Antisense ablation of type I metabotropic glutamate receptor mGluR1 inhibits spinal nociceptive transmission

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Electrophysiological and behavioral studies point to a role of group I metabotropic glutamate receptors (mGluR\(_1\) and mGluR\(_5\)) in mediating spinal nociceptive responses in rats. However, antagonists with a high degree of specificity for each of these sites are not yet available. We, therefore, examined the effects of antisense deletion of spinal mGluR\(_1\) expression in assays of behavioral analgesia and of electrophysiological responses of dorsal horn neurons. Rats treated with an mGluR\(_1\) antisense oligonucleotide reagent, delivered continuously to the intrathecal space of the lumbar spinal cord, developed marked analgesia as measured by an increase in the latency to tail-flick (55°C) over a period of 4–7 d. This correlated with a selective reduction in mGluR\(_1\), but not mGluR\(_5\), immunoreactivity in the superficial dorsal horn compared with untreated control rats, in parallel with a significant reduction in the proportion of neurons activated by the mGluR group I agonist 3,5-dihydroxyphenylglycine (DHPG), whereas the proportion of cells excited by the mGluR\(_5\) agonist, trans-azetidine-2,4-dicarboxylic acid (t-ADA) remained unaffected. In contrast, rats treated with mGluR\(_1\) sense or mismatch probes showed none of these changes compared with untreated, control rats. Furthermore, multireceptive dorsal horn neurons in mGluR\(_1\) antisense-treated rats were strongly excited by innocuous stimuli to their peripheral receptive fields, but showed severe reductions in their sustained excitatory responses to the selective C-fiber activator mustard oil and in responses to DHPG.

**Key words:** metabotropic glutamate receptors; mGluR\(_1\); mGluR\(_5\); dorsal horn; nociception; antisense oligodeoxynucleotide probe
mGluR expression to gain an unequivocal assessment of its role in nociceptive processing.

MATERIALS AND METHODS

Animals and evaluation of analgesia. Adult male Wistar rats (weight 280–450 gm, Charles River, Kent, UK) were used. Measurements of tail-flick latency (using a modified Ugo Basile tail-flick unit made in-house) of each rat (35–55°C to the base of the tail) were made for 3 d before, and recommencing 4 d after implantation of an indwelling intrathecal silicone cannula connected to an osmotic minipump for the continuous, quantitative administration of sense, missense, and antisense mGluR1 oligonucleotides (see below).

Oligonucleotide probes and implantation of osmotic minipump. The 21-base antisense oligodeoxynucleotides endcapped with phosphorothioate linkages (at the positions marked by *) were designed according to the primary sequence of the rat mGluR1 cDNA (Houamed et al., 1991; Masu et al., 1991). The sequence of the mGluR1 antisense probe was: 5'-GCCCAGCTGGCAGAG*T*-3', targeted against the intron translation initiation site (nucleotides 11 to +10) and will clearly not differentiate between splice variants in the carboxyl tail region. The complementary mGluR1 sense probe used: 5'-T*C*TCGCCACAATGGTCCGG*C*-3', corresponds to the reverse order of nucleotides of the above. The mismatch probe was: 5'-T*C*TCGCCACAAATGGTCCGG*C*-3'. None of the oligonucleotide probes shows internal complementarity with any other known sequences according to the GenBank Database. Custom synthesis, HPLC-purification, and gel filtration were performed by Osewl DNA Service (Southampton, UK), and probes were dissolved and aliquoted in sterile 0.9% saline, pH 7.4, to give 0.25 µg/µl final concentration for infusion, before being stored at -20°C until use.

For continuous infusion into the intrathecal lumbar spinal cord region, each rat was implanted with a minipump with cannula attachment, which was assembled the day before surgery. It consisted of an osmotic minipump [Alza Minipump, model 2001 (Palo Alto, CA); nominal infusion rate, 1 µl/hr] attached to two lengths of sterile cannulae: first, to the pump, a length of -1 cm of vinyl cannula (internal diameter 0.76 mm, outer diameter 0.99 mm) and to this was fitted second, a length of -6 cm of silicone cannula (internal diameter 0.64 mm, external diameter 1.20 mm) (Degania Silicone). The pump and cannulae were filled with one of the above solutions (or saline control) under sterile conditions, and then the cannulae were joined to the pump, avoiding air bubble formation, before being placed in sterile saline at 37°C overnight.

