K⁺ channel TASK-1 knockout mice show enhanced sensitivities
to ataxic and hypnotic effects of GABAₐ receptor ligands

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Effects of GABA-A receptor ligands in TASK-1 and -3 KO mice.

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Non-standard abbreviations: ANOVA, analysis of variance; GABA, γ-aminobutyric acid; K2P, two-pore-domain background K⁺ channel; KO, knockout; LORR, loss of righting reflex.

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Abstract

TASK two-pore domain leak K+ channels occur throughout the brain. However, TASK-1 and TASK-3 knockout (KO) mice have few neurological impairments, and only mildly reduced sensitivities to inhalational anesthetics, contrasting with these channels’ anticipated functions and importance. TASK-1/-3 channel expression can compensate for the absence of GABA_A receptors in the GABA_A α6 KO mice. To investigate the converse, we analyzed the behavior of TASK-1 and -3 KO mice after administering drugs with preferential efficacies at GABA_A receptor subtypes: benzodiazepines (diazepam and flurazepam, active at α1βγ2, α2βγ2, α3βγ2 and α5βγ2 subtypes), zolpidem (α1βγ2 subtype), propofol (β2-3-containing receptors), gaboxadol (α4βδ and α6βδ subtypes), pregnanolone and pentobarbital (many subtypes). TASK-1 KO mice showed increased motor impairment in rotarod and beam walking tests after diazepam and flurazepam, but not after zolpidem. They also showed prolonged loss of righting reflex induced by propofol and pentobarbital. Autoradiography indicated no change in GABA_A receptor ligand binding levels. These changed behavioral responses to GABAergic drugs suggest functional upregulation of α2β2/γ2 and α3β2/γ2 receptor subtypes in TASK-1 KO mice. Additionally, female, but not male TASK-1 KO mice were more sensitive to gaboxadol, suggesting an increased influence of α4βδ or α6βδ subtypes. The benzodiazepine sensitivity of TASK-3 KO mice was marginally increased. Our results underline that TASK-1 channels perform such key functions in the brain that compensation is needed for their absence. Further, because inhalational anesthetics
activate GABA<sub>A</sub> receptors, the upregulation of GABA<sub>A</sub> receptor function in TASK-1 KO mice might produce an underestimate of TASK-1 channel’s significance as a target for inhalational anesthetics.
Introduction

Two-pore domain potassium (K2P) channels form leak conductances which regulate neuronal excitability (Duprat et al., 1997; Rajan et al., 2000). In mammals the K2P family comprises 15 differentially expressed genes (Talley et al., 2001; Aller and Wisden, 2008). K2P channels such as TASK-1 (kcnk3) and TASK-3 (kcnk9) are targets for many neurotransmitters and neuromodulators (Millar et al., 2000; Talley et al., 2000; Linden et al., 2006; Meuth et al., 2006). Regulating TASK-1 and -3 channels might, for example, influence arousal state and motor control. Additionally, inhalation anesthetics open TASK and some other K2P channels (Patel et al., 1999; Sirois et al., 2000; Berg et al., 2004; Franks, 2008); thus these channels are in vivo targets explaining some actions of inhalational anesthetics such as halothane and isoflurane (Heurteaux et al., 2004; Linden et al., 2006; 2007; Franks, 2008). Depending on cell type, the TASK-1 and -3 subunits assemble as hetero- or homomers (Aller et al., 2005; Berg et al., 2004; Berg and Bayliss, 2007).

Gene knockouts (KOs) showed that the TASK-1/-3 channels control adrenal gland development and regulate aldosterone secretion (Davies et al., 2008; Heitzmann et al., 2008); however behavioral studies on the TASK KO mice have discovered relatively few effects (Aller et al., 2005; Meuth et al., 2006; Linden et al., 2006; 2007; Mulkey et al., 2007). Male TASK-1 KO mice exhibit modest motor deficits in testing on an accelerating rotating rod and on narrow horizontal beams (Aller et al., 2005; Linden et al., 2006). TASK-3 KO mice have no motor phenotype, even though TASK-3 KO cerebellar granule cells can no longer sustain action potential firing at high frequencies (Brickley et al., 2007; Linden et al., 2007). Compensatory mechanisms and/or genetic degeneracy
(e.g. substitution by other K2P subunits) may explain the absence of strong motor phenotypes.

To examine compensatory events in the TASK KO mice we decided, based on our earlier findings (Brickley et al., 2001), to start with GABA\(\alpha\) receptors. The TASK-1, TASK-3 and THIK-2 K2P genes are upregulated in the cerebellar granule cells to compensate the genetic deletion of the GABA\(\alpha\) receptor \(\alpha6\) subunit (Brickley et al., 2001; Aller et al., 2005; Aller and Wisden, 2008), which is normally strongly enriched in this unique neuronal population (Laurie et al., 1992). Importantly, these \(\alpha6\) KO mice do not show any motor disabilities (Jones et al., 1997; Korpi et al., 1999), indicating full compensation of the missing GABAergic background conductance by the K2P channels.

GABA\(\alpha\) receptors are GABA-gated anion channels that produce fast (phasic) inhibition at central synapses and tonic inhibition (from ambient GABA) at extrasynaptic sites (Farrant and Nusser, 2005). The channels form as pentamers. The GABA\(\alpha\) receptor gene family contains 19 subunit genes; here we consider only \(\alpha1-6, \beta1-3, \gamma2, \text{ and } \delta\). These genes are differentially expressed in the brain and spinal cord (e.g. Laurie et al., 1992; Wisden et al., 1991; 1992), and receptor subtypes with these subunits largely account for how most classical GABAergic drugs act (Rudolph and Möhler, 2004). The \(\alpha\beta\gamma2\) receptor subtypes are enriched at synapses, although found also extrasynaptically, and the \(\alpha4\beta\delta\) and \(\alpha6\beta\delta\) subtypes are extrasynaptic (Farrant and Nusser, 2005).

