RGS proteins are negative regulators of signaling through heterotrimeric G protein-coupled receptors and, as such, are in a position to regulate a plethora of biological phenomena. However, those have just begun to be explored in vivo. Here, we describe a mouse line deficient for Rgs4, a gene normally expressed early on in discrete populations of differentiating neurons and later on at multiple sites of the central nervous system, the cortex in particular, where it is one of the most highly transcribed Rgs genes. Rgs4neoZ/neoZ mice had normal neural development and were viable and fertile. Behavioral testing on mutant adults revealed subtle sensorimotor deficits but, so far, supported neither the proposed status of Rgs4 as a schizophrenia susceptibility gene (by showing intact prepulse inhibition in the mutants) nor (unlike another member of the Rgs family, Rgs9) a role of Rgs4 in the acute or chronic response to opioids.

**MATERIALS AND METHODS**

**Targeting strategy.** The targeting vector, derived from a 129/OLA cosmid encompassing the Rgs4 locus (RZPD Resource Center, Berlin, Germany), consisted of an 11-kb 5' homology arm and a 1.12-kb 3' homology arm separated by an Fpl recognition target-flanked neo cassette itself preceded by a loxp/police-acceptor site/promotorless lacZ cassette (Fig. 1A). The neo and lacZ cassettes replaced a 1.2-kb region containing low-complexity sequences with no similarity to the human locus, located 0.6 kb downstream of the Rgs4 poly(A) signal. Upstream (4.5 kb) of these two cassettes, a second loxp site and a third FRT site were introduced in the second intron of Rgs4, so that recombination by the Fpl or Cre recombinase would interrupt the Rgs4 coding sequences before the RGS domain (Fig. 1A).

**Production of mice and genotyping.** A homologous recombinant clone of E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown). Injection of the clone into E14-1 embryonic stem (ES) cells (129/OLA) was obtained by electroporation and verified for the correct recombination event by Southern blotting using both internal and external probes (Fig. 1C and not shown).
FIG. 1. Generation of Rgs4 deficient mice. (A) Targeting strategy. (Top) Wild-type Rgs4 allele with exons 1 to 5 (boxes, coding sequences in dark shade, non coding sequences in light shade). The RGS domain is encoded by exons 3, 4, and 5. Selected sites for EcoRI (E), BamHI (B), KpnI (K), and SwaI (S) are indicated. (Middle) Targeting vector. A loxP/BamHI/FRT cassette has been inserted in the SwaI site. A cassette (loxP site/splice acceptor [Sa]/promoterless lacZ open reading frame/FRT site/neO open reading frame under the control of the pGK promoter/FRT site/three out-of-phase stop codons) replaces the KpnI/BamHI fragment downstream of the fifth exon. A thymidine kinase cassette under the control of the hsv promoter was added at the 3′ end of the construct. (Bottom) Recombined locus. (B) PCR genotyping strategy of the wild-type, targeted, and Cre recombined loci. Primers are represented by arrowheads. (C) Southern blot of the DNA of a recombinant (IIID3) and a wild-type (IIIE4) ES clone, digested by EcoRI and probed with the external probe depicted in panel A. floxed, recombined allele. (D) PCR analysis of tail DNA of wt, Rgs4loxP/RSF/lacZ+ (wt/lox), and Rgs4loxP/RSF/lacZ/RSF/lacZ (loxZ/loxZ) pups with the primers depicted in panel B. (E) PCR analysis of tail DNA of wild-type, Rgs4loxP/RSF/lacZ (wt/loxZ), and Rgs4loxP/RSF/lacZ (loxZ/loxZ) pups with the primers depicted in panel B (see Materials and Methods for details). (F) Transverse sections through wild-type and homozygous mutant E11.75 embryos, showing that the expression of Rgs4 mRNA is lost in the mutants, even though the ganglionic cells are present, as assessed by β-tubulin expression. nt, neural tube.

Histological methods. Embryos (the day of the vaginal plug was considered as embryonic day 0.5 [E0.5]) were stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as described by Knittel et al. (25) and for in situ hybridization combined or not combined with immunohistochemistry as described by Tiveron et al. (44). Adult tissues were treated as described by Grillet et al. (17). Golgi stains were done with the rapid GolgiStain kit (FD Neurotechnologies) according to the manufacturer’s instructions.

