was calculated ($T_2 - T_1$), mGluR7$^{-/-}$ as compared with mGluR7$^{+/+}$ mice had significantly reduced SIH ($F_{1,18} = 7.48$, $P = 0.014$), which is indicative of an attenuated anticipatory anxiety in mGluR7$^{-/-}$ mice (Fig. 5).

Locomotor activity in mGluR7$^{-/-}$ mice

As many of the behavioural parameters analysed are exploration based, we analysed the effects of mGluR7$^{-/-}$ mice on basal locomotor activity (Fig. 6). There was no difference in total locomotor activity between the genotypes over the entire 60-min period ($F_{1,25} = 0.98$, $P = 0.331$). However, there was a significant effect of time on locomotor activity ($F_{11,\ 275} = 144.90$, $P < 0.001$) and a significant time–genotype interaction ($F_{11,275} = 3.75$, $P < 0.001$). Post-hoc analysis revealed that mGluR7$^{-/-}$ mice had a higher activity level at the first two time-points tested only (5 and 10 min).

Normal passive avoidance learning in mGluR7$^{-/-}$ mice

Previous evidence suggested a deficit in amygdala-dependent memory processing in mGluR7$^{-/-}$ mice using a conditioned taste-aversion paradigm (Masugi *et al.*, 1999). To analyse whether the animals had any deficit in a different cognitive task, they were tested in a passive avoidance learning paradigm. On the training day, mGluR7$^{-/-}$ and mGluR7$^{+/+}$ mice showed no significant differences in behaviour, although it took most mGluR7$^{-/-}$ mice longer to enter the dark chamber ($F_{1,30} = 3.20$, $P = 0.084$), which is again consistent with an anxiolytic-like phenotype. On the retention test day (Fig. 7), both mGluR7$^{-/-}$ as well as mGluR7$^{+/+}$ mice showed equal avoidance of the dark compartment in which they had been previously shocked ($F_{1,30} = 0.25$, $P = 0.619$). These results indicate that mGluR7$^{-/-}$ mice successfully learn to avoid the aversive stimuli in this test. In agreement with the results in the staircase test (see above), during the retention test mGluR7$^{-/-}$ mice had a significantly reduced urination score as compared with mGluR7$^{+/+}$ mice (0.69 vs. 0.19).

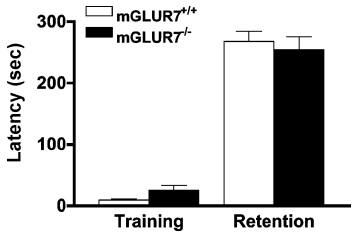

FIG. 7. mGluR7$^{-/-}$ mice have normal passive avoidance learning. On the training day, mGluR7$^{-/-}$ mice ($n = 16$) had a slightly reduced latency to enter the dark compartment compared with mGluR7$^{+/+}$ mice ($n = 16$). On the retention test day, both genotypes equally well avoided the compartment where they had previously been shocked. All bars represent mean values, with vertical lines indicating 1 SEM. *Groups that differed significantly from saline-treated animals.

Discussion

The present studies demonstrate that selective ablation of the group III mGlu receptor subtype mGluR7 is associated with changes in animal behavioural paradigms predictive of antidepressant and anxiolytic action. Our findings suggest that drugs acting at mGluR7 may provide novel treatments for psychiatric disorders such as depression and anxiety.

The forced swim test is the most widely used pharmacological model to assess antidepressant activity in rodents. This is largely due to its ease of use, reliability across laboratories and its ability to detect activity of a broad spectrum of clinically effective antidepressants (Cryan *et al.*, 2002). Furthermore, this test is the most widely used paradigm to assess depression and antidepressant-related phenotypes in genetically altered mice (Porsolt, 2000; Cryan *et al.*, 2002; Seong *et al.*, 2002). The magnitude of reduced immobility of the mGluR7$^{-/-}$ mice in this test is similar to that which we and others have reported for a variety of antidepressants, including selective monoamine reuptake or oxidase inhibitors (Porsolt *et al.*, 1977; Cryan *et al.*, 2001).