The function of the GABA\(\alpha\) receptor system can be probed with selective drugs (Korpi et al., 2002; Rudolph and Möhler, 2004). Drug specificity in vivo has been established using knock-in mice with point mutations in the GABA\(\alpha\) receptor subunit genes and subunit knockout mice (Rudolph and Möhler, 2004). Zolpidem acts
preferentially at $\alpha_1\beta_2$ receptors (producing sedation and hypnosis); diazepam and flurazepam act on $\alpha_1\beta_2$ (sedation, hypnosis and anterograde amnesia), $\alpha_2\beta_2$ and $\alpha_3\beta_2$ (anxiolysis and myorelaxation) and $\alpha_5\beta_2$ receptors (myorelaxation). Propofol acts largely at $\beta_2$- and $\beta_3$-containing $\text{GABA}_A$ receptors (Rudolph and Möhler, 2004). The $\text{GABA}$ mimetic gaboxadol exerts most of its effects (sedation, ataxia and analgesia) via $\alpha_4\beta_3$ receptors (Chandra et al., 2006). Steroids and barbiturates modulate many $\text{GABA}_A$ receptor subtypes (Korpi et al., 2002; Belelli and Lambert, 2005). Similar to TASK-1 and -3 channels, $\text{GABA}_A$ receptors are facilitated by inhalational anesthetics (Mihic et al., 1997). In the present study, we studied the sensitivity of TASK-1 and -3 KO mice to various $\text{GABA}$ergic drugs, using motor performance and sedation tests, and found a general increase in the effects of these compounds in the TASK-1 KO mice.
Methods

TASK-1 and TASK-3 KO mice. TASK-1 and TASK-3 KO mice, with the genetic background C57Bl/6J x 129S1/SvJ, were generated and genotyped as previously described (Aller et al., 2005; Brickley et al., 2007). Homozygous KO and control littermates were from heterozygous breeding. Mice were maintained in groups of 1-5 in plastic cages (37 x 21 x 15 cm, Tecniplast, Buguggiate, Italy) with food pellets (Harlan BV., Horst, Netherlands) and tap water available ad libitum. Lights were on from 6 a.m. to 6 p.m. All behavioral tests were performed during the light phase (motor training between 8 a.m. and 5 p.m. and pharmacological tests between 8 a.m. and 1 p.m.). When the same mice were used for several tests at least one week wash-out period was kept between experiments. The mice were 3-9 months old and weighing 26-40 g (males) or 22-35 g (females). All animal tests were approved by the Laboratory Animal Committee of the University of Helsinki and the Southern Finland Provincial Government.

Motor coordination. Effects of GABAergic drugs on motor coordination were analyzed as described earlier (Korpi et al., 1999). The mice were first trained for 7 days (6 trials a day) to walk on a rotating rod (dowel 4 cm in diameter; Rotamex 4/8, Columbus Instruments, Ohio, USA) for 180 s with the rotation speed accelerating from 5 to 40 rpm. The latency to fall from the rod was recorded and a daily average of 6 trials was calculated for each animal. The same mice were also trained to walk along 1-m-long wooden beams (1.2 and 0.8 cm in diameter; 84 cm above the floor) back to their home cages. Beam training was performed once a day for 7 days, immediately after the rotarod training. Time to traverse the beams and the number of falls were recorded. The mice were 15-21 weeks old during the training; TASK-1 KO males and females were on
average 2 and 4 weeks older than littermate controls, respectively, however their weights did not differ from those of control mice (p > 0.05, t-test).

**Drug-induced ataxia.** The mice were first subjected to motor training as described above and they all achieved similar levels of performance. On the days of pharmacological testing, the mice were injected (i.p.) with vehicle followed by a single dose or cumulative doses (at 30 min intervals) of test compounds. Fifteen min after each injection (unless otherwise stated), motor performance was tested first on the rotarod for 180 s (speed from 5 to 30 rpm) and immediately thereafter on the 1.2-cm-thick beam.

The effects of GABAergic drugs on motor performance were tested using the same animals in the following order (time after the training): flurazepam (1 day), pentobarbital (1 week), zolpidem (2 weeks), pregnanolone (3 weeks), gaboxadol (4 weeks) and diazepam + flumazenil (14 weeks). Between pharmacological tests, the mice were subjected to the rotarod and beam walking to maintain their motor skills. The mean weights of the test groups did not significantly differ between genotypes. The effect of propofol on the rotarod performance was tested using another batch of pretrained TASK-1 KO mice and their littermate controls of 11-16 weeks old; TASK-1 KO mice were on average one week older than wildtype mice and no weight difference was found between genotypes (p > 0.05).

The effect of flurazepam on the motor performance of TASK-3 KO mice was tested using pretrained 16-26 weeks old animals. TASK-3 KO mice were on average 4 weeks older than wildtype controls. The mean weight of TASK-3 KO males was significantly higher than that of wildtype males (35.9 ± 1.8 vs. 32.8 ± 0.6 g; p < 0.05), but the weights of females did not differ between genotypes.
Elevated plus-maze test. Anxiolytic-like effects of diazepam (1 mg/kg, s.c.) were tested on an elevated plus-maze test as described earlier (Linden et al., 2006). The mice were placed individually on a central platform facing an open arm and allowed free exploration of the maze for 5 min. The time spent in open and closed arms and crosses between the compartments were recorded using a CCD video camera above the maze and EthoVision Color-Pro 3.0 software (Ethovision System, Noldus Information Technology, Wageningen, Netherlands). The mice tested on the elevated plus-maze were 33-39 weeks old; TASK-1 KO mice were on average 3 weeks older than wildtype controls. The weights of KO males and females did not differ from those of wildtype mice (p > 0.05). The same mice were used two weeks later for testing sedative effects of diazepam in an open arena.

Locomotor activity in an open arena. Sedative effects of diazepam were tested by determining spontaneous locomotor activity in a novel arena. The mice were placed individually in a box made of grey plastic (50 x 50 cm with 27-cm high walls) 15 min after diazepam administration (3 mg/kg, s.c.), and their behavior (total movements in cm and duration of moving) was recorded for 5 min using EthoVision. The number of rearings was counted manually from a monitor placed in an adjacent room.

Drug-induced loss of righting reflex (LORR). Sensitivity to propofol and pentobarbital was determined by measuring the latency to and duration of LORR as described earlier (Saarelainen et al., 2008). After injection of propofol (200 mg/kg, i.p.) or pentobarbital (45 mg/kg, i.p.) righting reflex was tested every four min by placing the mouse on its back on a plastic V-shaped trough. When the animal could not right itself within 5 s in three successive test trials, the righting reflex was considered lost.
Thereafter the test was repeated every four min until the righting reflex was regained. Sufficient body temperature was maintained using a heat radiator placed above the mice or using a heating pad (37 °C). Propofol was tested with 14-19 weeks old mice, TASK-1 KO mice being on average one week older than wildtype mice. TASK-1 KO males were weighing significantly less than wildtype males (27.3 ± 0.9 vs. 31.3 ± 1.3 g; p < 0.05, t-test), but the weights of females did not differ between genotypes. Pentobarbital was tested with 12-18 weeks old mice; TASK-1 KO mice were on average 3 weeks older. The weights of TASK-1 KO males and females did not differ from those of wildtype mice (p > 0.05).

