Molecular Psychiatry (2008) 13, 970–979

www.nature.com/mp

ORIGINAL ARTICLE

mGluR7 facilitates extinction of aversive memories and controls amygdala plasticity

M Fendt[1,2,4], S Schmid[2,4], DR Thakker[1,4,5], LH Jacobson[1,4], R Yamamoto[1], K Mitsukawa[1], R Maier[1], F Natt[3], D Hüsken[3], PH Kelly[1], KH McAllister[1], D Hoyer[1], H van der Putten[1], JF Cryan[1] and PJ Flor[1]

[1]*Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland;* [2]*Tierphysiologie, Zoologisches Institut, Fakultät für Biologie, Universität Tübingen, Tübingen, Germany and* [3]*Genome and Proteome Sciences, Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland*

Formation and extinction of aversive memories in the mammalian brain are insufficiently understood at the cellular and molecular levels. Using the novel metabotropic glutamate receptor 7 (mGluR7) agonist AMN082, we demonstrate that mGluR7 activation facilitates the extinction of aversive memories in two different amygdala-dependent tasks. Conversely, mGluR7 knockdown using short interfering RNA attenuated the extinction of learned aversion. mGluR7 activation also blocked the acquisition of Pavlovian fear learning and its electrophysiological correlate long-term potentiation in the amygdala. The finding that mGluR7 critically regulates extinction, in addition to acquisition of aversive memories, demonstrates that this receptor may be relevant for the manifestation and treatment of anxiety disorders.
Molecular Psychiatry (2008) **13,** 970–979; doi:10.1038/sj.mp.4002073; published online 21 August 2007

Keywords: anxiety; fear learning; glutamate; metabotropic glutamate receptors

Introduction

Anxiety disorders are an enormous public health concern.[1] Current anxiolytic agents largely target GABA(γ-aminobutyric acid)ergic and serotonergic neurotransmission. These treatments are of limited efficacy in a significant proportion of patients and have side effects, underlining the need for developing novel treatments. Glutamate is the major excitatory neurotransmitter in the adult central nervous system. In recent years, there has been a resurgence of interest in the role of glutamate in anxiety disorders.[2,3] The fast actions of glutamate on neurotransmitter release and cell excitability are mediated by ionotropic receptors, whereas metabotropic glutamate receptors (mGluRs) mediate its slower modulatory actions.[4] It is becoming clear that manipulation of the glutamatergic system by selective activation or blockade of mGluR subtypes can lead to altered anxiety and fear responses.[2] Further, in both rodents and humans, pharmacological modulation of glutamatergic neurotransmission enhances the extinction of learned fear,[5,6] suggesting it could be a useful adjunct to behavioural therapy for certain anxiety disorders. This possibility is of particular interest since classical anxiolytics impair the responsiveness to behavioural therapy.[7]

mGluR7, the most highly conserved mGluR subtype across mammalian species,[8] is localized in brain regions that are known to be critical for the processing of emotional behaviour including the amygdala.[9] mGluR7 is located presynaptically, within all major amygdaloid and periamygdaloid nuclei.[9] Studies on mGluR7-deficient mice have pointed towards a key role for this receptor in models of innate fear as well as in learned, amygdala-dependent anxiety-related responses.[10–12] Further progress in understanding this receptor's role in the acquisition and/or extinction of conditioned fear has been hampered by the lack of pharmacological tools. Recently, we developed the first mGluR7-selective allosteric agonist AMN082, which we have shown to be a potent activator of human mGluR7.[13] *In vivo*, AMN082 was demonstrated to modulate stress parameters in an mGluR7-dependent manner.[13]

Given the crucial role of the amygdala in emotional learning and the manifestation of anxiety disorders,[14] we hypothesized that mGluR7 might be crucial in amygdala-dependent fear learning. We, therefore, used novel pharmacological and genetic techniques

Correspondence: Current address. Dr JF Cryan, Department of Pharmacology & Therapeutics, School of Pharmacy, University College Cork, Cork, Ireland.
E-mail: j.cryan@ucc.ie or
Dr PJ Flor, Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, CH-4002 Basel, Switzerland.
E-mail: peter_josef.flor@novartis.com
[4]These authors contributed equally to this work.
[5]Current address: Biosciences R&D, Science & Technology, Medtronic Inc., Minneapolis, MN, USA.
Received 1 November 2006; revised 20 May 2007; accepted 6 June 2007; published online 21 August 2007

to activate or impair mGluR7 activity in rats and mice. Subsequently, we tested the effects of these manipulations in different *in vivo* and *in vitro* models of amygdala-dependent conditioned fear, fear-potentiated startle (FPS) and conditioned taste aversion (CTA). First, we administered AMN082 systemically to enhance mGluR7 action. Second, we used a recently validated technique for the delivery of short interfering RNA (siRNA) to achieve widespread mGluR7 knockdown.[15,16]

Materials and methods

Ethical considerations

All experiments were conducted according to international guidelines for the care and use of laboratory animals and with respect to national laws on animal use. The local ethics committees (Kantonales Veterinäramt Basel, Basel, Switzerland; Regierungspräsidium Tübingen, Tübingen, Germany) approved all experiments.

Pharmacological characterization of AMN082 in vitro

Previously, we demonstrated AMN082's potent and selective activity on human mGluR7.[13] To support the rodent studies described in this paper, we characterize here the activity of AMN082 on rat mGluR7 and closely related receptors. The CHO cell lines stably expressing rat mGluR2 or rat mGluR7a were a generous gift from Professor S Nakanishi (Kyoto University, Japan); pharmacological properties of these cell lines are described.[17,18] CHO cells stably expressing human mGluR4 were described previously and characterized.[19] Pharmacological parameters of AMN082, for example, potency, efficacy and selectivity, were determined using cAMP and GTPγS assays, essentially as published.[8,19,20] Briefly, measurements of cyclic AMP (cAMP) accumulation were performed using the cAMP scintillation proximity assay (SPA) direct screening assay system (Amersham Biosciences, Freiburg, Germany) with cells seeded in 96-well plates at a density of $6.5-8.5 \times 10^3$ cells per well and grown for 1 day until a 40–70% confluent layer of cells was obtained. Cells were incubated for 20 min in hepes buffered saline (HBS) containing 1 mM 3-isobutyl-1-methylxanthine (IBMX). After washing cells with HBS, they were incubated with fresh HBS containing 30 μM forskolin, 1 mM IBMX and the test agents for 20 min. The reaction was stopped by aspirating off the drug-containing medium. The cAMP produced by the cells was released by adding 1% solution of dodecyltrimethylammonium bromide per well and the samples were shaken at room temperature for 5 min. Then, the immunoreagent solution containing equal volumes of [^{125}I]cAMP tracer, antiserum and SPA anti-rabbit reagent in lysis buffer was placed in a final volume of 200 μl per well. A standard curve was constructed in the range of 0.2–25.6 pmol/well of cAMP for each plate. The samples were incubated at room temperature for 15–20 h before the SPA beads were sedimented by centrifugation at 900 r.p.m. for 6 min at room temperature. The plates were then counted in a Packard TopCount (Instrument Company, Meriden, USA). The cAMP levels of the test wells were calculated relative to the standard curve.