Surgery was performed under sterile conditions, with Sagatal (Rhone Merieux, Harlow, Essex, UK: 0.06 ml/100 gm, i.p.) anesthesia, followed by a maintenance level of halothane (Zenech, UK) under the most rostral vertebra and for current controls. The resistance of all side barrels was 20 –30 MΩ.

To directly monitor the loss of receptor protein expression after 5 d of intrathecal treatment with the mGluR1 antisense oligonucleotide probe, immunohistochemical analysis of the lumbar spinal cord was performed. Animals taken for immunohistochemistry were deeply anesthetized with Sagatal (0.12 ml/100 gm, i.p.) and perfused transcardially with 0.1 M PBS (containing 3 mM sodium nitrite and 1000 U heparin, pH 7.4) before being perfused with 4% paraformaldehyde/0.1 M PBS. A laminectomy was then performed, and the spinal cord was removed. The position of the cannula with respect to the level of spinal segment was ascertained at the end of each experiment, and only data from animals with correct cannula placement (L3–L6) were used in analysis. No animal showing abnormal gait or paralysis during the 7 d period was included in the study.

Electrophysiological studies. To assess the physiological effects of the loss of receptor expression after mGluR1 antisense treatment, neuronal responsiveness to ionophoretically applied mGluR agonists was investigated. Experiments were performed on 60 rats. Under initial halothane anesthesia, the jugular vein and trachea were cannulated. Intravenous a-chloralose (60 mg/kg) and urethane (1.2 mg/kg) with supplementary doses of a-chloralose (10 mg/ml) were given throughout the experiment as required. Core body temperature was maintained at 37–38°C by means of a thermostatically controlled foil heater. Animals inspired oxygen-enriched air. The animal was placed in a stereotaxic frame, and the thoracolumbar spinal column was supported using three pairs of swan-necked clamps. A laminctomy was performed at L2–L5, and agar (2% in saline at 37°C) was injected under the most rostral vertebra and over the exposed cord to increase mechanical stability. Above the recording region, a section of the now solidified agar was removed, the dura was removed, and liquid paraffin (37°C) was poured over the exposed cord. Extracellular recordings were made from single neurons in laminae III–V through the center barrel of a seven-barrelled glass microelectrode filled with 4 M NaCl (pH 4.0–4.5, tip diameter 4–5 µm, DC resistance 5–8 MΩ). The bandwidth of the recording amplifier was 1 Hz to 7 kHz. The following drugs were ionophoresed from the side barrels of the electrode: group I mGluR agonist: 3,5-dihydroxyphenylglycine (DHPG), 10 mM aqueous, pH 4.5; mGluR1, agonist: trans-azetidine-2,4-dicarboxylic acid (t-ADA), 10 mM aqueous, pH 8.0–8.5; and the AMPA receptor agonist: AMPA, 10 mM aqueous, pH 8.0–8.5. All compounds were obtained from Tocris Cookson, Bristol, UK. Retention currents of 10 nA were used to minimize drug leakage between tests. A remaining barrel contained 1 M NaCl, pH 4.0–4.5, for automatic current balancing, using a Neurophore BH2 ionophoresis system (Medical Systems, Great Neck, NY) and for current controls. The resistance of all side barrels was 20–30 MΩ.

Recordings were made from any multisegmental neuron encountered at a depth from the dorsal surface corresponding to laminae I–IV, as shown in previous studies using electrophoretic deposition of dye (Fleetwood-Walker et al., 1988, 1995). The cutaneous receptive fields of neurons were delimited by innocuous brushing with a soft brush, and the distal hindlimb. The use of a mechanized rotating fine brush to stimulate hair follicles (AB) afferents has been described previously (Fleetwood-Walker et al., 1985) and was qualitatively insensitive to human skin. Further characterization was performed using noxious radiant heat (30–48°C, rise time 5 sec, plate temperature for 10 sec) and noxious pinch. Approximately 90% of all the cells tested in normal animals also showed sustained responses to mechanical stimulation. The responses to ionophoresed agonists were then explored with drug ejection currents being increased in a stepwise manner, every minute in steps of 10 nA from 5 to 45 nA. The response of neurons to the chemical allogen mustard oil (allyl isothiocyanate; Sigma, Poole, UK; 7.5% in paraffin oil) was observed after being repeatedly applied to the receptive field area (normal) every 5 sec, until sustained activation was achieved.