**Drugs.** Flurazepam dihydrochloride (Sigma, St. Louis, MO, USA), gaboxadol hydrochloride (H. Lundbeck A/S, Copenhagen, Denmark) and pentobarbital sodium (Sigma) were dissolved in physiological saline. Diazepam 5 mg/ml injection solution (solution: fractionated soybean oil 150 mg, acetylated monoglycerids 50 mg, egg lecithin 12 mg, glycerol 22.5 mg, ad 1 ml water, pH 8; Alpharma ApS, Copenhagen, Denmark) was diluted to 0.1-0.5 mg/ml with saline. Zolpidem tartrate (Sanofi-Synthelabo AB, Bromma, Sweden) was crushed from 10-mg Stilnoct® tablets and suspended in saline. Flumazenil (Tocris Bioscience, Bristol, UK) was suspended in 100% Tween80 and brought to the final concentration with saline (3% Tween80). Pregnanolone (5β-pregnan-3α-ol-20-one; Sigma) was dissolved first in 100% cremophor EL (polyoxyethyleneglycerol triricinoleate 35; Sigma) and diluted with saline to the final concentration (20% cremophor). Vehicle control animals were injected with Intralipid® emulsion (Fresenius Kabi SB, Uppsala, Sweden) diluted with saline to 2-10% (vehicle for diazebam), 20% cremophor EL (vehicle for pregnanolone), 3% Tween80 (vehicle for
flumazenil) or with saline (all other drugs). All substances were injected in a volume of 10 ml/kg.

**GABA<sub>A</sub> receptor ligand autoradiography.** We used the ligands [^3H]Ro 15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-α]1,4-benzodiazepine-3-carboxylate), [^3H]muscimol and t-butyl-bicyclophosphoro[^35S]thionate ([^35S]TBPS) as described previously (Mäkelä et al., 1997; Korpi et al., 2002). Sections (14-μm-thick) were cut from frozen brains and spinal cords of TASK-1 KO (females n = 5-6 and males n = 5-6) and littermate control (females n = 5-6 and males n = 5-6) mice with a Leica CM 3050S cryostat (Leica Microsystems, Benheim, Germany), thaw-mounted onto gelatin-coated slides, and stored at -80 °C until experiments.

Sections were preincubated for 15 min for [^3H]Ro 15-4513 and [^3H]muscimol binding in ice-cold 50 mM Tris-HCl (pH 7.4) + 120 mM NaCl and 170 mM Tris-HCl (pH 7.4), respectively. Flumazenil-sensitive benzodiazepine binding sites were labeled during 60 min in similar, ice-cold buffer with 15 nM [^3H]Ro 15-4513 (PerkinElmer, Life and Analytical Science, Boston, USA) with and without 10 μM diazepam (Orion, Espoo, Finland; dissolved in dimethylsulphoxide). GABA-binding sites were labeled with 10 nM [^3H]muscimol (NEN Radiochemicals, PerkinElmer, Boston, USA) for 30 min. Non-specific binding was determined by adding 10 μM flumazenil (Hoffmann-La Roche, Basel, Switzerland; dissolved in dimethylsulphoxide) to [^3H]Ro 15-4513 assays and 100 μM GABA (Sigma) to [^3H]muscimol assays. After incubations, the sections were washed in ice-cold incubation buffers twice for 60 s ([^3H]Ro 15-4513 assay) or twice for 30 s ([^3H]muscimol assay), dipped into water and air-dried.
For labeling picrotoxin-sensitive $[^{35}S]$TBPS sites, the sections were preincubated for 15 min in ice-cold 50 mM Tris-HCl (pH 7.4) before a 90-min incubation at room temperature with 6 nM $[^{35}S]$TBPS (NEN Radiochemicals, PerkinElmer; diluted with cold TBPS to radioactivity level of ~100 cpm/μl) in 50 mM Tris-HCl (pH 7.4) supplemented with 120 mM NaCl. Non-specific binding was determined using 100 μM picrotoxinin (Sigma). After the incubation the sections were washed in ice-cold 10 mM Tris-HCl (pH 7.4) for 3 x 2 min, dipped in distilled water and air dried.

For the analysis of the population of GABA$A$ receptors at which GABA is a partial agonist (so called “GABA-insensitive” $[^{35}S]$TBPS binding sites; Sinkkonen et al., 2001; Saarelainen et al., 2007) brain sections were preincubated in ice-cold 50 mM Tris-HCl (pH 7.4) supplemented with 120 mM NaCl for 15 min. Final incubation was done with 2 nM $[^{35}S]$TBPS (radioactivity ~950 cpm/μl) in the absence (basal) or presence of saturating 1 mM GABA at room temperature for 90 min. Non-specific binding was determined using 100 μM picrotoxinin. After incubation the sections were washed 3 x 30 min in ice-cold 10 mM Tris-HCl (pH 7.4), dipped into water and air-dried.

The slides were exposed with plastic $^3$H- or $^{14}$C-radioactivity standards (GE Healthcare, Little Chalfont, Buckinghamshire, UK) to Biomax MR films (Eastman Kodak, Rochester, NY) for up to 8 weeks. The autoradiographs were quantified using MCID M5 image analysis devices and programs (Imaging Research Inc., St. Catharines, ON, Canada) and converted to nCi/mg or nCi/g, respectively.