Measurements of GTPγ[^{35}S] binding were performed with membranes prepared according to Maj *et al*.[19] Membrane fractions were diluted in assay buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 10 mM $MgCl_2$, 100 mM NaCl, 2 mM ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*,-tetraacetic acid (EGTA), 20 μM GDP, pH 8.0), homogenized briefly using a Polytron and incubated for 10 min at 30°C. Following pre-incubation, assay mixtures were prepared in 96-well microtitre plates. The composition of the assay mixtures in a final volume of 200 μl/well was as follows: 20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, 2 mM EGTA, 20 μM GDP, pH 8.0, 15–35 μg membrane protein (pre-treated as described above), 1.5 mg WGA SPA beads, 0.05–0.2 nM GTPγ[^{35}S] and the test compounds (agonists, modulators and/or antagonists) at the appropriate concentrations. Non-specific binding was measured in the presence of unlabelled GTPγS in excess (10 μM). The samples were incubated at room temperature for 40–60 min (with shaking) before the SPA beads were sedimented by centrifugation at 200 *g* for 10 min at room temperature. The plates were then counted in a Packard TopCount.

Acquisition of FPS

FPS is one of the most widely used models for assessing amygdala-dependent Pavlovian associative learning. Although it can be applied to other species, it is most widely used in the rats.[21] We have previously used the rat FPS to assess the effects of various mGluR ligands in fear learning;[22,23] therefore, this was an appropriate paradigm to examine effects of AMN082. Forty-nine male Sprague–Dawley rats (250–350 g; Charles River GmbH, Sulzfeld, Germany) were treated either with vehicle, one of two different doses of AMN082 (10 or 20 mg/kg, orally (p.o.), dissolved in 0.5% methyl cellulose), or the benzodiazepine anxiolytic diazepam (20 mg/kg, p.o.). Diazepam was included as a positive control.[24] After 30 min, they were fear conditioned in separate conditioning boxes using 10 pairings of light stimulus (15 W; 3.7 s duration) and a 0.8 mA foot shock that was presented in the last 0.5 s of the light stimulus. On the following day, the animals were tested for FPS (pre-extinction test). After 5 min acclimation to the startle apparatus without any stimuli, 10 startle stimuli (10 kHz, 20 ms duration including 0.4 ms rise and fall time, 100 dB sound pressure level (SPL)) were presented to habituate the startle response. Then, 20 further startle stimuli were given, half of them co-terminating with the now conditioned stimulus light, half of them alone (in a pseudorandomized order). The peak magnitudes of the acoustic startle responses were measured by an accelerometer in a time window of 80 ms after the startle stimulus onset.

Electrophysiology

One of the most widely used cellular correlates of conditioned fear learning in rodents is long-term potentiation (LTP) in thalamo-amygdala synapses.[25,26] Pharmacological and genetic perturbations that specifically block this form of amygdala LTP generally also interfere with fear learning and expression. Excitatory postsynaptic currents (EPSCs) in principal neurons of the lateral amygdala were evoked by presynaptic stimulation of thalamic and cortical afferent fibres in rat (male Sprague–Dawley; 2–5 weeks old; Charles River GmbH, Sulzfeld, Germany) brain slices as described previously.[22,23] Rats were chosen for these experiments as it allows direct correlations with data emerging from FPS experiments. Briefly, the 400-μm thick slices were placed in a holding chamber containing standard artificial cerebrospinal fluid (ACSF) (124 mM NaCl, 26 mM $NaHCO_3$, 1.3 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2 mM $CaCl_2$, 2 mM KCl, 10 mM glucose, equilibrated with 95% O_2 and 5% CO_2; all chemicals from Sigma, Munich, Germany) and 100 μM picrotoxin (Sigma, Munich, Germany; to block inhibitory $GABA_A$ receptors). Then, cells were voltage clamped at -80 mV and stimulated via the thalamic or cortical afferents through concentric bipolar stainless-steel electrodes (SNEX 100, Science Products, Hofheim, Germany). Stimulation duration was 150 μs, and stimulus intensity was kept low (0.2–1.0 mA) to avoid non-monophasic responses and spiking after potentiation. Thirty EPSCs were averaged (control) and then 1 μM AMN082 was added to the bath solution. After 5 min wash-in time, another 30 EPSCs were averaged (AMN082). Seal quality and series resistance were monitored throughout the experiment. In LTP experiments, cells were held at -80 mV by a DC current in the current clamp mode. Excitatory postsynaptic potentials (EPSPs) were elicited every 6 s by thalamic fibre stimulation with paired pulses (50 ms interstimulus interval). Ten EPSPs within 1 min were always averaged. After a control measurement for at least 5 min, LTP was induced by a pairing protocol: 10 pairings of presynaptic stimuli with 5 ms postsynaptic depolarizations (0.6 nA) at 30 Hz were repeated 15 times at 0.2 Hz. Depolarizations were precisely timed to the peaks of the EPSPs. Thereafter EPSPs were again measured every 6 s for at least 30 min.

Extinction of FPS

Without any treatment, 81 Sprague–Dawley rats (250–350 g; Charles River GmbH, Sulzfeld, Germany) were fear-conditioned and tested for FPS (pre-extinction test), as described for acquisition. On the following day, the rats were treated either with vehicle, one of the three different doses of AMN082 (3, 10 and 20 mg/kg, p.o.), or diazepam (20 mg/kg, p.o.). Diazepam was included in the design to compare with the acquisition experiments. Further, while there are studies on the effects of diazepam on the extinction of conditioned contextual fear responses,[27,28] there are few data on its effects on extinction of a cue-induced conditioned fear response. After 30 min, the rats were put in the conditioning box, and the conditioned stimulus light was presented 30 times with an interstimulus interval of 1 min (extinction training[29]). On the next day, FPS was measured again as described above. To exclude unspecific effects of AMN082, further 18 rats were trained and tested as described, but no conditioned stimuli were presented in the extinction session, that is no extinction training should occur.

CTA following AMN082

CTA in common with other conditioned fear paradigms is dependent on amygdala function, albeit with an underlying neural circuitry that differs considerably from FPS,[30,31] but clearly including the insula,[32] an area that is repeatedly shown to be important in human anxiety.[33] In this test, animals learn to associate a novel flavour, for example that of normally highly palatable saccharin, with delayed visceral malaise, such as that induced by lithium chloride (LiCl), and subsequently reduce their saccharin consumption. CTA develops rapidly, often after a single conditioning trial.[12,31] The emotional aspects of CTA have recently been highlighted and it has been proposed as a useful model of certain anxiety states.[34] Further, CTA is a suitable paradigm for assessing the extinction of fear memories, given that the rate of extinction is relatively slow (in the order of days). Therefore, we evaluated whether mGluR7 activation would facilitate learning and/or extinction of CTA memories. Thus the use of CTA, allows the investigation of the processes involved in both the acquisition and extinction of aversive memories.[30,35]

