

Supplementary Materials and Methods

**Primary behavioral testings.** Undisturbed normal behavior: Each animal was placed in a glass viewing jar of 17 cm height and 21 cm diameter for 5 min. On the bottom of the jar a sheet of white absorbing paper was placed. The jar was placed in a room with red lights. The spontaneous behaviors were recorded without disturbing the animal and the amount of urination or defecation was recorded at the end of the observation period. Incidents of bizarre or stereotyped behavior and convulsions were recorded during this period. Scored items: Agressivity (when the animal was handled); Convulsions (when the animal was handled); Body position; Spontaneous activity; Respiration rate; Tremor; Stereotypies.

**Spontaneous motor activity.** Afterwards, each animal was transferred from the viewing jar to a Plexiglas (52 x 52 x 40 cm) open-field which floor is divided into 9 equal squares. The open-field was placed in a dark room with red light. The stop watch was started and the immediate reaction to the new environment was recorded. Scored items: Transfer arousal; Palpebral closure; Locomotor activity; Tail elevation; Touch escape; Startle response; Positional passivity

**Reflexes and autonomic functions.** The animal was removed from the open-field and gripped tail between the thumb and the forefinger. Scored items: Visual placing; Grip strength; Body tone; Pinna reflex; Corneal reflex; Righting reflex; Skin color. Locomotor activity in Open Field: A video track was placed over a plexiglass open field (length 52 cm x 52 cm; height 40 cm) and recorded the activity of the animal during 10 min. The floor of the open-field was divided into 9 equal squares. The locomotor activity was expressed in terms of mean number of crossed squares per min.

**Light/dark boxes test.** The apparatus consisted of two polyvinylcarbonate boxes (20 x 20 x 14 cm) covered with Plexiglas. One of these boxes was darkened. Light from a 100 W desk lamp illuminated the other box (about 4’400 Lux). An opaque
plastic tunnel (5 x 7 x 10 cm) separated the dark box from the illuminated one. The animals were placed individually in the lit box, with head directed towards the tunnel. The time spent in the lit box (TLB) and the number of transitions between the two boxes were recorded over a 5-min period after the first entry of the animal in the dark box. Object recognition test: The object recognition task was performed in a Plexiglas (52 x 52 x 40 cm) open-field. The floor was divided into 9 equal squares. The open-field was placed in a dark room. The objects to be discriminated were a marble and a dice. The animals were first accustomed to the open-field for 30 min. The next day, they were submitted to a 10-min acquisition trial (first trial) during which they were individually placed in the open-field in presence of an object A (marble or dice). The locomotor activity (reflected by the number of squares crossed) and the time taken by the animal to explore object A (when the animal’s snout was directed towards the object at a distance ± 1 cm) were recorded. A 10-min retention trial (second trial) was carried out 3 h later. For this trial, the object A and another object B were placed in the open-field, the locomotor activity and the times tA (for exploration of object A) and tB (for exploration of object B) were recorded. A recognition index (RI) defined as \([tB/(tA + tB)] \times 100\) was calculated. Recognition is considered to correlate with a RI significantly higher than 50 %. Data analysis: a global analysis of the data was performed using a Student’s \(t\) test or a Mann-Whitney test. The level of significance was set at \(P< 0.05\). Results are expressed as mean ± SEM.

**Generation of TRAAK-deficient mice**

TRAAK genomic clones were isolated from a 129 mouse BAC genomic library by using a TRAAK cDNA probe and subcloned into pBluescript SK (Stratagene). Clones spanning the KCNK4 gene locus were sequenced and mapped to design the targeting vector and PCR primers. The targeting vector contained about 9 kb of
genomic homology dispatched into a 3 and 6 kb PCR amplified fragments upstream and downstream of exon 2 respectively (5’ arm primers: AGCCAGGTCTACAAGGTCTC / CTGGTGCTGCTTTACTTGGT; 3’ arm primers: TGTGTGAGCCAGAAGAGCCT / GGATTGCTGCGAGTTTAGAC). Gene disruption was achieved by replacement of 100 bp of exon 2, encoding most of the TM1 domain of the channel, by the IRES-geo cassette. The cassette insertion was located 54 bp after the start codon of the TRAAK protein coding sequence. The presence of stop codons in the three reading frames upstream the IRES-geo cassette sequence, allowed interruption of subsequent translation of mRNA of the native protein, 18 amino acids after beginning. The PGK-neomycin resistance gene contained in the IRES-geo cassette and a copy of the Diphteric Toxin subcloned adjacent to the homologous region allowed positive/negative selection of the ES clone.
**Supplementary Data: Figures 1 and 2**

**FIGURE 1. Disruption of the KCNK4 gene.** (A), Targeting vector and mutated locus after homologous recombination. External probes used to characterize homologous recombination are designated as P1 and P2. Arrowheads (1, 2 and 3, 4) display locations of the couple of primers used for PCR analysis of the different products. Double headed arrows indicate expected size of restriction fragments for Southern blot analysis (b = BamH1; e = EcoR1; n = Not 1). (B), Southern blot analysis of BamH1 and EcoR1 digested tail DNA from wild-type (+/+), heterozygous (+/-) or homozygous (-/-) KCNK4 mice probed with P1 and P2, respectively. (C), Western Blot of the TRAAK protein (58 kDa) in brain from +/+, +/- and -/- mouse brain homogenates using specific anti-TRAAK antibody (Lauritzen et al, 2000).

**FIGURE 2. Stimulation of TREK-1 channel activity by halothane and sevoflurane in human TREK-1-transfected COS cells (A and B) and Effects of isoflurane on LORR and MAC in Trek1+/+ and Trek1-/- mice (C).** (A) and (B), Effect of 2 mM Halothane (A) or Sevoflurane (B) on the I-V curve of TREK-1 in whole-cell configuration. I-V curve in steady-state control condition and after a 5 min application of Halothane or Sevoflurane (2.0 mM). I-V curve was elicited by a voltage ramp (1s duration from -130 mV to +100 mV). (C), Loss of righting reflex (LORR) measurements after inhalation of isoflurane. Latency to LORR is defined as the period of time (s) from inhalation to the loss of the righting reflex. Concentration for LORR (atm %) corresponds to average concentrations of isoflurane for the recovery from loss of the righting reflex. Data represent mean ± SEM (n=20 per genotype). Statistical significance (Student’s t test) ** when P<0.0001. Logistic regression probability of no movement fitted for isoflurane concentrations. The inspired MAC (minimum alveolar anesthetic concentration: 50% probability of no movement to tail-clamp reflex) and its 95% confidence interval (horizontal line) are shown on the graph.
FIGURE 1

A

Native Locus

14 kb
8.4 kb

1 kb

Targeting Vector

n

atg

b

DTA

pBSK

Mutated Locus

3.6 kb

8.1 kb

P1

P2

B

C

+/-  +/-  -/-

8.4 kb

3.6 kb

BamH1 / P1

+/-  +/-  -/-

14 kb

8.1 kb

EcoR1 / P2

+/-  +/-  -/-

62

47.5