Statistics. The results are given as means ± standard error. We used SPSS Software (SPSS 12.0.1, SPSS Inc., Chicago, IL, USA) or GraphPad Prism (Prism 5.0, GraphPad Software Inc., CA, USA) for repeated measures ANOVA or two-way ANOVA followed
by Newman-Keuls post hoc test. For two-mean comparisons Mann-Whitney test or Student’s t-test were used. The significance level was p < 0.05.
Results

Pretraining of mice in the motor tests. To examine if GABA_A receptors were functionally upregulated in the TASK KO mice, we tested the mice’s motor ability using the rotarod and horizontal beam while they were under the influence of benzodiazepine drugs. Before pharmacological tests the mice were trained to perform the motor tasks. During training, every mouse learned to stay on the rod for the 180 s, although on the first training day the male TASK-1 KO mice fell off earlier (82 ± 4 s, n = 9) than the control littermate males (116 ± 9 s, n= 8) (genotype × sex interaction F_1, 32 = 6.26, p = 0.018 and genotype effect F_1, 15 = 8.01, p = 0.013; full data not shown). This is in agreement with our previous study with other batches of TASK-1 KO male mice (Aller et al., 2005). In TASK-1 KO females, motor performance and learning on the rotarod were not affected. For the horizontal beam, as in the rotarod training, all animals learned to quickly traverse the beams by the end of the training period. However, during the first 5 days of training the genotype effect became significant (F_1, 32 = 15.61, p < 0.001) TASK-1 KO mice falling more frequently from the 0.8-cm beam than their control littermates as in our previous study (Aller et al., 2005). The female TASK-1 KO mice crossed the 1.2-cm beam slower and they fell from the 1.2- and 0.8-cm beams more frequently than the wildtypes (training x genotype interaction in time: F_4, 68 = 8.32, p < 0.001, in falls from the 1.2-cm beam: F_4, 68 = 4.89, p < 0.01; genotype effect in falls from both beams: F_1, 17 > 10.91, p ≤ 0.004) (data not shown).

Increased sensitivity of TASK-1 KO mice to the ataxic effects of benzodiazepines. To avoid any basal motor differences affecting drug responses the test
conditions were easier than during the training: the acceleration of the rod was from 5 to 30 rpm in 3 min and only the thicker 1.2-cm beam was used. Having pretrained the drug-naïve mice, we found that cumulative dosing of the benzodiazepine flurazepam (5 + 15 + 25 mg/kg, i.p., at 30 min intervals, total dose 45 mg/kg, testing 15 min after each dose) produced greater ataxia in TASK-1 KOs than control littermates (Fig. 1A). After the last dose, the TASK-1 KO mice could stay on the rotating rod only for 74 ± 8 s, whereas the control littermate mice reached 129 ± 10 s before falling. The genotype difference was significant both in the rotarod (dose × genotype interaction $F_{3, 99} = 5.70, p = 0.001$; genotype effect $F_{1, 33} = 18.16, p < 0.001$) and beam walking (time: dose × genotype interaction $F_{3, 99} = 11.70, p < 0.001$ and genotype effect $F_{1, 33} = 16.71, p < 0.001$; falls: dose × genotype interaction $F_{3, 99} = 13.44, p < 0.001$ and genotype effect $F_{1, 33} = 29.58, p < 0.001$) tests (Fig. 1A). Since no significant sex effect was observed, the data of males and females were combined.

To confirm that the enhanced benzodiazepine sensitivity in the TASK-1 KO mice was mediated by the GABA$_A$ receptor benzodiazepine site, we used another benzodiazepine agonist, diazepam, with and without the selective benzodiazepine antagonist flumazenil (15 mg/kg, s.c.). The effect of diazepam on motor performance was completely blocked by the pretreatment with flumazenil (drug x pretreatment interaction $F_{3, 90} = 41.39, p < 0.001$ and pretreatment effect $F_{1, 30} = 70.10, p < 0.001$, Fig. 1B). The effect of diazepam was also affected by the genotype (drug x genotype interaction $F_{3, 90} = 6.65, p < 0.001$ and genotype effect $F_{1, 30} = 12.16, p = 0.002$) TASK-1 KO mice being more sensitive to the motor impairing effects of diazepam when pretreated with vehicle but not when pretreated with flumazenil (drug x genotype x pretreatment interaction $F_{3, 90}$...
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\[ t = 7.95, \ p < 0.001 \] and genotype x pretreatment interaction \( F_{1, 30} = 10.87, \ p = 0.003; \) Fig. 1B). Essentially similar results were obtained from the beam walking test (Fig. 1B).

To distinguish between the \( \alpha\beta\gamma2 \) type GABA\(_A\) receptors likely to be upregulated in the TASK-1 KO mice, we repeated the motor tests with zolpidem, a benzodiazepine site agonist with preferential affinity at \( \alpha1\beta\gamma2 \) GABA\(_A\) receptors. Cumulative dosing with zolpidem \((1 + 2 + 6 \text{ mg/kg, i.p.})\) significantly shortened the latency to fall from the rod \((F_{3, 96} = 159.2, \ p < 0.001)\) and increased the time to traverse the beam and the number of falls \((\text{time: } F_{3, 96} = 35.49, \ p < 0.001; \ \text{falls: } F_{3, 96} = 12.58, \ p < 0.001)\). Importantly, the effects were similar between the TASK-1 KO and control littermate mice (no significant dose x genotype interaction or genotype effect, Fig. 1C).

**Flurazepam-induced ataxia in the TASK-3 KO mice does not differ from littermate controls.** We repeated the flurazepam motor tests on TASK-3 KO mice. Previously, we showed that the motor phenotype of the TASK-3 KO mice on the rotarod was identical with littermate controls, but their walking on the beams was slightly impaired on the first day of the training (Linden et al., 2007). In the present study, flurazepam similarly impaired the rotarod performance in both TASK-3 KO and littermate control mice \((\text{dose effect } F_{3, 102} = 34.48, \ p < 0.001, \ \text{no dose x genotype interaction or genotype effect})\). In the beam test, the TASK-3 KO mice showed a modest increase in flurazepam sensitivity by falling more frequently from the 1.2-cm-thick beam than the littermate control mice after 20 and 45 mg/kg doses \((\text{dose x genotype interaction } F_{3, 105} = 4.70, \ p = 0.004, \ \text{genotype effect } F_{1, 35} = 10.69, \ p = 0.002, \) Fig. 2). Although, TASK-3 KO males were slightly heavier than their littermate controls, this appeared not to affect the results since the weights of females did not differ between genotypes, and we
found no dose \times genotype \times sex interaction (F_{3, 99} = 0.70, p = 0.55) in repeated measures two-way ANOVA. In another parameter measuring beam walking, time to cross the beam, the flurazepam effect was similar in both TASK-3 KO and their littermate controls (dose effect F_{3, 105} = 47.08, p < 0.001, no dose \times genotype interaction or genotype effect, Fig. 2).