We have established that BALB/c mice are a useful strain for assessing CTA,[36] so we used this strain for our initial pharmacological studies. Also, the time course of extinction in this mouse strain is relatively long (approximately 4–5 days) and, therefore, amenable to assess potential facilitatory responses. Sixty mice (BALB/cByJIco, 30 ± 0.2 g; Charles River, L'Arbresle, France) arrived in the lab 2 weeks before experimentation began. After a 1-week settling period, mice were housed singly for a further week and were then trained over 5 days to drink water from 15 ml plastic drinking tubes in two 30-min intervals per day. On the sixth day, mice were given vehicle (0.5% methyl cellulose) or AMN082 at 3 or 6 mg/kg (p.o.) 30 min before the initial presentation of a 0.5% saccharin solution in the drinking tubes. After 30 min the saccharin solution was removed, and following a further 30-min interval, the mice were then given either saline (unconditioned mice) or a malaise-inducing dose of LiCl (intraperitoneal, 6 mEq/kg, dose was chosen based on our previously published dose–response studies[36]). To determine the acquisition of an aversion, and the time course of habituation or extinction in unconditioned and conditioned mice, respectively, mice were presented with a choice of two drinking tubes containing either water or saccharin solution in the morning drinking sessions

for the following days. The weight of water or saccharin consumed was used to calculate an aversion index (AI% = water intake/(water + saccharin intake) × 100). Water alone was presented in the afternoon drinking sessions throughout the experiment.

CTA following siRNA-induced knockdown
siRNA is evolving as a very useful strategy to assess the effects of genes in neuropsychiatric disorders.[37] We have validated a method for siRNA-induced knockdown in the mouse.[16] As many of the initial studies of mGluR7 have relied on information from mGluR7 knockout animals,[10,12,38,39] which are bred on a C57BL/6 background, we chose to conduct our siRNA studies on this background. Seventy-four male C57BL/6 mice (23–31 g; Charles River, France) were housed and surgeries performed as described previously,[15,16] by stereotaxically placing the cannula for continual infusions into the dorsal third ventricle from a subcutaneously implanted osmotic minipump reservoir. Infusions performed were of vehicle alone, two different siRNAs specifically targeting the mGluR7 mRNA (GenBank accession no. BC080315.1) at nt 1301–1321 (siRNA-1) or 2377–2397 (siRNA-2), and corresponding three nucleotide-mismatch siRNAs (mmRNAs) with guide sequences 5′-UGAAU UAAGAAAUCCAAUCCdTdT-3′ (mmRNA-1) or 5′-UA AACGGGAUGUAAGUGCCdAdG-3′ (mmRNA-2), respectively. siRNAs used *in vivo* were selected from a standardized *in vitro* mRNA fusion-construct screening of 11 different constructs with other target mGluR7 sequences at nt 1243–1263, 1442–1462, 1605–1625, 1998–2018, 2196–2216, 2260–2280, 2304–2324, 2445–2465 and 2739–2759 (data not shown[40]). Starting day 7 of intracerebroventricular infusions, mice were trained to drink water from 15 ml Falcon tubes (cut at the base for an outlet) for 30-min sessions per day, between 900 and 1000 (morning session), and between 1630 and 1730 (evening session). Conditioning (test day 1) and subsequent testing of habituation or extinction of aversion (test days 2–6) were performed as described in the earlier section, during the morning drinking session on infusion day 12 onwards. The only modification was that the positions of the two tubes (~1 inch apart), containing saccharin solution or water, were alternated on each subsequent test day to avoid any confounding place preference. The duration of analysis of extinction was restricted to 6 days as this is the duration for which the pumps are able to reliably maintain infusion.

Processing of the brain for gene expression analysis
Mice were decapitated within an hour of their final testing session. Brains were removed and serial coronal sections of 10 μM thickness were obtained at the following anterior-posterior (AP) coordinates, in millimetres, relative to Bregma;[41] 3.56 (granular layer of the olfactory bulb), 2.46 (prefrontal cortex and anterior olfactory nucleus), 1.18 (caudate putamen and nucleus accumbens), −0.46 (to confirm the site of injection), −1.58 (cerebral cortex, hippocampus, thalamus, hypothalamus and amygdala), −4.36 (dorsal raphe) and −5.34 (locus coeruleus). Sections were thaw-mounted onto poly(L-lysine)-coated slides such that each slide contained at least four sections spread across the rostrocaudal axis for each brain region. Slides were coded for a blind mRNA analysis. *In situ* hybridization was performed and quantitated as described,[42] using ^{35}S-labelled antisense riboprobes, to detect mGluR7 or neuron-specific enolase (NSE) mRNA on adjacent brain sections. The DNA templates, for riboprobe synthesis, were generated from cDNA fragments of mouse mGluR7 (nt 1265–1724; GenBank accession no. XM144986) or NSE (nt 2089–2290; GenBank accession no. X52380). At the end of the *in situ* hybridization procedure, slides were dipped in liquid nuclear emulsion and the optical density (OD) of silver grains, positive for the probed mRNA, was quantified as described previously.[42] In brief, densitometric quantification of mRNA-positive grains in each brain region is expressed as percent OD values relative to the values in corresponding brain regions from vehicle-treated mice.

Drugs
AMN082 was synthesized internally, at Novartis Pharma AG, Basel, Switzerland. Doses were chosen based on pharmacokinetic profile of the drug in the brain following oral administration in rats and mice (PJ Flor and A Enz, unpublished results). Remarkably, lower doses are required in mice than rats to reach equivalent brain levels, hence doses were adjusted accordingly between species. L-glutamate, L-AP4 and DL-AP4 were purchased from Tocris Biosciences (Bristol, UK). Lithium and diazepam (Diazepam–Lipuro emulsion) were obtained from Sigma and Braun Melsungen AG (Melsungen, Germany), respectively. Doses were chosen based on previous experiments in our laboratories.[24,36]

Statistical analysis
In all experiments, analysis of variance or Student's *t*-test were used. For *post hoc* analysis, Dunnett's test (*in vitro* pharmacology, FPS data), Fisher's least significant difference (LSD) tests (CTA data) or Tukey's tests (densitometric analysis) were used. $P < 0.05$ was used to indicate statistical significance.

