**Supplementary Information**

## Supplementary Experimental Procedures

### Mice

*Scn1a* mutant mice were generated by targeted deletion of the last exon encoding domain IV from the S3 to S6 segment and the entire C-terminal tail of $Na_V1.1$ channels as described previously[1]. Mutant mice were generated on a congenic 129/SvJ background and backcrossed to C57BL6/J background to at least the F10 generation. The animals used in this study were generated by crossing heterozygous mutant males of C57BL/6J background with WT C57BL/6J females; from this cross wildtype and heterozygote mice were born in 1:1 ratio. Mice were genotyped as described previously[2]. Floxed *Scn1a* mice were generated by replacing the endogenous exon-25 of *Scn1a* with a targeting vector containing the exon flanked by LoxP sites and an FRT flanked neomycin-selection cassette, which was removed prior to experiments. Floxed *Scn1a* homozygous (F/F) and heterozygous (F/+) mice maintained on a C57BL/6J background were indistinguishable from WT littermates. In the Dlx1/2-I12b-Cre transgenic mouse, an intergenic Dlx1 and Dlx2 enhancer drives expression of Cre recombinase specifically in forebrain GABAergic neurons, including interneurons in cerebral cortex and hippocampus. All behavioral tests were done with age-matched littermate pairs of male mice, aged 6 to 10 months. All experiments with animals were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of Washington Institutional Animal Care and Use Committee.

### Immunocytochemistry

An antibody that specifically recognizes the α1 subunits of Type I sodium channels (anti-Nav1.1, rabbit polyclonal) was used in this study to detect differences in expression levels in WT and $Scn1a^{+/-}$ mice.

This antibody was generated against residues 465-481 of Nav1.1. The generation, purification, and characterization of these antibodies have been reported previously[3]. An anti-GABA (gamma amino butyric acid, guinea pig polyclonal) antibody was obtained from Abcam (Cambridge, MA) and used as a marker for GABAergic interneurons.

Adult mice were anesthetized with isoflurane and intracardially perfused with 4% paraformaldehyde and the brain was removed immediately. The tissue was postfixed for 2 h, successively placed in 10% and 30% sucrose in 0.1 M phosphate buffer (PB) overnight for each sucrose solution and cut on a sliding microtome (40 μm) and stored in 0.1 M PB containing 0.02% sodium azide. Free-floating sections were then processed for immunocytochemistry. Briefly, the tissue was rinsed in 0.1 M Tris buffer (TB) for 15 min, and rinsed in 0.1 M Tris buffered saline (TBS) for 15 min. For double labeling, the tissue was then incubated in affinity purified anti-Nav1.1 antibody (diluted 1:150) and anti-GABA (diluted 1:600) in TBS containing 0.1% Triton X-100 and 1% normal goat serum for 36 hrs at 4$^{o}$C. The tissue was then rinsed in TBS for 1 h, incubated in anti-rabbit IgG tagged with Alexa 488 (diluted 1:400, Invitrogen) and anti-guinea pig IgG tagged with Alexa 555 (diluted 1:400, Invitrogen) for 3 h at 37$^{o}$C. The tissue was finally rinsed in TBS for 10 min, rinsed in TB for 20 min, mounted on gelatin subbed slides, cover slipped with Vectashield (Vector), sealed with nail polish and viewed under the microscope. For controls, the primary antibody was omitted, replaced with normal rabbit serum, or preincubated in control peptide (15-20 μM) overnight at 4$^{o}$C before being applied to the tissue sections.

Following completion of staining, digital images were collected on a Leica SL confocal microscope located in the Keck Imaging Facility at the University of Washington. For quantification purposes, tissue slices from 3 WT and 3 *Scn1a*$^{+/-}$ mice were processed for immunocytochemistry simultaneously and imaged in the same confocal session using the same gain, offset, and laser intensity. A one-in-ten series of sections was analyzed from each animal. The digital images were opened in Photoshop 7 and blind cell counts were made to determine the number of cells double-labeled for Nav1.1 and GABA from various

regions of the brain. Intensity of cell staining was analyzed using the Region-of-Interest function in the Igor Pro software (Wavemetrics).

**Open-field test**

Open field test was performed as previously described[4]. Briefly, each individual mouse was placed near the wall-side of 38 x 42 cm open field arena, and the movement of the mouse was recorded by USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology) for 10 min. The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0, Noldus Technology). Total distance traveled and time in center (15 x 15 cm imaginary square) were measured. The open field arena was cleaned with 70 % ethanol and wiped with paper towels between each trial. All data shown are means ± s.e.m. and analyzed using Student's two-tailed, unpaired t-test.

**Stereotyped behavior**

During a 10-min open field test period, the amount of time spent grooming was measured manually, assisted by the manual scoring function in the video tracking software (Ethovision XT 7.0, Noldus Technology). The observer was blind to the genotype. Circling behavior was scored automatically by the video tracking software (EthoVision XT 7.0, Noldus Technology). A complete 360-degree turn of nose angle with respect to the body center was counted as one circling event. All data shown are means ± s.e.m. and analyzed using Student's two-tailed, unpaired t-test.

**Nesting behavior**

Single-housed mice were transferred into a new cage with nest-building material, a 5 x 5 cm square of white compressed cotton pads (Nestlets TM; Ancare, Bellmore, NY) in a random corner. After 6, 24, and 48 h, nest building was scored on a scale of 0-3, as previously described[5]. All data shown are means ± s.e.m. and analyzed using two-way ANOVA, with time and genotype as levels, with Bonferroni's post hoc comparisons.

**Elevated plus maze test**

The elevated plus maze is a plus-shaped maze that is elevated 60 cm above the floor. It consists of two closed arms surrounded by 15-cm high transparent walls and two open arms (5 x 25 cm) with a small ledge along the side of two open arms to prevent falling from the maze during the test. Each mouse was placed in the center (5 x 5 cm) of the maze facing one of the closed arms. During the 10-min test period, the movement of the mouse was recorded by a USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology). The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0). Times spent in closed arms, center, and open arms were measured. The maze was cleaned with 70 % ethanol and wiped with paper towels between each trial. All data shown are means ± s.e.m. and analyzed using Student's two-tailed, unpaired t-test.