Statistical analysis of the proportion of cells activated by agonists in the different groups of rats (normal, antisense reagent-treated, or sense reagent-treated rats) was performed by Mann–Whitney U test.

Immunohistochemistry. To directly monitor the loss of receptor protein expression after 5 d of intrathecal treatment with the mGluR1 antisense oligonucleotide probe, immunohistochemical analysis of the lumbar spinal cord was performed. Animals taken for immunohistochemistry were deeply anesthetized with Sagatal (0.12 ml/100 gm, i.p.) and perfused transcardially with 0.1 M PBS (containing 3 mM sodium nitrite and 1000 U heparin, pH 7.4) before being perfused with 4% paraformaldehyde/0.1 M PBS. A laminectomy was then performed, and the spinal cord was removed, with the brain, which were then post-fixed in the same solution for a further 4–5 hr, before being incubated in 25% buffered sucrose overnight (4°C) and then stored in cryoprotective solution (30% ethylene glycol and 20% glycerol, in 0.05 M PBS, pH 5.5) at -70°C. Transverse microtome sections (50 µm) were then cut from the frozen tissue, through lumbar segments L3–L6, and suitable sections of brain tissue were used as positive controls for the antibodies used. Tissue sections were stored in cryoprotectant at -20°C, until use. Sections were removed from the cryoprotectant as required, for processing for either mGluR1 or mGluR5 immunoreactivity. Unless otherwise stated, all solutions were made up in 0.1 M PBS, and all incubations were performed at room temperature with gentle agitation. In all steps involving antibodies, the tissue sections were washed twice, for 10 min each, with PBS between succeeding steps. Sections were incubated in 1% hydrogen peroxide (30 min; Sigma) to remove any endogenous peroxidase activity, followed by incubation in normal goat serum (1 hr) to block nonspecific binding. They were then incubated with polyclonal anti-antipetide antibodies raised to rat mGluR1 (1180-1199) (0.25 µg/ml, 48 hr, 4°C; Chemicon, Temecula, CA) or rat mGluR5 (1130-1139) (1 µg/ml, 48 hr, 4°C; Upstate Biotechnology, Lake Placid, NY) followed by biotinylated goat anti-rabbit IgG antibody (1: 200 in PBS, 1 hr; Vector Laboratories, Peterborough, UK). Sections were then incubated for 90 min with an avidin–biotin complex (Vectorstain Elite ABC kit, Vector Laboratories). A further wash in PBS was performed, followed by 1% hydrogen peroxide (30 min) and the addition of 1% diaminobenzidine tetrachloride (DAB; 0.2 mg/ml; Sigma) in the presence of 3% hydrogen peroxide (1 µg/ml) to enable visualization of the receptor protein precipitate. After a final wash in PBS, the sections were mounted onto poly-l-lysine-coated microscope slides, allowed to air dry
before dehydration through ascending concentrations of alcohol, and then cleared in xylene (Sigma) and mounted in DePex mountant (BDH). Further immunohistochemical controls consisted of replacing the primary antibodies with nonimmune goat serum, or for preabsorption controls, see below. To overcome any potential problems caused by variable development of the DAB reaction, batches of control, sense, mismatch, and antisense-treated spinal sections were processed simultaneously, meaning that direct comparisons between them could be confidently made.

As a preabsorption control for specificity of the mGluR1 antibody, aliquots were incubated with membranes from COS 7 cells overexpressing the rat mGluR1α receptor from a construct in pcDNA 1 (a gift from S. Nakaniishi, Kyoto University, Japan) or with membranes from control COS 7 cells. Transfections were performed using DEAE dextran, and cells were harvested 72 hr later (Lutz et al., 1993). Cells were homogenized, and the crude particulate fraction was washed twice in ice-cold 20 mM HEPES-NaOH, pH 7.2, with phosphatase and peptidase inhibitors. Antibody aliquots were incubated with membranes (16 hr rolling at 4°C) before use (at twice the usual concentration). Preincubation with normal COS 7 cell membranes had no detectable effect, showing staining in normal dorsal horn apparently identical to that with untreated antibody, whereas the mGluR1-expressing membranes caused virtually complete loss of immunostaining (see Fig. 3E).