Results

Pharmacological characterization of AMN082 in vitro
Previously, we have reported the *in vitro* characterization of AMN082 on cloned human glutamate receptors.[13] To support the rodent behavioural studies with AMN082 disclosed in this study, we consider it important to describe the activity of AMN082 on rat mGluR7 and closely related receptors. Using the classical cAMP assay for rat mGluR2 and −7a, we show that AMN082 potently stimulates rat mGluR7 (effector concentration for half-maximum response

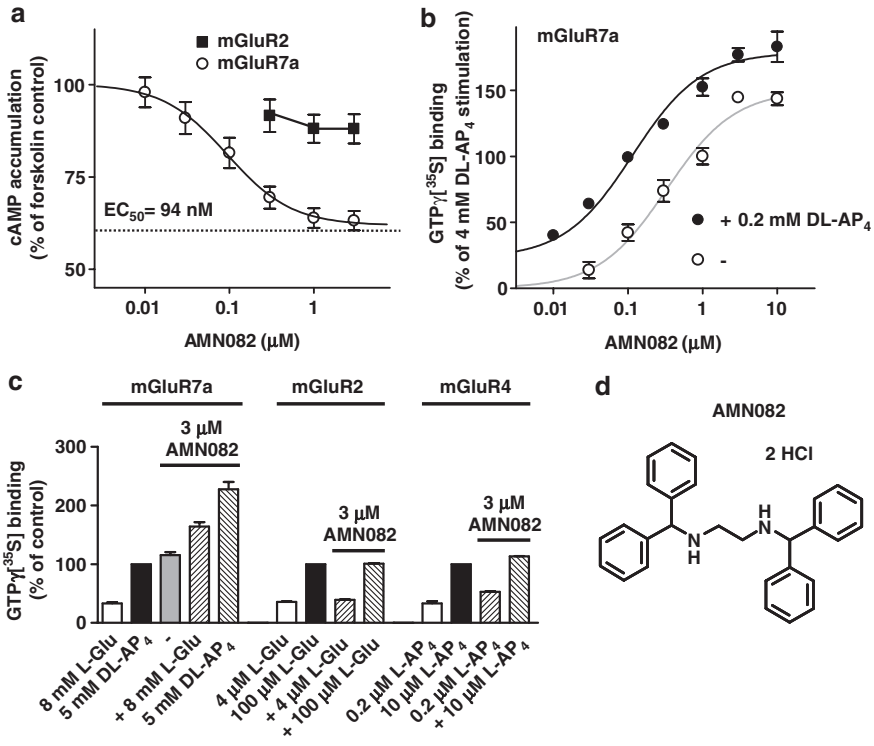

Figure 1 Potency, mGluR7 selectivity and efficacy of the novel agonist AMN082. (**a**) AMN082 (0.01–3 μM) inhibition of forskolin-stimulated cyclic AMP (cAMP) accumulation in CHO cells stably expressing rat mGluR7a was dose dependent and comparable in efficacy to DL-AP_4 (4 mM, dotted line). In contrast, AMN082 had no significant effect on cAMP in rat mGluR2 stably expressing cells. The results are expressed as % of control (30 μM forskolin-stimulated cAMP levels). (**b**) Concentration–response curves for AMN082 in the presence and the absence of a submaximal DL-AP_4 concentration using GTPγ[^{35}S] binding on rat mGluR7a expressing CHO cell membranes; the effector concentration for half-maximum response (EC_{50}) values for AMN082 were 115 and 330 nM, respectively. (**c**) GTPγ[^{35}S]-binding experiments using membranes from CHO cells stably expressing rat mGluR7a, rat mGluR2 and human mGluR4; the indicated concentrations of AMN082, DL-AP_4 and L-glutamate (L-Glu) were applied alone and in combination. Black bars indicate control stimulations by orthosteric agonists (set to 100%). Stimulating effects of AMN082 and the orthosteric ligands were additive on CHO-mGluR7a membranes; AMN082 was inactive at rat mGluR2 and human mGluR4. All data points shown (**a–c**) are from at least six measurements obtained on 2–4 independent experimental days, and expressed as means with s.e.m. (**d**) Chemical structure of AMN082.

(EC_{50}) = 94 nM), with full agonist efficacy comparable to DL-AP4, while showing no effect on rat mGluR2 (Figure 1a). In addition, GTPγ[^{35}S] binding was performed to determine concentration–response curves for AMN082 in the presence and the absence of a submaximal DL-AP_4 concentration on rat mGluR7a expressing CHO cell membranes; the EC_{50} values for AMN082 were 115 and 330 nM, respectively (Figure 1b), similar to the value found in the cAMP assay (above). Using this GTPγ[^{35}S] binding assay, we demonstrate again full agonist efficacy of AMN082 on rat mGluR7a, while showing no effect on rat mGluR2 or mGluR4 (see Figure 1c). Moreover, agonist effects at rat mGluR7 of AMN082 were additive to those of L-glutamate and DL-AP4 (Figures 1b and c) indicating that AMN082 acts on a different site on mGluR7 than the orthosteric ligands L-glutamate and DL-AP4. This was also previously described for human mGluR7, where AMN082 (Figure 1d) interacts with an allosteric site within the transmembrane or C-terminal domain of the receptor.[13]

mGluR7 activation retards acquisition of conditioned fear and amygdaloid LTP

The FPS paradigm in rats measures the fear that is induced by an exposure to an otherwise innocuous stimulus (light) following its association, through repeated pairings, with an innately aversive stimulus (foot shock). The degree of conditioned fear can be readily quantified through potentiation of the startle response that rats exhibit in response to a sudden and intense stimulus (loud acoustic pulse). Here, we demonstrate that pre-conditioning treatment with the mGluR7 allosteric agonist AMN082, similar to the benzodiazepine anxiolytic agent diazepam, prevents the acquisition of conditioned fear (AMN082: $F_{3,35} = 3.27$, $P = 0.03$; diazepam: $F_{1,18} = 4.87$, $P = 0.04$; Figure 2a). In the rat amygdala slice preparation, AMN082 had no effect on the EPSC amplitude evoked by thalamic or cortical fibre stimulation ($t < 0.82$, $P > 0.22$; Figure 2b), while inducing a marked attenuation of LTP in thalamo-amygdala synapses in the lateral amygdala (Figure 2c). Specifically, using a

pairing protocol in standard ACSF plus 100 μM picrotoxin, EPSPs evoked by thalamic fibre stimulation were significantly potentiated to 145% of initial amplitude. AMN082 added to the bath solution prevented induction of this type of LTP (interaction stimulation condition × treatment: $F_{1,67} = 30$, $P < 0.0001$).

mGluR7 activation facilitates extinction of conditioned fear

Using the FPS paradigm, here we demonstrate that treatment with AMN082 not only retards acquisition but, when given immediately before extinction training, also facilitates extinction of conditioned fear ($F_{3,61} = 3.26$, $P = 0.027$, Figure 3a). In contrast, diazepam has no effects on extinction ($F_{1,30} = 0.002$, $P = 0.97$). To control for the possibility that the more rapid reduction in startle is caused by effects of AMN082 on forgetting the tone–shock association, another group of rats received no conditioned stimuli in the extinction session, that is, no extinction training. As shown in Figure 3b, these animals showed no reduction in conditioned fear (factor test day: $F_{1,16} = 0.06$, $P = 0.81$; interaction day × treatment $F_{1,16} = 0.38$, $P = 0.55$) showing that AMN082 does not induce direct conditioned stimulus-unconditioned stimulus (CS–US) dissociation.