**Anxiolytic and sedative effects of diazepam in TASK-1 KO mice.** To test whether enhanced sensitivity to benzodiazepines was specific to motor-impairing effects, or seen also in other behavioral endpoints, the anxiolytic effect of diazepam was tested in the elevated plus-maze test. Overall, diazepam (1 mg/kg, s.c.) increased the time spent in open arms (treatment effect F_{1, 30} = 9.96, p = 0.004, two-way ANOVA; Fig. 3A). The time in the open arms was increased in the KO mice regardless of the treatment (genotype effect F_{1, 30} = 5.33, p = 0.028). No treatment \times genotype interaction was found.

Since we found a significant genotype effect in the main two-way ANOVA, the results were next analyzed using separate Mann-Whitney tests for wildtype and TASK-1 KO mice. These tests revealed that diazepam significantly increased the time spent in the open arms in the wildtype controls, but not in the TASK-1 KO mice (Fig. 3A). The number of open arm entries was not significantly affected by genotype or diazepam (F_{1, 30} \leq 3.82, p \geq 0.06; Fig. 3A). Diazepam significantly increased the total movements (treatment effect F_{1, 30} = 5.65, p = 0.024), but no significant genotype difference could be seen (Fig. 3A).

A higher dose of diazepam (3 mg/kg, s.c.) produced sedation (reduced locomotor activity in a novel arena) both in the TASK-1 KO and control littermate mice without any significant difference between the genotypes (Fig. 3B). Two-way ANOVA revealed a
significant drug effect on all parameters measured: total movements (F_{1, 30} = 7.41, p = 0.011), % duration of moving (F_{1, 30} = 19.42, p < 0.001) and number of rears (F_{1, 30} = 11.45, p = 0.002).

Motor-impairing effects of other GABAergic drugs in TASK-1 KO mice are sex-dependent. To further profile the GABA\textsubscript{A} receptor-mediated responses in TASK-1 KO mice, we compared the actions of the intravenous GABAergic anesthetics pregnanolone, propofol and pentobarbital and the GABA site agonist gadoxadol on rotarod performance in TASK-1 KO and littermate control mice. These drugs were administered as cumulative doses. The data from males and females are presented separately, because their responses differed to all drugs, except for pentobarbital (Fig. 4).

In males, a dose x genotype interaction was found in rotarod performance after cumulative doses of pregnanolone (F_{3, 36} =3.50, p = 0.025) with the KO mice falling significantly earlier from the rod than the controls after the dose of 20 mg/kg (p < 0.05, Newman-Keuls test). In females, pregnanolone produced ataxia similarly in both TASK-1 KO and control littermates (overall dose effect F_{3, 54} = 28.28, p < 0.001; no interaction or genotype effect, Fig. 4B). Gaboxadol affected the TASK-1 KO females more than control littermate females (dose x genotype interaction F_{3, 54} =5.88, p = 0.002, genotype effect F_{1, 18} = 12.23, p = 0.003; Fig. 4D). No genotype effect or interaction was found in gadoxadol’s effects on males (Fig. 4C). Propofol produced stronger ataxia in TASK-1 KO males than in control littermate males (dose x genotype interaction F_{3, 48} =6.56, p = 0.001, genotype effect F_{1, 16} = 5.19, p = 0.037; Fig. 4E). No genotype effect or interaction was found in propofol’s effects on females (Fig. 4F). A dose x genotype interaction was found in rotarod performance of males after cumulative doses of pentobarbital (F_{3, 39} =
17.45, p < 0.001) without a significant genotype effect. In females, pentobarbital produced ataxia similarly in both TASK-1 KO and control littermates (no interaction or genotype effect, Fig. 4H).

**Longer propofol- and pentobarbital-induced LORR in TASK-1 KO mice.** We previously found that TASK-1 KO mice exhibit reduced sensitivity to the volatile anesthetics halothane and isoflurane (Linden et al., 2006). We studied here whether LORR (unconsciousness, one component of anesthesia) produced by propofol (200 mg/kg, i.p.) and pentobarbital (45 mg/kg, i.p.) was affected in the TASK-1 KO mice. Both anesthetics significantly increased the hypnotic effect (i.e. prolonged the duration of LORR) in the TASK-1 KO mice compared with control littermates (p < 0.05, Fig. 5). The slightly lower body weight of the TASK-1 KO males compared with the wildtype males appeared not to affect the propofol’s hypnotic effects since no weight difference between genotypes was found in females, and we found no genotype-sex interaction in two-way ANOVA; only genotype effect F1, 26 = 5.83, p = 0.023. After propofol-induced LORR, the rectal temperatures did not differ between the littermate controls and TASK-1 KO mice (37.5 ± 0.2 vs. 37.7 ± 0.2 °C, respectively). After pentobarbital-induced LORR, the temperatures were slightly (p < 0.05, Student’s t-test) lower in the TASK-1 KO mice (37.1 ± 0.2 °C) than in the littermate controls (37.8 ± 0.2 °C).

**No change in GABA<sub>A</sub> receptor ligand binding levels in TASK-1 KO brain or spinal cord.** GABA<sub>A</sub> receptor subtype levels can be sensitively estimated by autoradiography (for example, Mäkelä et al., 1997; Korpi et al., 2002): picrotoxin-sensitive [35S]TBPS autoradiography assays the amounts of many receptor subtypes (ligand binds in the Cl⁻ channel pore); [3H]muscimol autoradiography selects for α4βδ,
α6βδ and α6βγ2 type receptors; the benzodiazepine [3H]Ro 15-4513 binds to all αβγ2-type GABA<sub>A</sub> receptors but in the presence of diazepam it selects for α6βγ2 in the cerebellar granule cell layer; finally, a GABA-insensitive [35S]TBPS binding component is formed of GABA<sub>A</sub> receptor populations in which GABA is a partial agonist (Saarelainen et al., 2008); these receptors are most likely extrasynaptic (Sinkkonen et al., 2001).