**Three-chamber test**

The test was performed as described previously[6] with minor modifications. The three-chamber apparatus is a non-transparent plexiglass box (25 x 50 cm) with two transparent partitions that make left, center, and right chambers (25 x 16.7 cm). Each partition has a square opening (5 x 5 cm) in the bottom center. A

cylindrical wire cage (10.5 cm diameter; Galaxy Pencil Cup, Spectrum Diversified Designs) was used as an inanimate object or the cage housing a stranger mouse. A cylindrical bottle filled with water was placed on the top of the wire cup to prevent the test mouse from climbing to the top of the cup. The three-chamber unit and wire cups were cleaned with 70% ethanol and wiped with paper towels between each trail. In the first 10-min session, a test mouse was placed in the center of the three-chamber unit, where two empty wire cages were located in the left and right chambers to habituate the test mouse. The mouse was allowed to freely explore each chamber. In the second 10-min session, an age- and gender-matched C57BL/6J mouse (M1) that had never been exposed to the test mouse, was placed in one of the two wire cages. The wire cage on the other side remained empty (E). Then, the test mouse was placed in the center, and allowed to freely explore the chamber for 10 min. The test mouse was removed and in the last 10-min session, a second age- and gender-matched C57BL/6J stranger mouse (M2) that had never been exposed to the test mouse, was placed in one wire cage, which previously served as a the empty cage. Thus, the test mouse would now have the choice between a mouse that was already familiar (M1) and a new stranger mouse (M2). The test mouse was placed in the center, and allowed to freely explore the chamber for 10 min. The movement of the mouse was recorded by a USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology). The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0). Time spent in each chamber, and time spent within a cm radius proximal to each wire cage were measured. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis and one-way ANOVA with Tukey's post hoc analysis.

**Social interaction test**

The social interaction test was performed as described previously[7] with minor modifications. The test was performed in the open field arena that was used for the open field test. An age- and gender-matched

C57BL/6J mouse (M) caged in a rectangular wire mesh cage (6 x 6 x 10 cm), was used as a social cue. The stranger mouse had never been exposed to the test mouse. The test mouse was placed in the open field arena with an empty wire cage for 10 min for habituation. Following the 10 min session, a stranger mouse was placed in the same cage and the test mouse was allowed to explore the arena for another 10 min. The empty cage or caged stranger mouse was placed in the center of one quadrant of the arena, and immobilized on the floor of the arena with double-sided tape. For the social choice test, an inanimate object and the caged stranger mouse were placed simultaneously in the opposite side of the quadrant of the arena, then the test mouse was allowed to choose either an inanimate object or the caged stranger mouse. The movement of the mouse was recorded with a USB webcam (LifeCam HD-6000, Microsoft) and PC-based video-capture software (WinAVI Video Capture, ZJMedia Digital Technology). The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0). Time spent in the cage-containing quadrant, and time spent in the area 5 cm proximal to the cage were measured. Immobilization behavior was also measured by video tracking software. Immobilization was defined as the time when the mean velocity of a mouse was continuously less than 1 cm/s during a 10-s interval. The open field arena and the cage were cleaned with 70 % ethanol and wiped with paper towels between each trial. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis.

**Reciprocal interaction test**

A test mouse and an age- and gender-matched stimulus mouse that was marked on the tail using a black permanent marker were introduced in a neutral cage with fresh bedding. The cage was used only once. Mice were socially naïve with each other. The social interactions of mice were recorded by USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology) for 10 min. Time spent in aggressive interactions, such as attacking, wrestling, and

biting the dorsal surface, and time spent in non-aggressive interactions, such as nose-to-nose sniffing, anogenital sniffing, and grooming were measured manually using the event-recording function in the video-tracking software by a researcher who was blind to the genotype of test mice. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis.

**Olfactory discrimination tests**

The olfactory discrimination ability of mice was examined with the modified three-chamber test. Instead of wire cage or caged mouse used for social preference test, tightly sealed petri dishes containing food pellets were used as non-social olfactory cues. A dish with holes to release food odor was placed in one chamber, and another dish with no holes was placed in the other chamber. Alternatively, the bedding from 3-day-used male or female cages was used as a social odor instead of food pellets, and clean bedding as control, to examine the ability of mice to discriminate social odor. Fox urine was also used as a control, aversive social odor. The three-chamber and wire cups were cleaned with 70 % ethanol and wiped with paper towels between each trial. A test mouse was placed in the center chamber, and allowed to explore the chambers for 10 min. Alternatively, a Y-maze was also used for olfactory choice test. The Y-maze is a Y-shaped maze composed of 3 equal-sized transparent plexiglas arms (30 x 20 x 10 cm) with removal gates in each entry. Two cotton tipped swabs, one with odor and the other without odor, were placed at the end of the right and left arms, and the test mouse was placed in the central arm. During a 5-min trial, the test mouse was only allowed to explore the right and left arms by closing the gate of the central arm. The movement of the mouse was recorded by USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology). The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0, Noldus Technology). Time spent in each chamber, time spent in the area 5 cm proximal to each wire cage, the number of entries in each chamber, and the latency to access each chamber were measured. For the Y-maze olfactory

choice test, time spent in each arm was measured. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis and one-way ANOVA with Tukey's post hoc analysis.

**Olfactory habituation/dishabituation test**

The test was performed as described previously[8] with minor modifications. All the tests were done in a home cage, where the test mouse was singly housed. Odor stimulants were delivered with a cotton tipped swab, which was located in the center of the cage, 7 cm above the bedding through a hole in the cage top. After 30 min of habituation by applying a cotton tipped swab without odor stimulant, the test mouse was stimulated by serial application of odorants: Water, Banana flavor 1:100 diluent (Kroger, Cincinnati, OH), C57BL/6J male urine 1:100 diluent, and finely ground food pellets with each 2 min duration and 1 min inter-trial interval. The sequence of the odor stimulation was described as followed: Water1, Water2, Water3, Banana1, Banana2, Banana3, Urine 1, Urine 2, Urine 3,, Food1, Food2, Food3. Time spent sniffing the odorants were measured by manual observation with stopwatch. Sniffing was only scored when the test mouse's nose was closer than 1 cm with the swab. Digging behavior was also measured by manual observation of the test mouse in response to each odorant during each trial. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis and one-way ANOVA with Tukey's post hoc analysis.