In situ hybridization probes were pKS-mRgs4 (17), dopamine β-hydroxylase (Dbh) (44), class III β-tubulin (kind gift of C. W. Ragsdale), and lacZ, and antibodies were specific for Phox2a (44), Phox2b (36), or Islet1/2 (kind gift of T. Jessell).

General health assessment and gross neurological examination were performed, with slight modifications, as previously described (19, 20) on 11 wt and 12 Rgs4loxP/RSF/lacZ male littermates, 13 to 15 weeks old, born from matings of heterozygotes, themselves descendants of three backcrosses on C57BL/6 of the original (mixed 129/B6D2) Cre-deleted mouse. All experiments were carried out in accordance with the European Community Council Directive (24 November 1986) at the Institut Clinique de la Souris (Illkirch, France) except for the
morphine withdrawal test, which was carried out in the authors’ lab. A more comprehensive description of each protocol can be found at http://www-mci.u-strasbg.fr/service_4.html.

Behavioral tests. (i) Rotating rod test. After 60 s. habituation trials on a rotating rod (Bioseb, Chaville, France) (one trial at 0 rpm and 2 trials at 4 rpm), mice were subjected to three testing trials (separated by 5- to 10-min intervals) during which the rotation accelerated from 4 to 40 rpm in 5 min and the time from latency to falling off was measured.

(ii) String test. In three consecutive trials (separated by 10-min intervals), the animals were suspended by their forepaws on a horizontally stretched wire, and the time they took to catch the wire with their hind paws was recorded.

(iii) Grip test. In three consecutive trials, the animals were allowed to grasp a grid connected to an isometric dynamometer (Bioseb) and slowly moved backwards until they released it. The maximal strength developed was expressed in grams per gram.

(iv) Tail flick test. The tail of the animal was placed under a heat source (a shutter-controlled lamp (Bioseb)) and heated three times at different sites at intervals of 1 min. The time taken by the animal to flick its tail was recorded (cutoff point at 25 s).

(v) Hot-plate test. In two trials, the mice were placed on a hot plate adjusted to 52°C (Bioseb), and the latency to the first reaction (licking, moving the paws, or leaping) was recorded. The test was ended if the mouse did not react within 30 s. In morphine experiments, the second trial duration was increased to 180 s maximum.

(vi) Shock threshold test. Mice were allowed to habituate for 30 s to a fear conditioning chamber. Behavioral responses to a 1-s foot shock (beginning at 0.05 mA and increased in 0.05-mA steps at 30-s intervals) were recorded. When both flinch (any detectable response) and vocalization were induced, shocks were increased in 0.1-mA steps (cutoff point at 1 mA) until a jump (defined as the two hind paws leaving the ground) was induced.

(vii) Open field test. Mice were tested in an automated open arena (Panlab, Barcelona, Spain) divided into central and peripheral regions homogeneously illuminated at 150 lx. Mice placed at the periphery of the open field were allowed to freely explore the apparatus for 30 min. The distance traveled, the number of rearings, and the time spent in the central and peripheral regions were recorded.

(viii) Tail suspension test. Mice were fitted in a tail suspension device (MED Associates Inc., St. Albans, Vermont). The latency to the first immobilization was determined, and the immobilization time was monitored during a 6-min period in blocks of 2 min.