In mice, when AMN082 is given just once prior to CTA training (pairing of LiCl-induced malaise and saccharin flavour), it does not affect acquisition of CTA ($P > 0.1$, Figure 4a), but does have a marked facilitatory effect on CTA extinction (interaction test day × treatment: $F_{16,269} = 2.97$, $P < 0.001$, Figure 4a). In fact, mice that received a single AMN082 (6 mg/kg) treatment demonstrated full extinction within 4 days compared to at least 8 days in animals treated with vehicle (cf. behaviour of unconditioned mice, Supplementary Figure 1a online).

mGluR7 downregulation blocks extinction of conditioned fear

To assess further the role of mGluR7 in CTA extinction, we used a recently validated technique for the delivery of siRNA to achieve widespread gene knockdown in the adult mouse brain.[15,16] We studied two different *in vitro* validated siRNAs, which target two different parts of the mGluR7 gene, or corresponding three-nucleotide (nt) mmRNA; infusion was over a period of 17 days into the ventricle. Starting day 12 of infusion, these animals were tested for acquisition and extinction of CTA. Infusion of either of the two different mGluR7-targeting siRNAs did not affect the acquisition of CTA (Figure 4b) but completely blocked extinction as compared to mice infused with

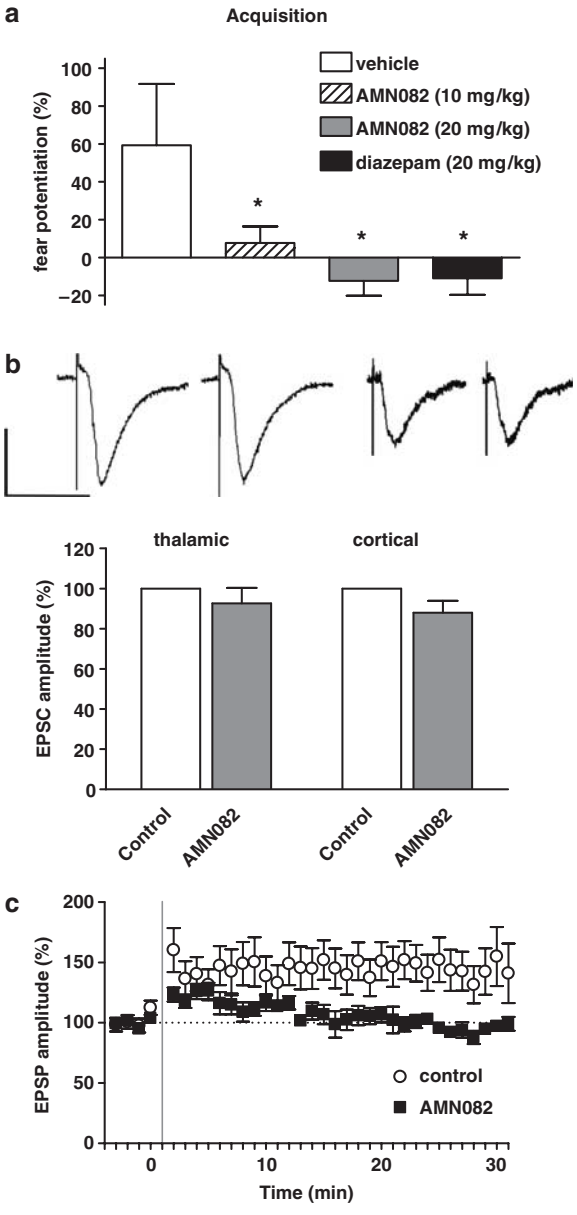

Figure 2 AMN082 blocked acquisition of fear-potentiated startle (FPS) and modifies amygdala synaptic function. (**a**) AMN082 and the anxiolytic diazepam were administered per os 30 min before fear conditioning (10 pairings of a 3.7 s light stimulus with a 0.8 mA foot shock, delivered in the last 0.5 s of the light). FPS was tested without treatment on the following day: both AMN082 and diazepam block the acquisition of FPS. Bars represent the percent fear potentiation by the conditioned stimulus light and are expressed as means with s.e.m. (*$P < 0.05$, *post hoc* Dunnett's *t*-test, comparison with the vehicle group) ($n = 9$–10 per group). (**b**) Patch-clamp recording in the whole-cell modus in amygdala brain slices: excitatory postsynaptic currents (EPSCs) in principal neurons of the lateral amygdala were evoked by presynaptic stimulation of thalamic and cortical afferent fibres in rat brain slices (*top*); scale bars indicate 25 ms (horizontal) and 50 pA (vertical). The application of AMN082 did not alter EPSC amplitudes. Bars represent % EPSC amplitude and are expressed as means with s.e.m. (**c**) AMN082 blocked associative long-term potentiation (LTP) of thalamically evoked EPSPs 20–30 min after application of the pairing protocol (10 pairings of EPSP with postsynaptic depolarizations at 33 Hz (15 × at 0.2 Hz)).

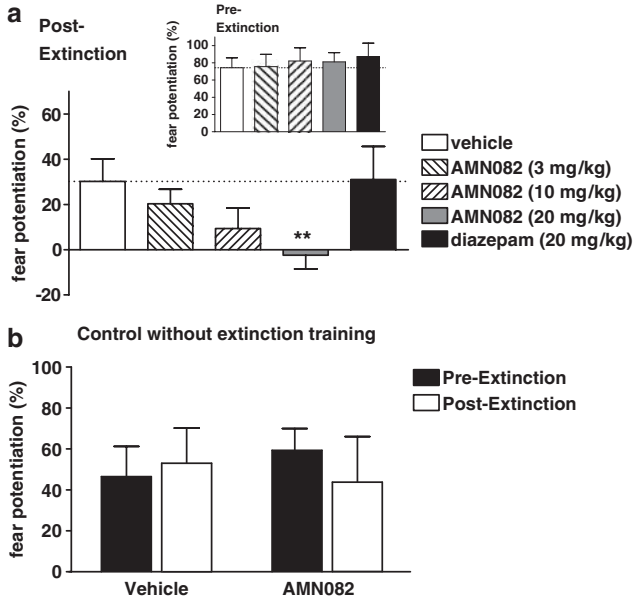

Figure 3 Effects of AMN082 on extinction of conditioned fear measured with the fear-potentiated startle (FPS) paradigm. (**a**) Animals were fear conditioned as described in the legend of Figure 2. The different groups of animals clearly did not differ in percent FPS in the pre-extinction test on the following day (insert). One day later, the animals were treated (p.o.) with AMNO82, and FPS and an extinction training were carried out (30 presentations of the light CS). In the post-extinction test (day 4), FPS was reduced after all treatments demonstrating successful extinction of conditioned fear. AMN082 dose dependently facilitates extinction of conditioned fear, whereas diazepam has no effects on extinction ($n = 16$–17 per group; $^{**}P < 0.01$, *post hoc* Dunnett's *t*-test, comparison with the vehicle group). (**b**) In the forgetting trial where no conditioned stimuli were presented during the extinction sessions, no extinction of conditioned fear was observed in these animals and there are no effects of AMN082 (20 mg/kg, p.o.) in this experiment ($n = 8$–10 per group).