**Novel object recognition test**

The arena used for the novel object recognition test was a rectangular cage (25 x 50 cm) covered with fresh bedding. The arena was used only once for one mouse. In the habituation session, a test mouse was placed in the arena and allowed to explore for 10 min. Following habituation, two objects of similar size,

but different shape and color, were placed in the opposite corners of the arena, 10 cm from the side walls, and then the test mouse was placed in the center of the arena, and allowed to explore the arena including the two novel objects for 10 min. After 24 h, one object was replaced with another novel object, which was of similar size but different shape and color than the previous object. Then, the same test mouse was placed in the center, and allowed to explore the arena and the two objects. The movement of mice was recorded by USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology) for 10 min. The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0, Noldus Technology). Time in each object area (10 x 10 cm) was measured. All data shown are means ± s.e.m. and analyzed using Student's two-tailed, paired t-test.

**Barnes circular maze test**

The Barnes circular maze is a circular planar white Plexiglas platform (92 cm diameter) 1 m elevated from the floor with 20 evenly-spaced holes (7 cm diameter), located 5 cm from the perimeter. A black escape box (15 x 7 x 7 cm) was placed under one hole. Spatial cues with distinct patterns and shapes were placed on the wall of the testing room. A 500 lux light was turned on during the trial. An experimenter was positioned in the same place with minimal movements throughout the trials. The platform and the escape box were cleaned thoroughly with 70% ethanol and paper towels between each trial to remove olfactory cues. One day before the training trials began, test mice were habituated in the target box for 3 min. The training trials were repeated for 4 consecutive days, and 3 trials were performed each day with 20 min inter-trial intervals. At the beginning of each trial, a test mouse, placed in the cylindrical holding chamber (10 cm diameter) was located in the center of the maze. After 10 s of holding time, a mouse was allowed to search for the target hole for 3 min. If the mouse failed to find the target hole in 3 min, it was gently guided into the target hole by the experimenter's hands. When the mouse entered the escape box,

the light was turned off and the mouse remained undisturbed for 1 min. The movement of the mouse was recorded, and the number of errors made and the latency to find the target hole were measured during the training trials by video tracking software. On the day 5, the probe trial was performed with each mouse. The escape box was removed during probe trials, and the test mouse was allowed to find the target hole freely for 90 s. During the probe trial, total moved distance and the latency to find the target hole were measured. % correct pokes and % time in the target area were also measured. All data shown are means ± s.e.m. and analyzed using two-way ANOVA with Bonferroni's post hoc analysis and Student's two-tailed, unpaired t-test.

**Contextual fear conditioning test**

The contextual fear conditioning chamber was a square arena (25 x 25 cm) with clear Plexiglas walls and the metal grid floor that consisted of a circuit board that delivers shocks to metal grids (Coulbourn Instruments). An analog camera, as a part of a whole fear conditioning system was attached on the top of the chamber. The camera and the circuit board were connected to a personal computer, and its software (Freeze Frame 2.0, Actimetrics) controlled the circuit and recorded the data. The chamber was cleaned with 70% ethanol and wiped with paper towels between each session. In the habituation session, a test mouse was placed in the chamber and allowed to explore for 2 min[9-12]. Immediately after habituation, the test mouse received single mild foot shock (2 s, 0.5 mA). After staying in the chamber one more min, the mouse was removed from the chamber. For the 30-min short-term memory test, the mouse was returned to the context 30 min after the end of the training session. For 24-hr long-term memory test, the mouse was returned to the context 24 hr after the training session. The movement of mice was recorded by a USB webcam (LifeCam HD-6000, Microsoft) and PC-based video capture software (WinAVI Video Capture, ZJMedia Digital Technology) for 2 min. The recorded video file was further analyzed by off-line video tracking software (EthoVision XT 7.0, Noldus Technology). The freezing scores were calculated by

dividing the test session into 1 min bouts and averaging together all 2 min for each animal. All data shown are means ± s.e.m. and analyzed using Student's two-tailed, unpaired t-test.

**Brain slice electrophysiology**

Hippocampal slices were prepared from P21–P25 mice using standard procedures modified from those previously described[13]. Briefly, mice were deeply anesthetized with isoflurane and decapitated. The brain was quickly removed and horizontal hippocampal slices (400 μm) were cut with a modified Vibratome (Pelco 101 series 1000; Ted Pella, Inc., Redding, CA) in chilled (0–4°C) slicing solution containing 75 mM sucrose, 87 mM NaCl, 25 mM $NaHCO_3$, 25 mM D-glucose, 2.5 mM KCl, 1.25 mM $NaH_2PO_4$, 0.5 mM $CaCl_2$, 7.0 mM $MgCl_2$, and pH 7.3. The slices were transferred to a storage chamber with fresh ACSF containing 126 mM NaCl, 2.5 mM KCl, 2.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 1.25 mM $NaH_2PO_4$, 26 mM NaHCO3, and 10 mM D-glucose, pH 7.3, and incubated at 37 $^oC$ for 45 min. The slices were then incubated at room temperature for at least another 45 min before recording. All solutions were saturated with 95% $O_2$ and 5% $CO_2$.

Whole-cell voltage-clamp recordings were performed on CA1 pyramidal neurons within hippocampal slices visualized under differential interference contrast (DIC) optics, and near infrared (bandpass 750–800 nm) illumination was used to identify individual neurons in a recording chamber located on an upright microscope (Axioskop; Zeiss, Oberkochen, Germany). Patch electrodes were pulled from 1.5 mm outer diameter thin-walled glass capillaries (150F-4; World Precision Instruments, Sarasota, FL) in three stages on a Flaming-Brown micropipette puller (model P-97; Sutter Instruments, Novato, CA). In the recordings of inhibitory postsynaptic currents (IPSCs), the patch electrodes were filled with intracellular solution containing CsCl (135 mM), *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES; 20 mM), ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA; 2 mM), Mg-ATP (2 mM), Na-GTP (0.5 mM), pH 7.25. Kynurenic acid (KA, 1 mM) was included in the

perfusion solutions to block the excitatory synaptic transmission in IPSC recordings. In the recordings of excitatory postsynaptic currents (EPSCs), CsCl was replaced by Cs-methanesulfonate. Where indicated, TTX (1 μM) was applied in the perfusion solutions to block action potentials and allow recording of miniature IPSCs and miniature EPSCs. When filled with intracellular solution, patch electrode resistance ranged from 3 to 5 MΩ. Recordings were obtained through a Multiclamp 700A amplifier (Axon) by the data-acquisition software (pCLAMP 8.0; Axon). Access resistance was continuously monitored for each cell. Only the cells with access resistance less than 20 MΩ were recorded, and recordings were terminated/discarded when a significant (>10%) increase occurred. Data from electrophysiology experiments were analyzed using Clampfit 9.0 (Axon) and Mini Analysis (Synaptosoft).