(ix) Auditory startle reflexivity and prepulse inhibition. Acoustic startle reactivity and prepulse inhibition of startle were assessed using a standard startle chamber (SR-Lab startle response system; San Diego Instruments) and 10 different trials: acoustic startle pulse alone (120 dB); prepulse trials with 70-, 80-, 85- or 90-dB stimuli presented either alone or preceding the pulse, and one trial in which only the background noise (65 dB) was presented to measure the habituation for 2 min, and presented with light/auditory cues for 2 min later. Alternations were defined as successive entries into each of the three arms. Performance was defined as the ratio of actual alternations to possible alternations. (x) Pavlovian fear conditioning. Pavlovian fear conditioning was assessed in 18.5- by 18.5- by 21.5-cm operant chambers (Coulbourn Instruments, Allentown, PA) with aluminum side walls and Plexiglas rear and front walls. A loudspeaker and a bright light, inserted on opposite walls, provided the cues during conditioning and cue testing. The activity of animals was monitored by infrared cells in the ceiling and recorded using the Graphic State software (Coulbourn). After a 4 min acclimatization, a light/tone (10 kHz) (conditional stimulus) was presented for 20 s and coterminated by a mild (1 s, 0.4 mA) foot shock (unconditional stimulus). Another conditional stimulus-unconditional stimulus pairing was presented 2 min later, and mice were returned to their home cages 2 min later. Twenty-four hours after the conditioning session, mice were tested for context by placing them back into the operant chamber and allowing them to explore it for 6 min. Testing for the cue was done 5 h after testing for context: mice were placed in a new chamber (with different wall color, odor, and floor texture), allowed to habituate for 2 min, and presented with light/auditory cues for 2 min. This sequence was repeated once.

(xii) Morphine withdrawal. Mice were injected intraperitoneally twice a day, 10 h apart, for 5 days with escalating doses of morphine (two injections of 20, 40, 60, 80, and 100 mg/kg of body weight on days 1, 2, 3, 4, and 5, respectively). On the sixth day, withdrawal was precipitated 2 h after one administration of morphine at 100 mg/kg, by subcutaneous injection of naloxone at 1 mg/kg. Withdrawal behaviors were monitored and scored over a period of 30 min according to Celerer et al. (4).

Statistical analysis. For quantitative parameters showing normal distribution, data were analyzed using the unpaired Student t test or repeated measures of analysis of variance (ANOVA) with one between factor (genotype) and one within factor (time). Qualitative parameters (e.g., some clinical observations) were analyzed using the χ2 test. The level of significance was set at a P value of <0.05. Morphine analgesia and withdrawal scores were analyzed using two-way ANOVA with genotype and treatment as factors of variation. When appropriate, one-way ANOVA and Student-Newman-Keuls post hoc analysis were performed.

RESULTS AND DISCUSSION

Generation of Rgs4 mutant mice. Since Rgs4 is expressed at gastrulation in Xenopus laevis (48), potentially reflecting an embryonic early vital requirement in vertebrates, we modified the Rgs4 locus so as to allow for tissue-specific knockouts. We sought to circumvent the potential mosaicism or incompleteness of the tissue-specific Cre-mediated recombination by the detection of effectively recombined cells. We thus designed the targeting vector so that (i) the action of a Cre recombinase on the conditional allele (Rgs4<sup>loxP/loxP</sup>) would generate an in-frame fusion with the lacZ reporter (Rgs4<sup>loxP/lacZ</sup>) (Fig. 1, A and B) and (ii) the Flp recombinase could be used to generate a simple null allele (Rgs4<sup>ΔEC</sup>) (Fig. 1A) which, in combination with the conditional one, insures that all lacZ<sup>+</sup> cells are indeed Rgs4<sup>−/−</sup>. In the present study, we analyzed the Rgs4 null phenotype in Rgs4<sup>loxP/lacZ/ΔEC</sup> mice. A homologous recombinant ES clone (Fig. 1C) was used to generate a chimera which gave rise to Rgs4<sup>loxP/ΔEC</sup> mice (Fig. 1D). These in turn were crossed with PGK-Cre mice (26) to produce Rgs4<sup>ΔEC</sup> offspring (Fig. 1E) which were viable and fertile. Intercrosses of Rgs4<sup>loxP/lacZ/ΔEC</sup> animals produced homozygous mutants in which the disruption of the Rgs4 locus was further verified by the fact that Rgs4 was undetectable by in situ hybridization (Fig. 1F).