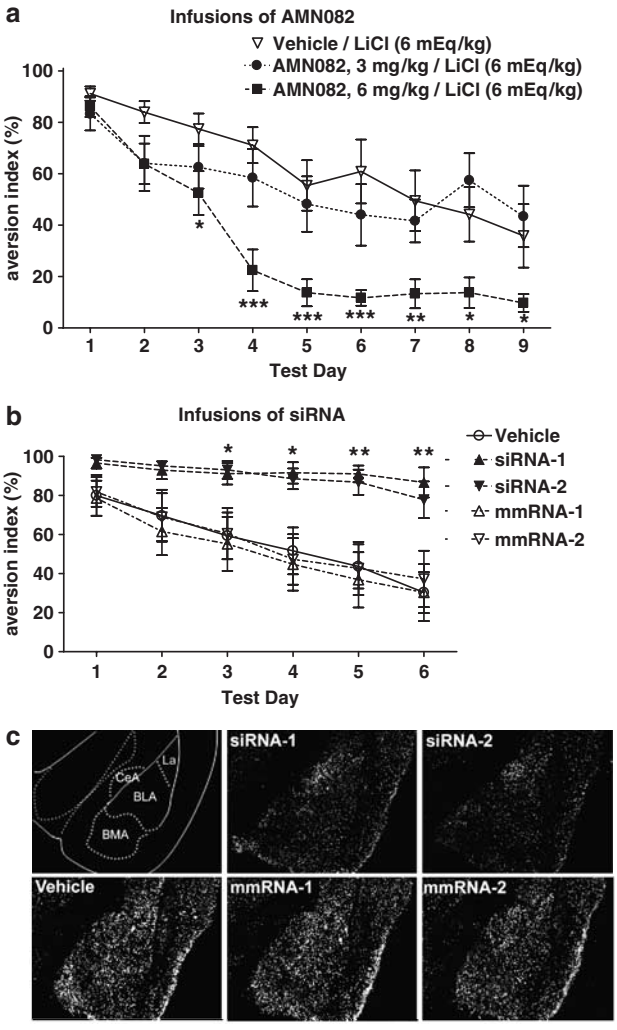

Figure 4 Effects of mGluR7 activation by AMN082 (p.o.) and siRNA-induced mGluR7 knockdown on extinction of conditioned taste aversion (CTA). (**a**) AMN082 did not influence acquisition of an aversion to a novel saccharin solution paired with a lithium chloride (LiCl)-induced malaise, but hastened the extinction of the aversion in conditioned mice ($n = 10$ per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Fishers LSD *post hoc* analysis). (**b**) Two distinct siRNAs specifically targeting mGluR7 mRNA (GenBank accession no. BC080315.1) at nt 1301–1321 (siRNA-1) or 2377–2397 (siRNA-2), corresponding three nucleotide-mismatch siRNAs (mmRNA-1 and -2) or vehicle were infused over 17 days via osmotic mini-pumps into the dorsal third ventricle. mGluR7-specific siRNAs had no effect on the acquisition of CTA on test day 1, but abolished extinction of the acquired aversion on subsequent test days ($n = 7$–8 per group $^{*}P < 0.05$, $^{**}P < 0.01$; both siRNA groups significantly differed from vehicle or mmRNAs on the same day, using two-way repeated measure analysis of variance followed by Fisher's *post hoc* test). (**c**) Representative low magnification, dark-field photomicrographs illustrating siRNA-mediated knockdown of mGluR7 mRNA in all amygdaloid nuclei (La, lateral; BLA, basolateral; BMA, basomedial) except the central amygdala (CeA).

vehicle or mmRNAs (interaction test day × treatment: $F_{20,233} = 1.75$, $P = 0.03$, Figure 4b; for the behaviour of unconditioned mice see Supplementary Figure 1b online). Interestingly, robust mGluR7 knockdown (ca. 15–20%) was not widespread but significantly observed in certain brain regions, including the basal and lateral (but not central) parts of the amygdala and the hippocampus (Figure 4c, Supplementary Figure 2 online) known to be involved in fear and anxiety. Other brain regions affected include the cerebral cortex, thalamus and regions of the hypothalamus; mGluR7 receptors located, and/or synthesized, in any of these brain regions could, therefore, play a key role in the CTA extinction process.

Discussion

Understanding the molecular and cellular basis of conditioned fear may open up novel avenues for the development of therapeutics for anxiety disorders.

Here, we demonstrate that mGluR7 activation reduced the acquisition of conditioned fear and enhances its extinction, whereas mGluR7 downregulation blocked the extinction of conditioned fear. The FPS paradigm in rats measures the fear that is induced by an exposure to an otherwise innocuous stimulus (light) following its association, through repeated pairings, with an innately aversive stimulus (foot shock). The degree of conditioned fear can be readily quantified through potentiation of the startle response that rats exhibit in response to a sudden and intense stimulus (loud acoustic pulse). Evolution has favoured this form of stimulus–stimulus learning as a means of rapidly ascertaining which environmental stimuli signal danger, and it is an essential component of mammalian defensive behaviour systems.[43,44] One of the most widely used cellular correlates of conditioned fear learning in rodents is LTP in thalamo-amygdala synapses.[25,26] Pharmacological and genetic perturbations that specifically block this form of amygdala LTP generally also interfere with fear learning and expression. Here, we demonstrate that pre-conditioning treatment with the mGluR7 allosteric agonist AMN082, similarly to the benzodiazepine anxiolytic agent diazepam, prevents the acquisition of conditioned fear. Furthermore, AMN082 induce a marked attenuation of LTP in thalamo-amygdala synapses in the lateral amygdala. Surprisingly, however, acquisition was not affected by mGluR7 activation in the CTA paradigm. This may reflect different neural structures governing these two conditioned learning responses, with FPS acquisition being mainly dependent upon amygdaloid plasticity,[44] whereas CTA acquisition not only involves amygdala regulation but also plasticity in the insular cortex.[45] Future studies are needed to clarify the electrophysiological correlates of CTA acquisition within the insula and amygdala and how mGluR7 receptors can modulate such responses.

Further, our CTA experiments suggest that memories formed and consolidated while mGluR7 is stimulated are easier to extinguish. The fact that CTA learning is very efficient, with all groups having an AI of more than 80% (Figure 4), gives rise to the possibility that our protocol may not be sensitive to any potential memory-enhancing effects of our manipulations. However, this is unlikely given that AMN082 actually impairs acquisition in the FPS paradigm, nonetheless we cannot rule out that there may be a ceiling effect in our CTA experiments, which may preclude us uncovering a pro-cognitive effect of AMN082 in this paradigm. Interestingly, AMN082 blocked the habituation of neophobia (Supplementary Figure 1) in unconditioned mice, which would also argue against it having memory-facilitating properties. In a similar vein, we cannot totally exclude that siRNA-induced knockdown of mGluR7 can give rise to a facilitation of CTA acquisition, as the protocol gives rise to near-maximal aversion.

As for the acquisition of conditioned fear, its extinction is also believed to be a learning process that results from the formation of new memories as opposed to simple forgetting.[46] The possibility of accelerating extinction learning offers the hope of facilitating extinction-based exposure therapy used to treat, for example, patients suffering from post-traumatic stress disorder.[5,47] In behavioural models, mGluR7 activation facilitated (both FPS and CTA tested), whereas mGluR7 downregulation retarded the extinction of conditioned fear (CTA). Of particular note is the fact that mice that received a single AMN082 (6 mg/kg) treatment demonstrated full extinction within 4 days compared to at least 8 days in animals treated with vehicle in the CTA paradigm. Such delayed effects on CTA extinction by a single administration of a drug have been previously reported for ligands with other mechanisms of action including $GABA_A$ receptor agonists.[48] Our findings of retarded extinction subsequent to siRNA-induced mGluR7 knockdown is in agreement with recent studies demonstrating that mice deficient in mGluR7 have deficits in the extinction of a conditioned emotional response.[11] Furthermore, our siRNA data also confirm that such deficits are unlikely to be due to developmental compensations resulting from lifelong absence of mGluR7.