Compared with littermate control mice, the amount and distribution of the binding sites for the benzodiazepine site ligand [3H]Ro 15-4513 was unaltered in the TASK-1 KO forebrain, cerebellum and spinal cord (Fig. 6A, Table 1). The amount of the diazepam-insensitive [3H]Ro 15-4513 binding (and thus of α6βγ2 receptors) was similar in the littermate control and TASK-1 KO cerebellar granule cell layer (Fig. 6A, Table 1). We found a significant sex effect in [3H]Ro 15-4513 binding to the cerebellar granule cell layer (F<sub>1,22</sub> = 9.52, p < 0.01, two-way ANOVA); binding in females being lower than that in males (females: wildtype 35 ± 3 and KO 33 ± 1 nCi/mg vs. males: wildtype 42 ± 2 and KO 38 ± 3 nCi/mg; mean ± standard error, n = 6). Because no other sex effect was found we pooled the binding results obtained from male and female brain sections (Table 1).

[3H]Muscimol binding to TASK-1 KO and control littermate brain sections was similar (Table 1), suggesting no change in the levels of α4βδ and α6βδ receptors. Brain sections from both TASK-1 KO and control mice bound rather similarly [35S]TBPS in all brain regions examined (Table 1). In the cerebellar granule cells, TASK-1 KO brains showed slightly reduced basal binding (Fig. 6B and Table 1). We also analyzed the basal [35S]TBPS binding to thoracic spinal cord sections (Table 1). No difference between the genotypes was found. Finally, we analyzed the proportion of “GABA-insensitive”
[35S]TBPS binding in the thalamus and cerebellar granule cell layer of TASK-1 KO and wildtype mice, but found no difference between the genotypes (Table 1).

We were particularly interested in the reticular nucleus of the thalamus because TASK-1 is highly expressed there (Linden et al., 2006). The location of this thin neuronal layer was first determined by comparing basal and GABA-insensitive [35S]TBPS binding: high concentrations of GABA (1 mM) fully displaced [35S]TBPS from this nucleus, but not from thalamic nuclei medial to it. The basal level of [35S]TBPS binding in the thalamic reticular nucleus did not differ between genotypes (KO 291 ± 13 vs. wildtype 269 ± 13 nCi/g, mean ± standard error, n = 9-10, Student’s t-test). The level of [3H]Ro 15-4513 binding was also similar in the reticular thalamus of TASK-1 KO and control mice (KO 7.6 ± 0.4 vs. wildtype 7.3 ± 0.4 nCi/mg, mean ± standard error, n = 12).
Discussion

This study shows that the motor-impairing actions of the non-selective benzodiazepines, diazepam and flurazepam, and the hypnotic actions of propofol and pentobarbital are greater in TASK-1 KO mice than in their littermate controls. However, the sedative effects of diazepam were unaltered in TASK-1 KO mice. The enhanced effects of benzodiazepines were blocked by flumazenil (Ro15-788, a selective antagonist of the benzodiazepine binding site on GABA<sub>A</sub> receptors), confirming that the effects are mediated via the benzodiazepine site of GABA<sub>A</sub> receptors (αβγ2-type receptors). Only minor enhancement was observed in the TASK-3 KO mice (this study; but see Linden et al., 2007 for unchanged propofol effect on LORR in TASK-3 KO mice). Although there were enhanced behavioral changes in the TASK-1 KOs to GABA<sub>A</sub> receptor ligands, the amounts of GABA<sub>A</sub> receptor subtypes examined using autoradiography were unchanged in all brain areas examined (except for a small decrease in [35S]TBPS binding over the cerebellar granule cell layer). Thus the upregulation in GABA<sub>A</sub> receptor function in the TASK-1 KO mice likely reflects a functional modification of the receptors in an unknown brain region (e.g. altered phosphorylation or amounts of receptor on the cell surface). A precedent comes from analysis of changed GABA<sub>A</sub> receptor function in protein kinase C (PKC) ε KO mice (Hodge et al., 1999). PKCε KO mice have a strongly changed function of their GABA<sub>A</sub> receptors as assessed by behavioral experiments with GABA<sub>A</sub> receptor ligands, but unchanged GABA<sub>A</sub> receptor protein levels. A decreased phosphorylation of the γ2 subunit might explain these functional changes in PKCε KO mice (Qi et al., 2007).
The enhanced sensitivity of TASK-1 KO mice to the benzodiazepine diazepam (5 mg/kg) was observed only in the rotarod and beam walking motor-coordination tests. Enhancement was not observed when sedation or anxiolysis was analyzed after doses of 3 mg/kg and 1 mg/kg of diazepam, respectively, suggesting that sedation and anxiolysis produced by diazepam are mediated by such brain structures/receptors which are not affected by TASK-1 deletion. In fact, in the elevated-plus maze anxiety test, diazepam significantly increased the open arm activity only in the wildtype mice, not in the TASK-1 KO mice. It is also possible that the increased sensitivity of TASK-1 KO mice is seen only when relatively high doses of GABAergic drugs are used and that the enhancement in TASK-1 KO mice requires doses above a certain threshold. However, this hypothesis remains to be tested in dose-response experiments of e.g. diazepam’s effects on motor-coordination versus locomotor activity. Nevertheless, the increased sensitivity of TASK-1 KO mice was also found when high doses of propofol and pentobarbital were used to analyze their hypnotic properties supporting the idea that the increased sensitivity of TASK-1 KO mice to GABAergic drugs may require strong pharmacological enhancement of GABA_A receptor function. In which brain region(s) does this change in GABA_A receptor function of TASK-1 KO mice take place and what receptor subtypes are these likely to be? The increased propofol sensitivity of TASK-1 KO mice with respect to hypnosis implicates mostly the β3 subunits (Jurd et al., 2003). Pentobarbital-induced LORR is increased in the TASK-1 KO mice, and this effect is also likely to partially require β3-containing GABA_A receptors (Zeller et al., 2007). The changed effects of diazepam, but not zolpidem, on motor performance suggest the involvement of α2β2/3γ2, α3β2/3γ2, or α5β2/3γ2.
receptors (Rudolph and Möhler, 2004). However, the involvement of α1β2/3γ2 receptors remains uncertain because the profound hypnotic activity of zolpidem may have confounded its possible motor-impairing effects in the TASK-1 KO mice. In those regions where TASK-1 is expressed at very low levels (e.g. cortical regions, hippocampus and amygdala) (Wisden et al., 1992; Talley et al., 2001, Linden et al., 2006), these receptors are less likely to be affected. More probable targets for TASK-1 KO-induced alterations are regions responsible for motor control because TASK-1 is strongly expressed in many key motor regions (cerebellum, brain stem motor nuclei and spinal cord) and the sensitivity of TASK-1 KO mice to GABAergic drugs was especially clear in motor tests.