Rgs4<sup>ΔEC</sup> embryos and adults examined by X-Gal staining or lacZ in situ hybridization displayed a pattern of lacZ expression matching and refining the previously described pattern of Rgs4 expression during development (17) and in the adult nervous system (10) (Fig. 2A to E and data not shown). A previously undescribed neuronal site was a subset of ganglion cells in the retina (Fig. 2F). The main discrepancy between our observations and published expression data concerns reports of Rgs4 mRNA in heart tissue, cardiomyocyte primary cultures (6, 24, 51), and even individual atrial myocytes (11). Based on this expression and its modulation during congestive heart failure as well as on gain-of-function experiments, Rgs4 has been proposed to mediate a counterregulatory mechanism during cardiac hypertrophy (29, 33, 35, 39, 41). However, we could not detect any lacZ expression in the heart muscle of Rgs4<sup>ΔEC</sup> adult mice (Fig. 2G), nor could we detect Rgs4 mRNA expression in situ hybridization (not shown). This, barring some species difference between mouse and rat or human (which were studied in previous reports), suggests that Rgs4 expression, if real, is extremely low and represents an improbable pharmacological target in cardiomyocytes. In contrast, we detected high levels of expression in the walls (presumably smooth muscle cells) of the large vessels of the heart and coronaries (Fig. 2G and H and not shown), which could
underlie at least part of the findings cited above. This strong vascular expression, also found at other sites (Fig. 2I) (and also reported for Rgs5 [1] and Rgs2 [6]) suggests an involvement in normal vascular function and the regulation of blood pressure, as was shown for Rgs2 (42).

Rgs4lacZ/+ mice were intercrossed to produce homozygous mutants which were recovered at a lower than Mendelian frequency (16%; n = 136). Roughly the same proportion of homoyzgotes were recovered at E8.5 (17%; n = 36) as at later stages. These figures, contrasted with the full viability of homoyzgotes resulting from intercrosses of Rgs4loxFRTlacZ/+ mice (26%; n = 83) suggests an early, incompletely penetrant lethality of Rgs4 null embryos. However, such a lethality would be dependent on the DBA/2 background (brought by the deleter mouse) and/or the E129 background (brought by the recombinant ES cell) since, after three backcrosses on a C57BL/6 background, a Mendelian frequency was obtained.

Rgs4 mutants had lower body weight than their wt littermates (t<sub>21</sub> = 4.46, P < 0.001) (Table 1) but overall were in good general health (normal body temperature, well-groomed coat, and normal body posture and righting reflex) (Table 1 and not shown). Gross neurological examination of animals revealed no sign of modified sensory functions as assessed in basic tests of vision, audition, olfaction, and touch sensitivity. However, it should be noted that most wt mice (8/11) but only

### Table 1. Body weight, temperature, and neurological tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Avg value for indicated mouse group&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Rgs4loxFRTlacZ/+</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>33.7 ± 0.8</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>Time to fall off rotating rod</td>
<td>111.3 ± 4.9</td>
</tr>
<tr>
<td>Time to complete string test (s)</td>
<td>16.7 ± 5.8</td>
</tr>
<tr>
<td>Grip strength (g/g)</td>
<td>6.7 ± 0.3</td>
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</table>

<sup>a</sup> Asterisks indicate significant difference from corresponding wild-type values (unpaired Student t test). **, P < 0.01; ***, P < 0.001.
2/12 mutants vocalized during handling ($\chi^2 = 7.3; P < 0.01$). Rgs4 mutants had poorer performance in the rotating rod test than wt mice ($t_{21} = 3.62; P < 0.01$) (Table 1), while they performed correctly in the grip and string tests (Table 1), suggesting an alteration in balance/motor coordination. It is of note that inactivation of either Gq or G12, both potential targets of RGS4, also leads to coordination deficits, which are, however, much more pronounced.

A host of biological roles for Rgs4 have been proposed based on the expression of the gene and on the in vitro cell biological properties of the protein. In this study we have explored several of those, mostly with negative results.