It is generally accepted that the ventromedial prefrontal cortex plays an important role in the extinction of conditioned fear[49–51] and the abundance of mGluR7 within the prefrontal cortex is relatively high.[9] However surprisingly, mGluR7 activation attenuated fear acquisition whereas fear extinction was enhanced. The reason for this interesting difference is currently unknown, but is absolutely unique for a single pharmacological mode of action. A possible explanation might be that mGluR7 differently affects the functionality of the amygdala and the prefrontal cortex, the two brain sites mainly responsible for acquisition and extinction, respectively. Specifically, it is possible that there are differential effects of mGluR7 functional activation because of distribution pattern of mGluR7 on both excitatory and inhibitory neuron terminals in these regions. This could potentially result in the amygdala being more inhibited while the prefrontal cortex becomes more activated with AMN082. Future studies involving site-specific injections into the prefrontal cortex and amygdala will aid in the further elaboration of the question whether there is regional basis of the effects of AMN082 on acquisition and extinction.

Alternatively, net pharmacological actions of AMN082 may be responsible for possible different actions of AMN082 within the amygdala as compared to the prefrontal cortex. For example, it has been recently shown that AMN082 and other mGluR-selective agonists facilitate mGluR7 receptor internalization[52,53] in addition to stimulating the receptor. This could induce a functional blockade of mGluR7 subsequent to its activation. Further studies are needed to discern if the magnitude and/or time course of such processes differ among various brain

regions. Moreover, it is currently unclear whether any of the physiological effects induced by AMN082 that were observed in the present study are influenced by such a mechanism.

Although we do not fully understand, yet, the intriguing finding that AMN082 impairs acquisition but enhances extinction of conditioned fear, it has to be noted that this is a potentially important finding, distinguishing mGluR7 activation from other pro-extinction mechanisms such as D-cycloserine, CB1 agonists, L-type voltage-gated calcium channel agonists and D2 dopamine receptor antagonists.[47] Since current concepts of anxiety disorders are based not only on pathological fear acquisition but also on pathological fear extinction, this unique property of AMN082 could be of high relevance for clinical use. Further, our data clearly demonstrate that mGluR7 agonists affect conditioned fear responses in a manner qualitatively different than benzodiazepine anxiolytics do (see Figure 3), with the latter having no effect on extinction. Previous studies using contextual fear conditioning have even shown that diazepam and other benzodiazepines have a state-dependent impairing effect on extinction.[27] Taken together, these data give credence to the clinical findings that these drugs impair outcomes in behavioural therapy programmes.[54] The finding that only a single administration of AMN082 is sufficient to facilitate extinction is in agreement with other pharmacological studies[55] and highlights the malleability of aversive extinction processes and suggests the potential for the development of drug therapies suitable for intermittent dosing alone or in combination with behavioural therapy.[56]

Taken together, our data demonstrate that activation of mGluR7 using the novel agonist AMN082 has specific effects on amygdala physiology and *in vivo* function including blockade of the acquisition of FPS and a facilitation of the extinction of FPS memory. In the CTA paradigm, mGluR7 activation accelerated, whereas siRNA-mediated downregulation of mGluR7 blocked CTA extinction. These findings clearly indicate that mGluR7 is a very promising target to develop treatments for anxiety disorders such as those with deficits in the ability to extinguish fear memories including post-traumatic stress disorders and specific phobias.

Acknowledgments

We acknowledge Professor P Herrling and Dr G Bilbe for giving continuous support and for critical, very helpful discussions. We are deeply grateful to Professor S Nakanishi (Kyoto University, Japan) for providing CHO cell lines stably expressing rat mGluR2 and rat mGluR7a. We thank Drs HR Olpe, CR Pryce and A Lüthi for critically reading the manuscript. Also, we thank H Bürki, S Leonhard, D Fehlmann, E Müller and S Lukic for their expert technical help.

References

1 Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov* 2005; **4**: 775–790.
2 Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat Rev Drug Discov* 2005; **4**: 131–144.
3 Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav* 2002; **71**: 379–392.
4 Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* 1997; **37**: 205–237.
5 Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E *et al.* Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry* 2004; **61**: 1136–1144.
6 Hofmann SG, Meuret AE, Smits JA, Simon NM, Pollack MH, Eisenmenger K *et al.* Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Arch Gen Psychiatry* 2006; **63**: 298–304.
7 Marks IM, Swinson RP, Basoglu M, Kuch K, Noshirvani H, O'Sullivan G *et al.* Alprazolam and exposure alone and combined in panic disorder with agoraphobia. A controlled study in London and Toronto. *Br J Psychiatry* 1993; **162**: 776–787.
8 Flor PJ, van der Putten H, Ruegg D, Lukic S, Leonhardt T, Bence M *et al.* A novel splice variant of a metabotropic glutamate receptor, human mGluR7b. *Neuropharmacology* 1997; **36**: 153–159.
9 Kinoshita A, Shigemoto R, Ohishi H, van der Putten H, Mizuno N. Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study. *J Comp Neurol* 1998; **393**: 332–352.
10 Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ, van der Putten H. Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. *Eur J Neurosci* 2003; **17**: 2409–2417.
11 Callaerts-Vegh Z, Beckers T, Ball SM, Baeyens F, Callaerts PF, Cryan JF *et al.* Concomitant deficits in working memory and fear extinction are functionally dissociated from reduced anxiety in metabotropic glutamate receptor 7-deficient mice. *J Neurosci* 2006; **26**: 6573–6582.
12 Masugi M, Yokoi M, Shigemoto R, Muguruma K, Watanabe Y, Sansig G *et al.* Metabotropic glutamate receptor subtype 7 ablation causes deficit in fear response and conditioned taste aversion. *J Neurosci* 1999; **19**: 955–963.
13 Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S *et al.* A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters *in vivo*. *Proc Natl Acad Sci USA* 2005; **102**: 18712–18717.
14 Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 2005; **48**: 175–187.
15 Thakker DR, Natt F, Husken D, van der Putten H, Maier R, Hoyer D *et al.* siRNA-mediated knockdown of the serotonin transporter in the adult mouse brain. *Mol Psychiatry* 2005; **10**: 782–789.
16 Thakker DR, Natt F, Husken D, Maier R, Muller M, van der Putten H *et al.* Neurochemical and behavioral consequences of widespread gene knockdown in the adult mouse brain by using nonviral RNA interference. *Proc Natl Acad Sci USA* 2004; **101**: 17270–17275.
17 Tanabe Y, Masu M, Ishii T, Shigemoto R, Nakanishi S. A family of metabotropic glutamate receptors. *Neuron* 1992; **8**: 169–179.
18 Okamoto N, Hori S, Akazawa C, Hayashi Y, Shigemoto R, Mizuno N *et al.* Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *J Biol Chem* 1994; **269**: 1231–1236.
19 Maj M, Bruno V, Dragic Z, Yamamoto R, Battaglia G, Inderbitzin W *et al.* PHCCC, a positive allosteric modulator of mGluR4: characterization, mechanism of action, and neuroprotection. *Neuropharmacology* 2003; **45**: 895–906.