Relative quantification of immunoreactivity was achieved using an Improvision 1.49 image analysis package at 400×. An 80 × 30 μm region of interest (ROI) cursor was aligned consecutively, centered on laminae I, IIouter, IIinner, and III and, in ventral horn, 40 μm diameter ROIs were centered over individual motoneurons. Arbitrary gray scale units (throughout the range 1–200) were assigned to make optimal use of the range for the given sample set. Each section was corrected for the (low) nonspecific background levels recorded from white matter. Individual measurements were performed on 15 or 16 separate sections for each experimental condition.

**RESULTS**

The position of the implanted intrathecal cannula was verified 4–7 d after surgery to ensure that only those animals with correct L3–L6 placements were taken for subsequent electrophysiological recording experiments or immunohistochemistry. For the animals included in this study, cannulae were found to lie on the dorsal surface of the spinal cord.

**Measurement of behavioral nociceptive responses**

Tail-flick latency was measured for 2–3 d before, and also after, surgical implantation of an indwelling intrathecal cannula to the lumbar spinal cord, allowing an intermediate 3 d gap for recovery from surgery.

After continuous infusion of the mGluR1 antisense oligonucleotide probe, there was a marked increase in tail-flick latency reaching a peak at 4–7 d after surgery, which was statistically significant compared with presurgery levels (p < 0.05; Mann–Whitney U test, n = 9), saline-infused controls, mismatch-treated, or the sense-treated rats (Fig. 1). In the subsequent 7 d, the tail-flick latency recovered steadily to values approaching presurgery controls, with 36 ± 5%, 78 ± 12%, and 88 ± 4% recovery at days 10, 12, and 14 after surgery, respectively (n = 4). The mGluR1 antisense oligonucleotide-treated animals that were subsequently taken within 7 d after minipump and cannula implantation surgery, for either electrophysiological studies or immunohistochemical investigation, all displayed delayed behavioral nociceptive responses.

**Electrophysiological recording experiments**

**Responses to mGlu receptor agonists**

In all experimental rats that were taken for electrophysiological recording experiments, ionophoretic application of the mGluR agonists DHPG or t-ADA was performed at between 5 and 45 nA, at which every minute the ionophoretic current was increased by 10 nA increments until activation was observed, or if none was observed by 45 nA after 2 min, then the drug application was terminated. Activation of cells occurred within the 5 sec to 2 min after the drug had initially been applied. Most cells were activated within 50 sec of either of the drugs being increased to 25 nA.

In normal animals, the mGluR1 agonist DHPG, administered in this regimen, caused overt activation of a majority of multireceptive cells (56%), whereas the mGluR2 agonist t-ADA activated a significantly smaller proportion (23%).
Table 1. Effect of mGluR₁ antisense treatment on neuronal responses to DHPG and t-ADA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of cells excited by agonist</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DHPG</td>
</tr>
<tr>
<td>Control (n = 13)</td>
<td>56 (25/45)</td>
</tr>
<tr>
<td>Sense (n = 7)</td>
<td>53 (16/30)</td>
</tr>
<tr>
<td>Mismatch (n = 6)</td>
<td>55 (12/22)</td>
</tr>
<tr>
<td>Antisense (n = 7)</td>
<td>20 (13/64)**</td>
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Table 2. Effects of sense, mismatch, and antisense mGluR₁ oligonucleotide infusions on different sensory responses of dorsal horn neurons

<table>
<thead>
<tr>
<th>Stimulus-evoked response (Hz)</th>
<th>Normal reagent-treated</th>
<th>Mismatch reagent-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>Sense (n = 7)</td>
</tr>
<tr>
<td>Innocuous brush</td>
<td>17.2 ± 2.3</td>
<td>22.1 ± 5.7</td>
</tr>
<tr>
<td>Acute chemical nociception</td>
<td>30.7 ± 2.2</td>
<td>22.7 ± 7.7</td>
</tr>
<tr>
<td>Sustained activity caused by</td>
<td>10.1 ± 2.6</td>
<td>7.4 ± 1.8</td>
</tr>
<tr>
<td>repeated nociceptive</td>
<td>1.1 ± 0.5</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>stimuli</td>
<td>0.5 ± 1.8</td>
<td></td>
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</tbody>
</table>

The sensory responses monitored were the acute neuronal responses to innocuous brush (mean taken over 10 sec) and the chemical algogen mustard oil applied to the peripheral receptive field (mean taken over 10 sec, 20–40 sec after initial application), as well as the sustained sensitized activity caused by repeated application of mustard oil (mean taken over 10 sec, 15–18 min after the first three applications). Basal firing rates of neurons were all within the range 0–1.0 Hz and were no different between treatments. Values are means ± SEM with numbers of neurons in parentheses. *Indicates significantly different from normal, sense, or mismatch reagent controls; p < 0.05, Mann–Whitney U test.