**Drug administration**

Clonazepam at indicated concentrations (0.0625 mg/kg ~ 0.5 mg/kg, Sigma) diluted in the vehicle solution (PBS with 0.5% Methylcellulose) was administered by an intraperitoneal injection in a volume of 0.01 ml/kg 30 min before the behavioral tests.

**Statistical analysis**

All data are shown as mean ± s.e.m. and analyzed using Student's t-test, one-way ANOVA with Tukey's post hoc comparison, and two-way ANOVA with Bonferroni's post hoc comparison. All the statistical analyses were done using Prism 4 (GraphPad).

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## Supplementary Table 1.

| WT vs *Scn1a*$^{+/-}$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test** | **# of Animals** | **Measurement** | **Treatment** | **Genotype** | **Average** | **s.e.m.** | **Statistical Test** | ***p* value** | ***post hoc* Test** | ***p* value** | **Figure** |
| Open Field | WT = 10<br>*Scn1a*$^{+/-}$ = 12 | Total Distance Traveled (cm) | - | WT | 3111.42 | 293.98 | Unpaired two-tailed t-test | < 0.01 | - | - | 1a, b |
| | | | | *Scn1a*$^{+/-}$ | 5010.84 | 466.80 | | | | | |
| | | Time in Center (s) | - | WT | 33.28 | 5.97 | Unpaired two-tailed t-test | < 0.01 | - | - | |
| | | | | *Scn1a*$^{+/-}$ | 12.90 | 3.22 | | | | | |
| Grooming | WT = 10<br>*Scn1a*$^{+/-}$ = 11 | Time spent Grooming (s) | - | WT | 27.78 | 4.01 | Unpaired two-tailed t-test | < 0.01 | - | - | 1e |
| | | | | *Scn1a*$^{+/-}$ | 78.83 | 16.27 | | | | | |
| Circling | WT = 9<br>*Scn1a*$^{+/-}$ = 8 | Total Circling Number | - | WT | 22.27 | 2.75 | Unpaired two-tailed t-test | < 0.001 | - | - | 1f |
| | | | | *Scn1a*$^{+/-}$ | 52.67 | 7.39 | | | | | |
| Elevated Plus Maze | WT = 10<br>*Scn1a*$^{+/-}$ = 10 | Number of entries | Closed | WT | 20.20 | 3.01 | Unpaired two-tailed t-test | n.s. | - | - | 1c, d |
| | | | | *Scn1a*$^{+/-}$ | 23.90 | 3.42 | | | | | |
| | | | Open | WT | 9.20 | 1.33 | Unpaired two-tailed t-test | < 0.05 | - | - | |
| | | | | *Scn1a*$^{+/-}$ | 5.30 | 0.94 | | | | | |
| | | Time in Open Arms (s) | - | WT | 67.91 | 9.94 | Unpaired two-tailed t-test | < 0.01 | - | - | |
| | | | | *Scn1a*$^{+/-}$ | 31.43 | 7.95 | | | | | |
| Nest Building | WT = 9<br>*Scn1a*$^{+/-}$ = 10 | Nest Building Score (0 - 3) | 0 h | WT | 0.00 | 0.00 | Two-way ANOVA | Genotype, Time, Interaction < 0.001 | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | S4 |
| | | | | *Scn1a*$^{+/-}$ | 0.00 | 0.00 | | | | | |
| | | | 6 hr | WT | 2.22 | 0.43 | | | | < 0.001 | |
| | | | | *Scn1a*$^{+/-}$ | 0.00 | 0.00 | | | | | |
| | | | 24 hr | WT | 2.89 | 0.11 | | | | < 0.001 | |
| | | | | *Scn1a*$^{+/-}$ | 1.60 | 0.27 | | | | | |
| | | | 48 hr | WT | 3.00 | 0.00 | | | | < 0.05 | |
| | | | | *Scn1a*$^{+/-}$ | 2.10 | 0.28 | | | | | |
| 3-Chamber | WT = 10<br>*Scn1a*$^{+/-}$ = 12 | Time in Chamber (s) - Habituation | Empty 1 | WT | 227.67 | 15.02 | Two-way ANOVA | n.s. | Bonferroni's (E1 v E2) | n.s. | S6 |
| | | | Center | WT | 143.38 | 13.50 | | | | | |
| | | | Empty 2 | WT | 237.42 | 18.28 | | | | | |
| | | | Empty 1 | *Scn1a*$^{+/-}$ | 269.60 | 20.07 | | | Bonferroni's (E1 v E2) | n.s. | |
| | | | Center | *Scn1a*$^{+/-}$ | 110.95 | 11.11 | | | | | |
| | | | Empty 2 | *Scn1a*$^{+/-}$ | 240.28 | 18.94 | | | | | |
| | | Time in Chamber (s) - Social Preference | Empty | WT | 168.62 | 14.37 | Two-way ANOVA | Treatment < 0.001 Interaction < 0.01 | Bonferroni's (E v M) | < 0.001 | 1g |
| | | | Center | WT | 131.08 | 15.68 | | | | | |
| | | | Mouse | WT | 298.13 | 21.63 | | | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 255.18 | 22.54 | | | Bonferroni's (E v M) | n.s. | |
| | | | Center | *Scn1a*$^{+/-}$ | 100.26 | 8.18 | | | | | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 249.64 | 24.79 | | | | | |
| | | Time in Chamber (s) - Social Novelty | Mouse 1 | WT | 163.38 | 20.42 | Two-way ANOVA | Treatment < 0.001 Interaction < 0.01 | Bonferroni's (M1 v M2) | < 0.01 | 1h |
| | | | Center | *WT* | 158.42 | 15.38 | | | | | |
| | | | Mouse 2 | WT | 279.15 | 18.10 | | | | | |
| | | | Mouse 1 | *Scn1a*$^{+/-}$ | 257.29 | 35.89 | | | Bonferroni's (M1 v M2) | n.s. | |
| | | | Center | *Scn1a*$^{+/-}$ | 114.91 | 15.61 | | | | | |
| | | | Mouse 2 | *Scn1a*$^{+/-}$ | 228.91 | 28.77 | | | | | |