The tail suspension test is similar to the forced swim test in the constructs that it purports to assess (immobility) and for its ability to detect a broad spectrum of antidepressants (Steru *et al.*, 1985). Nonetheless, it is becoming clear that both tests are probably different from each other in terms of the biological substrates that underlie their observed behaviours (Bai *et al.*, 2001). Accordingly, it is believed that using both tests can give complementary and/or converging information on activities of novel potential antidepressants or molecular pathways, including those altered in genetically modified animals (Porsolt, 2000; Bai *et al.*, 2001; Conti *et al.*, 2002; Cryan *et al.*, 2003). In both tests, the behavioural responses

comprise a coping strategy (Thierry *et al.*, 1984) in which immobility behaviours represent the psychological concept of 'entrapment' described in clinical depression (Dixon, 1998; Gilbert & Allan, 1998; Lucki, 2001). From our current data it is clear that a lack of mGluR7 receptors alters one or more important molecular mechanisms underlying such behaviours. mGluR7 is highly abundant in areas such as nucleus locus coeruleus, lateral septal nucleus, frontal cortex, amygdala and hippocampus. All are thought to be critically involved in modulation of antidepressant activity in paradigms such as the forced swim and tail suspension tests and in clinically depressed patients. Therefore, the mGlu7 receptor may be an innovative target for developing therapeutic agents to treat major depression.

In the light–dark box test, mGluR7$^{-/-}$ mice had an increased number of transitions between dark and light sides in the box, which implicates a role for mGluR7 in anxiety-related behaviour. In further support of this notion, mGluR7$^{-/-}$ mice were also less inhibited at exploring the dark compartment and did not freeze as much as mGluR7$^{+/+}$ mice once placed in the apparatus. Likewise, mGluR7$^{-/-}$ mice displayed anxiolytic-like behaviour in the elevated plus maze. Whilst both tests rest upon similar naturalistic conflicts between the tendency to explore a novel environment and aversive properties of a brightly lit, open area, the elevated plus maze also includes two additional anxiety-provoking environmental parameters (height and a totally open area) (Crawley, 2000). The use of both the light–dark box and the elevated plus maze tests, as reported here, is thought to be important to better examine anxiety-like behaviour (Griebel *et al.*, 2000). In particular, it has been described that mice of the strain C57BL/6, onto which the mGluR7 mutant allele was backcrossed for 14 generations, can vary in baseline anxiety and differential sensitivity to anxiolytics between these two paradigms (Griebel *et al.*, 2000). The anxiolytic-like behaviour of mGluR7$^{-/-}$ mice in the elevated plus maze is not as robust as that seen in the light–dark box test, nor is it as pronounced as in animals treated with benzodiazepines (Griebel *et al.*, 2000). Further evidence for the anxiolytic-like activity of mGluR7$^{-/-}$ mice is from the staircase test. Here, we observed a reduction specifically in rearings, without any changes in the number of steps climbed by mGluR7$^{-/-}$ vs. mGluR7$^{+/+}$ mice. Such a behavioural pattern in this test is quite selective for anxiolytic agents. Stimulant drugs, for example, tend to affect both parameters (Simiand *et al.*, 1984). A further indicator of decreased anxiety was a reduction (albeit not quite statistically significant) in the number of faecal boli and urinations of mGluR7$^{-/-}$ mice as compared with mGluR7$^{+/+}$ littermates in this test. These parameters provide an independent index of the altered physiological response of mGluR7$^{-/-}$ mice to the aversive environment (Gray & Lalljee, 1974).

The SIH test offers a more parametric analysis of the physiological response to anxiety (in this case the anticipatory anxiety caused by an acute stressor) than that in the staircase test whereby physiological responses to stress are auxiliary to the behavioural readouts. Also, this test has been validated extensively as a preclinical paradigm useful to detect conventional and putative anxiolytics, including drugs acting through group I and group II mGluRs (Spooren *et al.*, 2002; Olivier *et al.*, 2003). Brodkin *et al.* (2002) have recently shown that the SIH test can detect anxiolytic-like effects in genetically modified animals. In these studies, mGluR5 knockout animals had a reduced SIH which is in accordance with the ability of mGluR5 receptor antagonists to reverse this hyperthermia (Brodkin *et al.*, 2002; Spooren *et al.*, 2002). In line with our observed effects in the above exploration-based anxiety models, mGluR7$^{-/-}$ mice also had significantly reduced SIH. By using multiple tests that are thought to model different facets of the anxiety syndrome, we believe to have minimized the contribution of major confounding external and internal factors (Griebel *et al.*, 2000; Holmes, 2001; Belzung, 2001) other than the mutant allele.