Drugs were ionophoresed at 5–45 nA for 5 sec to 2 min. The numbers of cells responding, of those tested, are shown in parentheses, and the numbers of animals in each case are shown after the respective treatments. (*p < 0.05; **p < 0.01 compared to control response to DHPG in control, sense, and mismatch; χ² test).

In normal, untreated animals (n = 8), eight of nine cells showed a sustained and incremental excitatory response to repeated, topical application of mustard oil to their peripheral receptive field (up to three applications, every 5 min). The average increase in ongoing activity of these neurons after topical mustard oil application was from 0.3 ± 0.1 Hz before mustard oil to 10.1 ± 2.6 Hz of sustained activity, measured as the mean over 10 sec taken 15–18 min after initial application (Fig. 2A). Similarly, in the mGluR₁ sense oligonucleotide-treated control animals (n = 5), seven of eight multireceptive cells showed a sustained excitatory response, after three topical mustard oil applications [from 0.2 ± 1.0 Hz before mustard oil application to 7.4 ± 1.8 Hz sustained activity measured 15–18 min after initial application (Fig. 2B)]. Six cells recorded from mismatch-treated animals (n = 6) (Fig. 2C) also showed marked ongoing activation as in normals (0.1 ± 0.8 Hz before repeated mustard oil application and 7.3 ± 1.6 Hz afterward). In contrast, in rats treated with the mGluR₁ antisense oligonucleotide, which displayed greatly attenuated behavioral nociceptive responses (n = 6), six of six dorsal horn multireceptive cells showed no significant sustained response to repeated topical application of mustard oil to their peripheral receptive field (even with up to five applications); the average increase in ongoing activity being from only 0.1 ± 0.1 Hz before mustard oil, to only 0.5 ± 1.8 Hz (Fig. 2D). The sustained mustard oil-induced firing in antisense oligonucleotide-treated animals was significantly less than that in control or sense-treated animals (p < 0.05 by Mann–Whitney U test). All of the cells from which we recorded full sensory responses in normals, sense, and mismatch animals also showed clear responses to DHPG. In the six cells from antisense animals, from which we were able to gain adequate records of both brush and some residual mustard oil response, we found none that responded to DHPG.

Immunohistochemistry and immunoblotting for mGluR₁ and mGluR₅

The specificity of antibody labeling was verified by analyzing the distribution of mGluR₁ and mGluR₅ immunoreactivity in the CNS. In the cerebellar cortex, the molecular and Purkinje layers were strongly immunoreactive for mGluR₅, whereas the granular layer was less intensely labeled. The mGluR₁ immunoreactivity in the hippocampus was highest in the molecular layer, whereas there were many unlabeled cell bodies in the CA1 and CA3 fields. These observations are entirely consistent with previously published observations (Shigemoto et al., 1992, 1993; Romano et al., 1995).

In lumbar spinal cord of control untreated animals, neural elements strongly labeled for mGluR₁ were distributed in laminae I and II of the dorsal horn, to a lesser extent in deeper dorsal horn, and also in the ventral horn around motoneurons (Fig. 3A,F). This pattern of immunoreactivity was unchanged in animals treated with the mGluR₁ sense or mismatch oligonucleotide probe (Fig. 3B,C). In contrast, animals treated with the antisense response (calculated as the mean value over 10 sec, 20–40 sec after initial application) was not significantly altered in sense or mismatch oligonucleotide-treated animals compared with untreated controls, whereas responses from antisense oligonucleotide-treated animals were markedly reduced (p < 0.05; Mann–Whitney U test; Table 2). These observations concur with the changes in acute thermal nociceptive responses (tail-flick) seen in the behavioral experiments. Mechanical nociceptive responses were not investigated quantitatively in the present study.