| | | Time in Close Interaction (s) - Habituation | Empty 1 | WT | 90.82 | 9.64 | Two-way ANOVA | n.s. | Bonferroni's (E1 v E2) | n.s. | S7a |
| | | | Empty 2 | WT | 122.80 | 15.24 | | | | | |
| | | | Empty 1 | *Scn1a*$^{+/-}$ | 137.52 | 14.13 | | | Bonferroni's (E1 v E2) | n.s. | |
| | | | Empty 2 | *Scn1a*$^{+/-}$ | 116.81 | 15.97 | | | | | |
| | | Time in Close Interaction (s) - Social Preference | Empty | WT | 57.44 | 7.13 | Two-way ANOVA | Treatment < 0.001 Interaction < 0.05 | Bonferroni's (E v M) | < 0.01 | S7b |
| | | | Mouse | WT | 149.15 | 20.82 | | | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 120.26 | 10.22 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 126.90 | 21.29 | | | | | |
| | | Time in Close Interaction (s) - Social Novelty | Mouse 1 | WT | 70.93 | 7.46 | Two-way ANOVA | Interaction < 0.01 | Bonferroni's (M1 v M2) | < 0.05 | S7c |
| | | | Mouse 2 | WT | 124.98 | 17.99 | | | | | |
| | | | Mouse 1 | *Scn1a*$^{+/-}$ | 136.69 | 26.28 | | | Bonferroni's (M1 v M2) | n.s. | |
| | | | Mouse 2 | *Scn1a*$^{+/-}$ | 96.86 | 12.88 | | | | | |
| Social Interaction | WT = 10<br>*Scn1a*$^{+/-}$ = 11 | Time in Quadrant (s) | Empty | WT | 315.93 | 20.54 | Two-way ANOVA | Treatment < 0.01 Interaction < 0.05 | Bonferroni's (E v M) | < 0.001 | - |
| | | | Mouse | WT | 415.37 | 16.14 | | | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 340.32 | 20.22 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 349.47 | 15.24 | | | | | |
| | | Time in Close Interaction (s) | Empty | WT | 132.07 | 14.70 | Two-way ANOVA | Treatment < 0.01 | Bonferroni's (E v M) | < 0.001 | 1i |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mouse | WT | 274.45 | 16.82 | | Interaction < 0.05 | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 190.61 | 24.27 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 183.33 | 12.62 | | | | | |
| | | Total Distance Traveled (cm) | Empty | WT | 2899.47 | 159.90 | Two-way ANOVA | Treatment, Interaction < 0.05 | Bonferroni's (E v M) | < 0.05 | 1j |
| | | | Mouse | WT | 2738.50 | 148.47 | | | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 3558.66 | 186.77 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 2973.83 | 216.42 | | | | | |
| | | Immobilization Time (s) | Empty | WT | 8.81 | 3.25 | Two-way ANOVA | Treatment < 0.05 | Bonferroni's (E v M) | < 0.05 | 1k |
| | | | Mouse | WT | 17.42 | 6.65 | | | | | |
| | | | Empty | *Scn1a*$^{+/-}$ | 26.56 | 6.21 | | | Bonferroni's (E v M) | < 0.01 | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 96.17 | 30.86 | | | | | |
| Social Choice | WT = 7 *Scn1a*$^{+/-}$ = 7 | Time in Quadrant (s) | Object | WT | 63.30 | 7.64 | Two-way ANOVA | Interaction < 0.01 | Bonferroni's (E v M) | < 0.01 | S9a |
| | | | Mouse | WT | 107.86 | 6.32 | | | | | |
| | | | Object | *Scn1a*$^{+/-}$ | 115.39 | 20.52 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 75.53 | 8.18 | | | | | |
| | | Time in Close Interaction (s) | Object | WT | 39.33 | 7.93 | Two-way ANOVA | Interaction < 0.05 | Bonferroni's (E v M) | < 0.05 | S9b |
| | | | Mouse | WT | 69.77 | 6.26 | | | | | |
| | | | Object | *Scn1a*$^{+/-}$ | 90.17 | 22.40 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 52.09 | 6.57 | | | | | |
| Olfactory discrimination | WT = 7 *Scn1a*$^{+/-}$ = 7 | Time in Chamber (s) - Food pellet | No Food | WT | 129.89 | 10.76 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (No Food v Food) | < 0.001 | S10a |
| | | | Center | WT | 151.91 | 21.30 | | | | | |
| | | | Food | WT | 286.95 | 23.07 | | | Bonferroni's (No Food v Food) | < 0.001 | |
| | | | No Food | *Scn1a*$^{+/-}$ | 152.52 | 14.45 | | | | | |
| | | | Center | *Scn1a*$^{+/-}$ | 134.32 | 20.33 | | | | | |
| | | | Food | *Scn1a*$^{+/-}$ | 335.12 | 23.31 | | | | | |
| | | Time in Chamber (s) - Social Odor (Male Bedding) | Clean Bedding | WT | 197.50 | 23.49 | Two-way ANOVA | Interaction , Treatment < 0.001 | Bonferroni's (Clean v Male) | < 0.001 | S11b |
| | | | Center | WT | 97.45 | 12.60 | | | | | |
| | | | Male Bedding | WT | 316.93 | 23.84 | | | Bonferroni's (Clean v Male) | < 0.05 | |
| | | | Clean Bedding | *Scn1a*$^{+/-}$ | 288.10 | 23.97 | | | | | |
| | | | Center | *Scn1a*$^{+/-}$ | 113.79 | 20.52 | | | | | |