**Normal neuronal differentiation in Rgs4lacZ/lacZ mice.** We previously showed an exquisitely specific pattern of Rgs4 expression in the developing nervous system, partially under the control of a well characterized determinant of neuronal identity, the homeobox gene Phox2b (17). In spite of the lack of a gross developmental phenotype in homozygous mutant mice, we looked for subtle differentiation anomalies or delays in Rgs4− mice. Using the neuronal transcription factors Phox2a (44), Phox2b (36), and Islet1/2 (43) or lacZ as markers, we could not detect any defect in shape, size, or timing of formation of the facial and trigeminal nuclei, the locus coeruleus (LC), sympathetic and parasympathetic ganglia, or cranial ganglia VII, IX, and X (Fig. 3A to C and data not shown). The noradrenergic differentiation of sympathetic ganglion cells or the locus coeruleus were unaffected (Fig. 3D and D′ and not shown) as well as sympathetic axonal projections examined on Rgs4lacZ/lacZ, Phox2alacZ/+; Phox2alacZ/+ embryos (21) (not shown).

At later stages of development and in the adult, Rgs4 is switched on in many telencephalic and diencephalic structures. The gross histological structure of the brain, the pattern of lacZ expression, and Nissl and Golgi stains on the cortex of heterozygous and homozygous mutants failed to reveal any difference between them (Fig. 3E and E′ and data not shown).

**No obvious interference of the Rgs4 mutation with opioid signaling.** Endogenous or exogenous RGS proteins have been shown in vitro to dampen signaling through the α-opioid receptor (9, 37, 38). It follows that Rgs4 could regulate antinociception by endogenous or exogenous opioids, both presynaptically in nociceptor neurons of the dorsal root ganglia and postsynaptically in second-order sensory neurons on which they project, two sites of strong Rgs4 expression (13, 17) (Fig. 2E). We therefore tested the sensitivity of wt mice and mutants first to pain and then to the analgesic effects of morphine treatment.

Mutant mice had normal pain sensitivity in the tail flick test and showed first-reaction latency comparable to that of wt mice in the hot-plate test (Table 2). In the shock threshold test, subtle differences between wt mice and mutants were detected: the shock levels at which mice first detected the shock (flinch) were comparable in wt and Rgs4lacZ/lacZ mice (Table 2), but the shock intensity required to induce jumping was significantly increased in Rgs4lacZ/lacZ mice compared to wt mice ($t_{21} = 2.76; P < 0.05$; Student t test) (Table 2). Thus, the proportion of mutants displaying a jumping reaction before the cutoff point (41.6%) was significantly reduced compared to wt mice (90.9%) ($\chi^2 = 6.1; P < 0.05$), suggesting an effect of the mutation on central processing of painful stimuli.

Systemic injection of morphine at the dose of 8 mg/kg induced comparable antinociceptive effects in wt mice and mutants in the tail flick assay (treatment effect, F(1,19) = 12.9 (numbers in parentheses represent degrees of freedom corresponding to each experiment) and P < 0.01; genotype effect, F(1,19) = 0.2 and P > 0.05; genotype × treatment interaction, F(1,19) = 0.6 and P > 0.05) as well as in the first reaction to the hot-plate test (treatment effect, F(1,19) = 17.5 and P < 0.001; genotype effect, F(1,19) = 0.4 and P > 0.05; genotype × treatment interaction, F(1,19) = 0.4 and P > 0.05).
Another potential role of Rgs4 connected to its biochemical capacity to regulate opioid signaling is suggested by its expression in the LC (16, 17). The LC, the main noradrenergic center of the brain, has a high level of opioid receptors and is hypothesized on numerous grounds to be a mediator of the opioid withdrawal syndrome (reviewed in reference 31), although the evidence has been extensively questioned (reviewed in references 8 and 46). We therefore tested a role for Rgs4 in somatic signs of abstinence to opioids according to the procedure described in reference 4 (see Materials and Methods). No difference between mutants and wt mice was found, except for an almost complete suppression of the sniffing reflex (Fig. 4). It is of note that sniffing, a minor sign of abstinence, also stood out in the D2 dopamine receptor knockout mice as being the only withdrawal symptom affected (exacerbated, in that case) (27).