Sustained activity caused by mismatch (n = 6)

Acute chemical nociception (n = 6)

Sense

Antisense (n = 7)

Drugs were ionophoresed at 5–45 nA for 5 sec to 2 min. The numbers of cells responding, of those tested, are shown in parentheses, and the numbers of animals in each case are shown after the respective treatments. (*p < 0.05; **p < 0.01 compared to control response to DHPG in control, sense, and mismatch; χ² test).
Figure 2. Ongoing neuronal activity records showing typical excitatory firing responses of dorsal horn neurons to innocuous brush stimuli (□), to ionophoresis of DHPG (■), and to noxious mustard oil stimulation (●). A shows untreated control, B shows mGluR1 sense oligonucleotide-treated, C shows mGluR1 mismatch-treated, and D shows mGluR1 antisense-treated animals. All animals were tested for tail-flick responses before electrophysiological recording and were found to conform to the pattern displayed in Figure 1.
Figure 3. Effects of mGluR₁ sense, mismatch, and antisense infusion on mGluR₁ and mGluR₅ immunoreactivity in lumbar spinal cord. A–D show typical representations of mGluR₁ immunoreactivity in dorsal horn in control (saline), sense, mismatch, and antisense reagent-treated animals. E shows the virtual lack of immunoreactivity in control dorsal horn when the mGluR₁ antibody was preabsorbed with membranes from mGluR₁-overexpressing cells. F and G show mGluR₁ immunoreactivity in ventral horn in control and mGluR₁ antisense-treated animals. These results are typical of at least five animals in each case. Scale bars, 1.0 mm. H shows immunoblots using mGluR₁ and mGluR₅ antibodies after gel electrophoresis of lysates from spinal cord segments L3–L6 of (1) control, (2) antisense, (3) sense, and (4) mismatch-treated animals. The running positions of the molecular weight markers are shown in kilodaltons. Results are typical of three separate experiments.
The statistically significant differences were determined by Mann–Whitney U test (n = 15–16). *p < 0.05, **p < 0.01 compared with control; ***p < 0.05 compared with sense; and ****p < 0.01 compared with mismatch.
prominent in motoneuron recordings (Cao et al., 1997). It is clear, therefore, that ventral horn effects of mGluR agents may potentially contribute to, or at least modify, the effects of mGluR manipulations in behavioral analgesia experiments. However, increased latencies or thresholds to nociceptive stimuli were measured in behavioral responses to intrathecally applied mGluR group I antagonists in the absence of any overt signs of motor insufficiency (Fisher and Codere, 1996a; Young et al., 1997). Similarly, although mGluR<sub>1</sub> knock-out mice display a disruption of complex coordination behaviors that may arise from cerebellar dysfunction, they possess well maintained muscle strength and can organize effective goal-oriented swimming behaviors as well as normal animals (Conquet et al., 1994). In the present study, there was no evidence for any deficit in motor coordination, gait, or locomotor activity in the mGluR<sub>1</sub> intrathecal antisense-treated animals, corresponding to the lack of change in ventral horn mGluR<sub>1</sub> immunoreactivity (Fig. 3) animals, corresponding to the lack of change in ventral horn mGluR<sub>1</sub> immunoreactivity (Fig. 3F,G) after dorsally directed infusion of oligonucleotide. So, although it is not possible to unequivocally exclude a contribution of ventral horn effects to the behavioral results (and reflex indices of function here not tested), it is clear that the effects of mGluR<sub>1</sub> ablation (focused in the dorsal horn; Fig. 3, Table 3), as defined in the electrophysiological experiments (Table 2, Fig. 2), would alone be quite sufficient to explain the behavioral changes that we observed (Fig. 1) and have similarly been described in mGluR<sub>1</sub> knock-out mice (Corsi et al., 1996).

In conclusion, the present results demonstrate that the localized antisense ablation of mGluR<sub>1</sub> in dorsal horn (without affecting the congener mGluR<sub>3</sub>) results in a selective abrogation of neuronal responses to noxious stimuli (and perhaps also sensitization) without equivalent changes in non-nociceptive responses. Correspondingly, reflex behavioral responses to noxious thermal stimuli are attenuated in rats with antisense deletion of mGluR<sub>1</sub> in lumbar dorsal horn, in the absence of any signs of generalized motor deficit.

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