The fact that we did not see similar changes in the GABA<sub>A</sub> receptor system of the TASK-3 KOs might also indicate that the altered GABA<sub>A</sub> receptor function happens in a place where only TASK-1 is used (so that TASK-3 does not compensate in a TASK-1 knockout; i.e. a TASK-1/TASK-3 heteromer becomes a TASK-3 homomer). This explanation might rule out the cerebellar granule cells (which express α1, α6, β2, β3, γ2, TASK-1 and -3 genes) and spinal cord motor neurons (which express α2, β3, γ2, TASK-1 and -3 genes) as the region of action (Wisden et al., 1991; 1992; Laurie et al., 1992; Talley et al., 2001; Berg et al., 2004; Linden et al., 2006). A good candidate locus would be the reticular thalamic nucleus that has abundant expression of TASK-1, but little of TASK-3, and uses mainly α3β3γ2 GABA<sub>A</sub> receptors (Wisden et al., 1992; Talley et al., 2001; Linden et al., 2006). The GABA<sub>A</sub> receptor changes, if any, might again be functional because the ligand binding levels to GABA<sub>A</sub> receptors in this nucleus were not affected in TASK-1 KO mice.
At concentrations used clinically, inhalational anesthetics activate recombinant TASK-1 and -3 channels (Patel et al., 1999; Sirois et al., 2000; Franks, 2008). But in both TASK-1 and -3 KO mice, there was only a relatively mild reduction in potencies of inhalational anesthetics (Linden et al., 2006; 2007); on the other hand, genetic removal of another K2P gene family member, TREK-1, produced a greater decrease in sensitivity of mice to inhalational anesthetics and no change in pentobarbital’s ability to induce and maintain LORR (Heurteaux et al., 2004). Some believe that rather than acting in many brain regions, anesthetic agents produce many of their effects by acting on a restricted number of brain nuclei (Franks, 2008). Thus in contrast to TREK-1, maybe the TASK-1 and -3 genes are not expressed, or not expressed enough to make a difference, in those (unknown) neurons relevant for inhalational anesthetic action. However, inhalational anesthetics also act on GABA\textsubscript{A} receptors (Mihic et al., 1997). Given that these receptors seem functionally upregulated in TASK-1 KO brains, this could explain the smaller than expected reductions in sensitivity to inhalation anesthetics in the TASK-1 KO mice (Linden et al., 2006). Thus, it could still be that TASK-1 channels are important anesthetic targets \textit{in vivo}, but that the genetic deletion has fogged the effects. TASK-3 KO mice may have developed another independent compensatory mechanism.

TASK channel deletions in mice often produce sex-dependent effects (Linden et al., 2006; 2007; Heitzmann et al., 2008). In addition to diazepam’s effects, other drugs binding to GABA\textsubscript{A} receptors, such as propofol, gaboxadol and pregnanolone also produced increased ataxic actions in the TASK-1 KO mice. However, these actions were seen only in either males or females [compared with same sex-littermates without knowing the phase of the estrus cycle in females (see Maguire et al., 2005)] suggesting
sex-dependent regulation of GABA$_A$ receptors interacting with the changes produced by the TASK-1 deletion. Gaboxadol’s effects are abolished in mice with no $\alpha_4\beta_3\delta$ GABA$_A$ receptors (Chandra et al., 2006). By sensing ambient GABA, these receptors specialize in producing tonic inhibition at extrasynaptic sites in places such as the thalamus, neocortex and caudate-putamen (Wisden et al., 1992; Farrant and Nusser, 2005). Thus, extrasynaptic GABA$_A$ receptors might be functionally upregulated specifically in female mice, even though the autoradiography with [3H]muscimol indicated no change in $\alpha_4\beta_3\delta$ receptor levels in the brain samples between TASK-1 KO and control mice nor between males and females with an undetermined phase of estrus cycle. Other factors to consider are that TASK-1 KO female mice have higher than normal blood levels of aldosterone, but unaltered corticosterone levels (Heitzmann et al., 2008). For the adrenal gland, only adult female TASK-1 KO mice are affected (Heitzmann et al., 2008); the males recover after puberty. It remains to be studied whether other adrenal steroids are elevated in the TASK-1 female mice. Especially interesting is deoxycorticosterone, a precursor for both aldosterone and 5-$\alpha$-tetrahydrodeoxycorticosterone (THDOC); the latter is a positive modulator of GABA$_A$ receptors (Belelli and Lambert, 2005).

We cannot say if the changes in the GABA$_A$ receptor system in TASK-1 KO mice are truly adaptive/compensatory. But we can conclude that the genetic removal of TASK-1 K$^+$ currents increases the functional impact of GABA$_A$ receptor activation. Our results highlight the plasticity in GABA$_A$ receptor function that can potentially cope with adverse brain effects. It also seems possible that such changes in the GABA$_A$ receptor system might mask the full effects of the TASK-1 genetic deletion in mice.
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References


Footnotes

These authors contributed equally to this work (A.M.L., M.I.A.).

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Legends for figures

Figure 1. TASK-1 KO mice exhibit enhanced sensitivity to flurazepam and diazepam, but not to zolpidem in rotarod and beam walking tests. A. TASK-1 KO (-/-) mice were more sensitive to the ataxic actions of flurazepam (5 + 15 + 25 mg/kg, i.p.) than the littermate control (+/+) mice (n = 17-18). B. TASK-1 KO mice were more sensitive to diazepam (5 mg/kg, i.p.) than the littermate control mice. Pre-treatment with flumazenil (15 mg/kg, s.c.) completely blocked the effects of diazepam. The mice were tested on the rotarod and beam after vehicle and at indicated time points after diazepam (n = 7-9). C. No significant difference was observed in sensitivities to zolpidem (1 + 2 + 6 mg/kg, i.p., n = 16-18). Cumulative doses of flurazepam and zolpidem were given at 30-min intervals and mice were tested on the rotarod 15 min and on the 1.2-cm-thick beam 20 min after each dose. The rotating rod was accelerated from 5 to 30 rpm during 3 min. Data from males and females were combined because no sex difference was observed in repeated measures two-way ANOVA. Data are means ± standard error. *** p < 0.001, ** p < 0.01, * p < 0.05, genotype difference; ### p < 0.001, ## p < 0.01, # p < 0.05, difference between flumazenil- and vehicle-pretreated mice within the same genotype; Newman-Keuls post hoc test.