20 Flor PJ, Lukic S, Ruegg D, Leonhardt T, Knopfel T, Kuhn R. Molecular cloning, functional expression and pharmacological characterization of the human metabotropic glutamate receptor type 4. *Neuropharmacology* 1995; **34**: 149–155.
21 Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends Pharmacol Sci* 1992; **13**: 35–41.
22 Fendt M, Schmid S. Metabotropic glutamate receptors are involved in amygdaloid plasticity. *Eur J Neurosci* 2002; **15**: 1535–1541.
23 Schmid S, Fendt M. Effects of the mGluR8 agonist (S)-3,4-DCPG in the lateral amygdala on acquisition/expression of fear-potentiated startle, synaptic transmission, and plasticity. *Neuropharmacology* 2006; **50**: 154–164.
24 Schulz B, Fendt M, Gasparini F, Lingenhohl K, Kuhn R, Koch M. The metabotropic glutamate receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) blocks fear conditioning in rats. *Neuropharmacology* 2001; **41**: 1–7.
25 Rogan MT, Staubli UV, LeDoux JE. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 1997; **390**: 604–607.
26 Maren S. Synaptic mechanisms of associative memory in the amygdala. *Neuron* 2005; **47**: 783–786.
27 Bouton ME, Kenney FA, Rosengard C. State-dependent fear extinction with two benzodiazepine tranquilizers. *Behav Neurosci* 1990; **104**: 44–55.
28 Kikusui T, Takeuchi Y, Mori Y. Pharmacological manipulations of the extinction process of fear-induced ultrasonic vocalization in rats. *J Vet Med Sci* 2001; **63**: 591–595.
29 Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci* 2002; **22**: 2343–2351.
30 Bahar A, Samuel A, Hazvi S, Dudai Y. The amygdalar circuit that acquires taste aversion memory differs from the circuit that extinguishes it. *Eur J Neurosci* 2003; **17**: 1527–1530.
31 Welzl H, D'Adamo P, Lipp HP. Conditioned taste aversion as a learning and memory paradigm. *Behav Brain Res* 2001; **125**: 205–213.
32 Cubero I, Thiele TE, Bernstein IL. Insular cortex lesions and taste aversion learning: effects of conditioning method and timing of lesion. *Brain Res* 1999; **839**: 323–330.
33 Paulus MP, Stein MB. An insular view of anxiety. *Biol Psychiatry* 2006; **60**: 383–387.
34 Guitton MJ, Dudai Y. Anxiety-like state associates with taste to produce conditioned taste aversion. *Biol Psychiatry* 2004; **56**: 901–904.
35 Bermudez-Rattoni F, Ramirez-Lugo L, Gutierrez R, Miranda MI. Molecular signals into the insular cortex and amygdala during aversive gustatory memory formation. *Cell Mol Neurobiol* 2004; **24**: 25–36.
36 Jacobson LH, Kelly PH, Bettler B, Kaupmann K, Cryan JF. GABA(B(1)) receptor isoforms differentially mediate the acquisition and extinction of aversive taste memories. *J Neurosci* 2006; **26**: 8800–8803.
37 Thakker DR, Hoyer D, Cryan JF. Interfering with the brain: use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. *Pharmacol Ther* 2006; **109**: 413–438.
38 Sansig G, Bushell TJ, Clarke VR, Rozov A, Burnashev N, Portet C *et al*. Increased seizure susceptibility in mice lacking metabotropic glutamate receptor 7. *J Neurosci* 2001; **21**: 8734–8745.
39 Mitsukawa K, Mombereau C, Lotscher E, Uzunov DP, van der Putten H, Flor PJ *et al*. Metabotropic glutamate receptor subtype 7 ablation causes dysregulation of the HPA axis and increases hippocampal BDNF protein levels: implications for stress-related psychiatric disorders. *Neuropsychopharmacology* 2006; **31**: 1112–1122.
40 Huesken D, Lange J, Mickanin C, Weiler J, Asselbergs F, Warner J *et al*. Design of a genome-wide siRNA library using an artificial neural network. *Nat Biotechnol* 2005; **23**: 995–1001.
41 Paxinos G, Franklin KBJ. *The Mouse Brain in Stereotaxic Coordinates*. Academic Press: London, 2001.
42 Bischoff S, Barhanin J, Bettler B, Mulle C, Heinemann S. Spatial distribution of kainate receptor subunit mRNA in the mouse basal ganglia and ventral mesencephalon. *J Comp Neurol* 1997; **379**: 541–562.
43 Davis M. The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. *Trends Pharmacol Sci* 1992; **13**: 35–41.
44 Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev* 1999; **23**: 743–760.
45 Escobar ML, Alcocer I, Chao V. The NMDA receptor antagonist CPP impairs conditioned taste aversion and insular cortex long-term potentiation *in vivo*. *Brain Res* 1998; **812**: 246–251.
46 Myers KM, Davis M. Behavioral and neural analysis of extinction. *Neuron* 2002; **36**: 567–584.
47 Barad M. Fear extinction in rodents: basic insight to clinical promise. *Curr Opin Neurobiol* 2005; **15**: 710–715.
48 Akirav I. NMDA partial agonist reverses blocking of extinction of aversive memory by GABA(A) agonist in the amygdala. *Neuropsychopharmacology* 2007; **32**: 542–550.
49 Mickley GA, Kenmuir CL, Yocom AM, Wellman JA, Biada JM. A role for prefrontal cortex in the extinction of a conditioned taste aversion. *Brain Res* 2005; **1051**: 176–182.
50 Quirk GJ, Garcia R, Gonzalez-Lima F. Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry* 2006; **60**: 337–343.
51 Sotres-Bayon F, Cain CK, LeDoux JE. Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex. *Biol Psychiatry* 2006; **60**: 329–336.
52 Pelkey KA, Lavezzari G, Racca C, Roche KW, McBain CJ. mGluR7 is a metaplastic switch controlling bidirectional plasticity of feedforward inhibition. *Neuron* 2005; **46**: 89–102.
53 Pelkey KA, Yuan X, Lavezzari G, Roche KW, McBain CJ. mGluR7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. *Neuropharmacology* **52**: 108–117.
54 Makoff A, Pilling C, Harrington K, Emson P. Human metabotropic glutamate receptor type 7: molecular cloning and mRNA distribution in the CNS. *Brain Res Mol Brain Res* 1996; **40**: 165–170.
55 Cammarota M, Bevilaqua LRM, Rossato JI, Ramirez M, Medina JH, Izquierdo I *et al*. Relationship between short- and long-term memory and short- and long-term extinction. *Neurobiol Learn Mem* 2005; **84**: 25–32.
56 Myers KM, Davis M. Mechanisms of fear extinction. *Mol Psychiatry* 2007; **12**: 120–150.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)