| | | | Male Bedding | *Scn1a*$^{+/-}$ | 204.74 | 23.02 | | | | | |
| | | Time in Chamber (s) - Social Odor (Female Bedding) | Clean Bedding | WT | 173.64 | 24.99 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (Clean v Female) | < 0.001 | S11a |
| | | | Center | *WT* | 113.53 | 19.54 | | | | | |
| | | | Female Bedding | WT | 321.18 | 42.00 | | | Bonferroni's (Clean v Female) | n.s. | |
| | | | Clean Bedding | *Scn1a*$^{+/-}$ | 217.30 | 23.07 | | | | | |
| | | | Center | *Scn1a*$^{+/-}$ | 133.85 | 17.22 | | | | | |
| | | | Female Bedding | *Scn1a*$^{+/-}$ | 256.65 | 17.42 | | | | | |
| | | Time in Chamber (s) - Food Pellet | No Food | WT | 47.66 | 5.80 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (No Food v Food) | < 0.001 | S10b |
| | | | Food | WT | 184.32 | 23.39 | | | | | |
| | | | No Food | *Scn1a*$^{+/-}$ | 59.23 | 9.87 | | | Bonferroni's (No Food v Food) | < 0.001 | |
| | | | Food | *Scn1a*$^{+/-}$ | 215.88 | 27.42 | | | | | |
| | | Number of Entries - Food Pellet | No Food | WT | 14.00 | 1.22 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (No Food v Food) | < 0.001 | S10c |
| | | | Food | WT | 33.75 | 3.89 | | | | | |
| | | | No Food | *Scn1a*$^{+/-}$ | 21.22 | 2.34 | | | Bonferroni's (No Food v Food) | < 0.001 | |
| | | | Food | *Scn1a*$^{+/-}$ | 32.11 | 4.27 | | | | | |
| | | Latency to Chamber (s) - Food Pellet | No Food | WT | 63.66 | 14.47 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (No Food v Food) | < 0.05 | S10d |
| | | | Food | WT | 16.15 | 6.18 | | | | | |
| | | | No Food | *Scn1a*$^{+/-}$ | 53.03 | 19.09 | | | Bonferroni's (No Food v Food) | < 0.05 | |
| | | | Food | *Scn1a*$^{+/-}$ | 7.43 | 3.45 | | | | | |
| | | Time in Chamber (s) - Social Odor (Male Bedding) | Clean Bedding | WT | 122.99 | 19.28 | Two-way ANOVA | Interaction < 0.001 Treatment < 0.05 | Bonferroni's (Clean v Male) | < 0.001 | S11d |
| | | | Male Bedding | WT | 242.32 | 19.49 | | | | | |
| | | | Clean Bedding | *Scn1a*$^{+/-}$ | 154.32 | 15.02 | | | Bonferroni's (Clean v Male) | n.s. | |
| | | | Male Bedding | *Scn1a*$^{+/-}$ | 125.54 | 14.15 | | | | | |
| | | Time in Chamber (s) - | Clean Bedding | WT | 64.66 | 16.53 | Two-way ANOVA | Interaction < 0.05 | Bonferroni's (Clean v | < 0.001 | S11e |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Social Odor (Female Bedding) | Female Bedding | WT | 241.03 | 44.85 | | Treatment < 0.001 | Female) | | |
| | | | Clean Bedding | *Scn1a*$^{+/-}$ | 113.30 | 11.76 | | | Bonferroni's (Clean v Female) | n.s. | |
| | | | Female Bedding | *Scn1a*$^{+/-}$ | 163.39 | 18.63 | | | | | |
| | | Interaction Time Difference (s) - Social Odor | Male - Clean | WT | 119.33 | 35.98 | Unpaired two-tailed t-test | > 0.05 | - | - | S11c |
| | | | Female - Clean | WT | 176.38 | 50.82 | | | | | |
| | | | Male - Clean | *Scn1a*$^{+/-}$ | -28.78 | 13.09 | Unpaired two-tailed t-test | < 0.01 | - | - | |
| | | | Female - Clean | *Scn1a*$^{+/-}$ | 50.09 | 17.60 | | | | | |
| | WT = 9 *Scn1a*$^{+/-}$= 9 | Time in Chamber (s) - Aversive Social Odor (Fox urine) | Fox urine | WT | 164.25 | 22.87 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (FU v W) | < 0.01 | S11f |
| | | | Center | WT | 51.47 | 6.96 | | | | | |
| | | | Water | WT | 89.18 | 17.12 | | | | | |
| | | | Fox urine | *Scn1a*$^{+/-}$ | 160.93 | 13.43 | | | Bonferroni's (FU v W) | < 0.001 | |
| | | | Center | *Scn1a*$^{+/-}$ | 66.56 | 10.74 | | | | | |
| | | | Water | *Scn1a*$^{+/-}$ | 77.17 | 8.02 | | | | | |
| Olfactory Habituation / Dishabituation | WT = 7 *Scn1a*$^{+/-}$= 7 | Total Sniffing Time (s) | Water 1 | WT | 8.50 | 2.82 | Two-way ANOVA | Treatment, Habituation < 0.001 | - | - | S12a |
| | | | Water 2 | WT | 4.86 | 1.82 | | | | | |
| | | | Water 3 | WT | 2.65 | 0.62 | | | | | |
| | | | Banana 1 | WT | 21.09 | 5.06 | | | Bonferroni's (W3 v B1) | < 0.05 | |
| | | | Banana 2 | WT | 6.18 | 1.29 | | | | | |
| | | | Banana 3 | WT | 4.48 | 1.08 | | | | | |
| | | | Male 1 | WT | 35.77 | 7.03 | | | Bonferroni's (B3 v M1) | < 0.01 | |
| | | | Male 2 | WT | 16.42 | 4.86 | | | | | |
| | | | Male 3 | WT | 11.21 | 3.97 | | | | | |
| | | | Food1 | WT | 27.37 | 5.32 | | | Bonferroni's (M3v F1) | < 0.01 | |
| | | | Food2 | WT | 8.64 | 2.21 | | | | | |