As many of the tests employed here have a motor component to their readout of antidepressant or anxiolytic activity, we examined basal locomotor activity of knockout and wild-type mice in a novel environment. Although there was no overall difference in locomotor activity over the 60-min testing period between the genotypes, mGluR7$^{-/-}$ mice were slightly more active during the initial period after placement in the apparatus. Previous data from Masugi *et al.* (1999) using an open field failed to demonstrate any effect of deletion of mGluR7 on basal locomotion. It could be argued that this mild increase in activity observed here may contribute to the observed anxiolytic/antidepressant-like phenotype of the mice, and indeed such an influence is impossible to rule out. However, the converse could also be reasoned, i.e. that a mild increase in activity may be due to the fact that the animals are less anxious and therefore behaviourally disinhibited upon presentation with novel stimuli. This latter assumption is strengthened by a number of assertions. First, both the clear reductions in SIH and the trend toward a significant reduction in the physiological readouts observed in the staircase and passive avoidance tests are independent of locomotor activity. Secondly, in the staircase test and elevated plus maze there were no significant differences between genotypes on indices of hyperactivity (number of steps climbed and closed arm entries, respectively). Thirdly, the magnitude of the antidepressant and/or anxiolytic effects is quantitatively much greater in most of the tests than that seen on locomotor activity, the exception being the elevated plus maze. Fourthly, mGluR7$^{-/-}$ mice show a reduction in rearing in the staircase test (present data) and a significant decrease in vertical activity in a novel environment (J. F. Cryan, unpublished observations) which is not consistent with hyperactivity. Fifthly, when the animals were observed in their home-cage for the POT, no overt change in locomotion was observed. Finally, in the passive avoidance apparatus, mGluR7$^{-/-}$ mice have a trend toward an increased training latency which can possibly be interpreted as being less anxious in the bright compartment, hyperactivity would more likely be manifested as a reduction in latency to enter the dark compartment. For these reasons, reductions in anxiety and alterations in coping behaviour appear to be a more plausible explanation for the pattern of results in the various tests than hyperactivity *per se*.

Of all group III mGluRs, mGluR7 is the most widely distributed presynaptic auto- and heteroreceptor in the brain. Like most other group III mGluRs, it is thought to provide mainly negative feedback limiting transmitter release at synapses in a frequency-dependent fashion (Sansig *et al.*, 2001; Bushell *et al.*, 2002; Losonczy *et al.*, 2002; for review, see Cartmell & Schoepp, 2000). Its widespread distribution in the brain, especially in regions known to be critical in emotional behaviours, makes it a challenge to understand whether the effects reported here are based on changes in a specific transmitter system, such as the noradrenergic, serotonergic, GABAergic, glutamatergic and/or dopaminergic system. Further, we cannot rule out that potential developmental compensatory changes have occurred in mGluR7$^{-/-}$ mice that may impact their behaviour. The development of selective mGluR7 ligands will significantly address such issues and aid in the dissection of the exact role of this receptor in depression and anxiety.

Further evidence for a role of mGluR7 in depression comes from the work of Neugebauer *et al.* (2000). They have shown a loss of sensitivity to the inhibiting effects of group III mGluR agonists following withdrawal from chronic cocaine, and they postulate that such a loss, which is similar to what we have artificially generated in

our mGluR7$^{-/-}$ mice, may counteract adaptive changes such as increases in anxiety and depression seen in cocaine withdrawal. Similarly, Kenny and colleagues (Kenny and Markou, personal communication) have shown that antagonists of presynaptic group II mGluRs may have anti-anhedonic effects similar to that of certain fast-acting antidepressant agents, further confirming that blockade of the presynaptic inhibition of glutamate may be a useful strategy for the development of novel antidepressant agents. Accordingly, our current data provide novel evidence that selective modulation of mGluR7 may provide clinical benefit in psychiatric disorders such as depression and anxiety.

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Abbreviations

ANOVA, analysis of variance; GABA, γ-aminobutyric acid; LAP-4, s-2-amino-4-phosphonobutyrate; L-Glu, glutamate; L-SOP, L-serine-O-phosphate; mGluR, metabotropic glutamate receptor; MSOP, (R,S)-alpha-methylserine-O-phosphate; POT, primary observation test; SIH, stress-induced hypothermia; SPF, specific pathogen-free.

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