Figure 2. Flurazepam sensitivity in TASK-3 KO mice. TASK-3 KO (-/-) mice were similarly sensitive to the ataxic effects of flurazepam administered with cumulative doses (5 + 15 + 25 mg/kg, i.p.) as the littermate control (+/+) mice in the rotarod test. In the walking beam test, the TASK-3 KO mice showed a slightly increased sensitivity to flurazepam. Cumulative doses were given at 30 min intervals. Pretrained mice were
tested on the rotating rod 15 min and on the 1.2-cm-thick beam 20 min after each dose. Data from males and females were combined because no sex difference was observed in repeated measures ANOVA. Data are means ± standard error, n = 17-18. *** p < 0.001, * p < 0.05 genotype difference, Newman-Keuls post hoc test.

**Figure 3.** Anxiolytic and sedative effects of diazepam in the TASK-1 KO (-/-) and littermate control (+/+). A. Behavior in the elevated plus maze test 30 min after diazepam injection (1 mg/kg, s.c.). B. Locomotor activity in the open arena 30 min after diazepam (3 mg/kg, s.c.). Data from males and females were combined after testing in three-way ANOVA which showed no sex effect. Data are means ± standard error, n = 6-9. Open bars denote vehicle-treated mice and hatched bars diazepam-treated mice. * p < 0.05, Mann-Whitney test.

**Figure 4.** Motor-impairing effects of pregnanolone, gaboxadol, propofol and pentobarbital on the rotarod performance in the TASK-1 KO (-/-) and littermate control (+/+). Male and female mice. Cumulative dosing was used for pregnanolone (10 + 10 + 10 mg/kg, i.p.), gaboxadol (6 + 3 + 6 mg/kg, i.p.), propofol (30 + 30 + 40 mg/kg, i.p.) and pentobarbital (15 + 15 + 45 mg/kg, i.p.) at 15-45 min intervals. Motor testing on the rotarod was performed 10-30 min after each dose. Data are means ± standard error, n = 6-10. ** p < 0.001, * p < 0.05 genotype difference, Newman-Keuls post hoc test.

**Figure 5.** The duration of loss of righting reflex (LORR) induced by propofol (200 mg/kg, i.p.) and pentobarbital (45 mg/kg, i.p.) in TASK-1 KO mice (-/-) and littermate
control mice (+/+). LORR was measured every 4\textsuperscript{th} minute. Data from males and females were combined because no sex difference was observed in two-way ANOVA. Data are means ± standard error, n = 15-18. * p < 0.05, Student’s t-test.

**Figure 6.** GABA\textsubscript{A} receptor ligand binding to TASK-1 KO (-/-) and littermate control (+/+) brain sections. **A.** Representative autoradiographs of total and diazepam-insensitive [$^{3}\text{H}$]Ro 15-4513 binding to the TASK-1 KO mouse forebrain and cerebellum. No alterations were found between the KO and wildtype mice (see Table 1). **B.** Representative autoradiographs of basal [$^{35}\text{S}$]TBPS binding. Binding was decreased in the granule cell layer of the cerebellum in the TASK-1 KO mice compared with the littermate control mice (see Table 1). All autoradiographs shown are from male mice. Ctx, cortex; Gr, granule cell layer of the cerebellum; Hi, hippocampus; Th, thalamus.
**Tables**

**Table 1. Quantitative autoradiography of GABA<sub>A</sub> receptor ligand binding in TASK-1 KO (-/-) and wildtype (+/+) littermate brain and spinal cord sections.**

<table>
<thead>
<tr>
<th>Region</th>
<th>+/+</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Ro 15-4513, nCi/mg (all γ2 subunit-containing receptors, αβγ2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>58 ± 3</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>55 ± 2</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>Thalamus</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>Cerebellar granule cell layer</td>
<td>39 ± 2</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Spinal cord, ventral horn</td>
<td>31 ± 1</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>Spinal cord, dorsal horn</td>
<td>78 ± 1</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Diazepam-insensitive [&lt;sup&gt;3&lt;/sup&gt;H]Ro 15-4513, nCi/mg (α6βγ2 receptors)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar granule cell layer</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Muscimol, nCi/mg (α4βδ and α6βδ receptors)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>14 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Thalamus</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>Cerebellar granule cell layer</td>
<td>80 ± 5</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>[&lt;sup&gt;35&lt;/sup&gt;S]TBPS, nCi/g (many GABA&lt;sub&gt;A&lt;/sub&gt; receptor subtypes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>259 ± 9</td>
<td>270 ± 11</td>
</tr>
</tbody>
</table>
Binding in the brain was analyzed from the cortical sections obtained approximately at the levels shown in Fig. 6. Cortex, all layers of the somatosensory cortex; Hippocampus, all layers of the dentate gyrus and CA1, CA2 and CA3 subfields; Thalamus, including midline and intralaminar nuclei, mediodorsal, laterodorsal, ventrolateral, ventromedial, ventral posterolateral/medial and posterior nuclei.* p < 0.05, Student’s t-test; n = 11-12. Data are means ± standard error.
Fig. 1

A

Flurazepam

Rotarod

Latency to fall (s)

Time to cross (s)

Number of falls

Dose (mg/kg)

B

Diazepam + flumazenil

Rotarod

Latency to fall (s)

Time to cross (s)

Number of falls

Time (min)

C

Zolpidem

Rotarod

Latency to fall (s)

Time to cross (s)

Number of falls

Dose (mg/kg)
Flurazepam

Rotarod

Latency to fall (s)

- +/+  
- TASK-3 -/-

Dose (mg/kg)
sal 5 20 45

Beam

Time to cross (s)

Dose (mg/kg)
sal 5 20 45

Number of falls

Dose (mg/kg)
sal 5 20 45

Fig. 2
[\textsuperscript{3}H]Ro 15-4513 binding
Total (all $\alpha\beta\gamma_2$ receptors)

Cctx

Hi

Th

Diazepam-insensitive ($\alpha6\beta\gamma_2$ receptors)

Gr

[\textsuperscript{35}S]TBPS binding
Basal (many GABA_\text{A} subtypes)

+/-

-/-

Gr

Fig 6