| | | | Food3 | WT | 5.24 | 2.05 | | | | | |
| | | | Water 1 | *Scn1a*$^{+/-}$ | 1.14 | 0.66 | Two-way ANOVA | Interaction, Treatment, Habituation < 0.001 | - | - | |
| | | | Water 2 | *Scn1a*$^{+/-}$ | 0.71 | 0.63 | | | | | |
| | | | Water 3 | *Scn1a*$^{+/-}$ | 0.23 | 0.23 | | | | | |
| | | | Banana 1 | *Scn1a*$^{+/-}$ | 2.16 | 1.59 | | | Bonferroni's (W3 v B1) | n.s. | |
| | | | Banana 2 | *Scn1a*$^{+/-}$ | 0.57 | 0.50 | | | | | |
| | | | Banana 3 | *Scn1a*$^{+/-}$ | 0.26 | 0.26 | | | | | |
| | | | Male 1 | *Scn1a*$^{+/-}$ | 8.57 | 5.28 | | | Bonferroni's (B3 v M1) | n.s. | |
| | | | Male 2 | *Scn1a*$^{+/-}$ | 3.38 | 2.68 | | | | | |
| | | | Male 3 | *Scn1a*$^{+/-}$ | 0.65 | 0.65 | | | | | |
| | | | Food1 | *Scn1a*$^{+/-}$ | 23.84 | 4.21 | | | Bonferroni's (M3v F1) | < 0.001 | |
| | | | Food2 | *Scn1a*$^{+/-}$ | 6.82 | 1.48 | | | | | |
| | | | Food3 | *Scn1a*$^{+/-}$ | 3.07 | 0.80 | | | | | |
| Digging behavior | WT = 7 *Scn1a*$^{+/-}$= 7 | Total Digging Time (s) | Water | WT | 0.00 | 0.00 | Two-way ANOVA | Genotype < 0.01 Treatment < 0.05 | Bonferroni's (W v B) (W v M) | n.s. | S12b |
| | | | Banana | WT | 3.04 | 1.62 | | | | | |
| | | | Male | WT | 0.64 | 0.36 | | | | | |
| | | | Water | *Scn1a*$^{+/-}$ | 4.06 | 2.59 | | | Bonferroni's (W v B) (W v M) | < 0.01 | |
| | | | Banana | *Scn1a*$^{+/-}$ | 34.17 | 9.36 | | | | | |
| | | | Male | *Scn1a*$^{+/-}$ | 25.19 | 10.22 | | | | | |
| Olfactory Choice | WT = 7 *Scn1a*$^{+/-}$= 7 | Time in arm (s)- WT | Water | Odor + | 77.53 | 8.81 | Unpaired two-tailed t-test | n.s. | - | - | S12c |
| | | | Water | Odor - | 59.65 | 9.40 | | | | | |
| | | | Banana | Odor + | 101.99 | 16.27 | | < 0.05 | - | - | |
| | | | Banana | Odor - | 55.62 | 7.97 | | | | | |
| | | | Male | Odor + | 119.36 | 15.23 | | <0.01 | - | - | |
| | | | Male | Odor - | 54.53 | 8.11 | | | | | |
| | | Time in arm (s)- *Scn1a*$^{+/-}$ | Water | Odor + | 90.68 | 4.42 | Unpaired two-tailed t-test | n.s. | - | - | S12d |
| | | | Water | Odor - | 73.81 | 11.69 | | | | | |
| | | | Banana | Odor + | 52.51 | 6.20 | | < 0.001 | - | - | |
| | | | Banana | Odor - | 114.61 | 16.80 | | | | | |
| | | | Male | Odor + | 53.29 | 4.35 | | <0.001 | - | - | |
| | | | Male | Odor - | 128.06 | 15.41 | | | | | |
| Reciprocal Interaction | WT = 12 *Scn1a*$^{+/-}$= 9 | Time in Aggressive Behavior (s) | Aggressive | WT | 87.45 | 14.95 | Two-way ANOVA | Genotype < 0.001 Treatment < 0.01 | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.001 | 1l |
| | | | | *Scn1a*$^{+/-}$ | 11.42 | 9.05 | | | | | |
| | | Time in Non-aggressive Behavior (s) | Non-aggressive | WT | 147.74 | 17.39 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.01 | |
| | | | | *Scn1a*$^{+/-}$ | 58.26 | 15.53 | | | | | |
| Novel Object Recognition | WT = 6 *Scn1a*$^{+/-}$= 6 | Time Spent with Object (s) | Familiar | WT | 81.00 | 14.88 | Two-way Repeated Measure | n.s. | Bonferroni's (F v N) | < 0.01 | 2a |
| | | | Novel | WT | 139.67 | 22.19 | | | | | |
| | | | Familiar | *Scn1a*$^{+/-}$ | 49.33 | 9.38 | | | Bonferroni's | < 0.05 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Novel | *Scn1a*$^{+/-}$ | 90.83 | 18.33 | ANOVA | | (F v N) | | |
| Contextual Fear Conditioning | WT = 5<br>*Scn1a*$^{+/-}$= 9 | % Freeze | Control | WT | 11.21 | 4.45 | Two-way ANOVA | Genotype, Time, Interaction < 0.001 | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | 2c |
| | | | | *Scn1a*$^{+/-}$ | 21.81 | 7.60 | | | | | |
| | | | Train | WT | 61.64 | 15.40 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | |
| | | | | *Scn1a*$^{+/-}$ | 55.92 | 10.95 | | | | | |
| | | | 30 min | WT | 73.87 | 9.65 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.01 | |
| | | | | *Scn1a*$^{+/-}$ | 26.10 | 5.53 | | | | | |
| | | | 24 hr | WT | 78.82 | 8.42 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.001 | |
| | | | | *Scn1a*$^{+/-}$ | 14.28 | 4.36 | | | | | |
| | | Distance moved (cm) | Control | WT | 404.76 | 25.00 | Two-way ANOVA | Interaction < 0.01 Genotype, Treatment < 0.001 | Bonferroni's (Control v 30 m) (Control v 24 hr) | < 0.001 | S13a |
| | | | Train | WT | 95.36 | 27.79 | | | | | |
| | | | 30 min | WT | 173.49 | 34.83 | | | | | |
| | | | 24 hr | WT | 140.05 | 22.50 | | | | | |