These data stand in stark contrast with the marked increase in morphine-induced analgesia and physical symptoms of withdrawal in Rgs9-2 knockout mice (49). The difference could be due to class-specific effects (RGS9, belonging to the C class of RGS proteins, possesses, unlike RGS4, DEP and GGL domains) or to tissue distribution differences (the site[s] of Rgs9-2 action on analgesia or withdrawal being presently unknown). Future studies should aim at exploring morphine tolerance (delayed in Rgs9 knockouts [49]) and unraveling either more subtle or more integrative aspects of responses to pain in Rgs4 mutants.

No defect in prepulse inhibition in Rgs4 null mice. Along a different line of research, significant association between the Rgs4 locus and schizophrenia has been reported in several studies (5, 7, 30, 47), and Rgs4 transcripts have been found to be reduced in cortices of schizophrenic patients (28; see reference 18 for a review). The consequent status of Rgs4 as a susceptibility gene for schizophrenia is strengthened by its

**TABLE 2. Pain sensitivity**

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<td>Time to complete tail flick test (s)</td>
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<tr>
<td>Time to complete hot plate test (s)</td>
<td>Wild type: 9.3 ± 0.6, Rgs4lacZ/lacZ: 8.8 ± 0.5</td>
</tr>
<tr>
<td>Shock threshold (mA)</td>
<td>Flinching: 0.05 ± 0.00, 0.05 ± 0.00</td>
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<td>Jumping: 0.57 ± 0.06, 0.62 ± 0.07*</td>
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* An asterisk indicates significant difference (P < 0.05) from the corresponding wild-type value (unpaired Student t test).

**FIG. 4.** Naloxone-precipitated morphine withdrawal syndrome in wild-type and Rgs4 null mice. Data are expressed as means ± standard errors of the means. Black stars, morphine- versus saline-treated animals of the same genotype; white stars, wild-type versus mutant groups receiving the same treatment (two-tailed Student t test). One star, P < 0.05; two stars, P < 0.01.

**FIG. 5.** Startle reactivity and prepulse inhibition in wild-type and Rgs4 null mice. (A) The startle amplitude is measured at the background noise (BN) level or at indicated intensities. (B) The PPI is measured as percent startle response to the 120-dB pulse for prepulses of indicated intensities. Values are expressed as means ± standard errors of the means.

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abundant expression in the cortex, where it could regulate dopaminergic and glutamatergic transmission, both invoked in models of schizophrenia (see references 18 and 23 for a review). We therefore tested mutant mice for PPI, a measure of sensorimotor gating abilities, whose decrease is one of the most widely accepted schizophrenia-related behaviors in rodents (see reference 14 for a review). Mutant mice displayed a normal startle response compared to wt mice at all tested acoustic stimulus intensities (Fig. 5A). They also showed a normal PPI across all prepulse intensities used (Fig. 5B). Another test of schizophrenia-like cognitive dysfunction in rodents (latent inhibition) should be performed in the future, as well as potential interactions of the Rgs4 null mutation with schizophrenogenic environmental paradigms (45).

We also tested for depression- and anxiety-related symptoms (by the tail suspension test and the open-field test, respectively) and for associative learning and working memory defects (by Pavlovian fear conditioning and Y-maze spontaneous alternation, respectively). Rgs4lacZ/lacZ mice did not show any difference in their performance compared to wt mice in any of these tests (not shown).

In conclusion, subtle positive symptoms in Rgs4 mutants (namely, lower weight, partially penetrant, genetic background-dependent early lethality, poorer sensorimotor coordination, and central integration of painful stimuli) point to developmental and physiological roles for Rgs4. Further purification of the genetic background may unmask other, presumably subtle, behavioral phenotypes by reducing the variability between individuals. On the other hand, combined inactivation of other Rgs genes, facilitated by the fertility of Rgs4 null mice, may reveal roles hitherto masked by redundancy. Finally, postnatal inactivation allowed by our conditional knockout scheme could unveil roles developmentally compensated for in Rgs4 null animals.

ACKNOWLEDGMENTS

This work was supported by grants from the European Community (QLG2-CT-2001-01467), Association Francaise contre les myopathies, the French Ministry of Research (ACI 2002), and Association pour la Recherche sur le Cancer (fellowsip to N.G.).

We thank H. Meziane and A.-M. Ouazzaz for helpful comments on the behavioral tests.

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