| | | | Control | *Scn1a*$^{+/-}$ | 402.18 | 42.56 | | | Bonferroni's (Control v 30 m) (Control v 24 hr) | n.s. | |
| | | | Train | *Scn1a*$^{+/-}$ | 101.79 | 19.30 | | | | | |
| | | | 30 min | *Scn1a*$^{+/-}$ | 327.73 | 31.44 | | | | | |
| | | | 24 hr | *Scn1a*$^{+/-}$ | 367.15 | 29.21 | | | | | |
| | | Mean velocity (cm/s) | Control | WT | 3.66 | 0.24 | Two-way ANOVA | Interaction < 0.05 Genotype < 0.001 Treatment <0.01 | Bonferroni's (Control v 30 m) (Control v 24 hr) | < 0.01 | S13b |
| | | | Train | WT | 1.83 | 0.53 | | | | | |
| | | | 30 min | WT | 1.56 | 0.31 | | | | | |
| | | | 24 hr | WT | 1.31 | 0.23 | | | | | |
| | | | Control | *Scn1a*$^{+/-}$ | 3.61 | 0.38 | | | Bonferroni's (Control v 30 m) (Control v 24 hr) | n.s. | |
| | | | Train | *Scn1a*$^{+/-}$ | 1.96 | 0.37 | | | | | |
| | | | 30 min | *Scn1a*$^{+/-}$ | 2.97 | 0.29 | | | | | |
| | | | 24 hr | *Scn1a*$^{+/-}$ | 3.32 | 0.26 | | | | | |
| | | Maxium velocity (cm/s) | Control | WT | 28.35 | 7.12 | Two-way ANOVA | Genotype < 0.001 | Bonferroni's (Control v 30 m) (Control v 24 hr) | < 0.05 | S13c |
| | | | Train | WT | 11.93 | 2.92 | | | | | |
| | | | 30 min | WT | 14.07 | 2.92 | | | | | |
| | | | 24 hr | WT | 12.52 | 1.42 | | | | | |
| | | | Control | *Scn1a*$^{+/-}$ | 27.77 | 5.16 | | | Bonferroni's (Control v 30 m) (Control v 24 hr) | n.s. | |
| | | | Train | *Scn1a*$^{+/-}$ | 11.90 | 2.38 | | | | | |
| | | | 30 min | *Scn1a*$^{+/-}$ | 21.32 | 1.72 | | | | | |
| | | | 24 hr | *Scn1a*$^{+/-}$ | 19.41 | 0.58 | | | | | |
| Barnes Circular Maze | WT = 9<br>*Scn1a*$^{+/-}$= 9 | Number of errors | Trial 1 | WT | 49.15 | 5.05 | Two-way ANOVA | Interaction < 0.01 Genotype < 0.001 Trial < 0.001 | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | 2d |
| | | | | *Scn1a*$^{+/-}$ | 46.30 | 3.73 | | | | | |
| | | | Trial 2 | WT | 33.85 | 3.86 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | |
| | | | | *Scn1a*$^{+/-}$ | 44.78 | 5.62 | | | | | |
| | | | Trial 3 | WT | 20.96 | 3.18 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.01 | |
| | | | | *Scn1a*$^{+/-}$ | 42.48 | 5.87 | | | | | |
| | | | Trial 4 | WT | 10.19 | 2.55 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.001 | |
| | | | | *Scn1a*$^{+/-}$ | 42.56 | 5.92 | | | | | |
| | | Escape latency (s) | Trial 1 | WT | 160.19 | 7.40 | Two-way ANOVA | Interaction , Genotype, Trial < 0.001 | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | 2e |
| | | | | *Scn1a*$^{+/-}$ | 167.48 | 5.83 | | | | | |
| | | | Trial 2 | WT | 148.96 | 8.18 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | n.s. | |
| | | | | *Scn1a*$^{+/-}$ | 157.85 | 7.93 | | | | | |
| | | | Trial 3 | WT | 109.81 | 10.79 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.01 | |
| | | | | *Scn1a*$^{+/-}$ | 148.81 | 9.08 | | | | | |
| | | | Trial 4 | WT | 52.70 | 6.97 | | | Bonferroni's (WT v *Scn1a*$^{+/-}$) | < 0.001 | |
| | | | | *Scn1a*$^{+/-}$ | 148.59 | 9.29 | | | | | |
| | | Total distance moved (cm) | - | WT | 373.35 | 54.50 | Unpaired two-tailed t-test | n.s. | - | - | 2f |
| | | | | *Scn1a*$^{+/-}$ | 330.60 | 42.63 | | | | | |
| | | Latency to target (s) | - | WT | 9.19 | 4.21 | Unpaired two-tailed t-test | < 0.001 | - | - | 2g |
| | | | | *Scn1a*$^{+/-}$ | 80.28 | 8.43 | | | | | |
| | | % Correct pokes | - | WT | 27.58 | 4.84 | Unpaired two-tailed t-test | < 0.001 | - | - | 2h |
| | | | | *Scn1a*$^{+/-}$ | 3.24 | 1.86 | | | | | |
| | | % Time in | - | WT | 52.50 | 8.98 | Unpaired | < 0.001 | - | - | 2i |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | target | | *Scn1a*$^{+/-}$ | 10.04 | 5.53 | two-tailed t-test | | | | |

## *Scn1a*$^{Flox/+}$ (Flox) vs *Dlx*$^{+}$-*Scn1a*$^{Flox/+}$ (Dlx)

| Test | # of Animals | Measurement | Treatment | Genotype | Average | s.e.m. | Statistical Test | *p* value | *post hoc* Test | *p* value | Figure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Open Field | Flox = 8<br>Dlx = 7 | Total Distance Traveled (cm) | - | Flox | 2556.28 | 205.85 | Unpaired two-tailed t-test | < 0.05 | - | - | 3a |
| | | | | Dlx | 3398.63 | 212.66 | | | | | |
| | | Time in Center (s) | - | Flox | 30.75 | 3.57 | Unpaired two-tailed t-test | < 0.01 | - | - | 3b |
| | | | | Dlx | 16.70 | 4.17 | | | | | |
| Circling | | Total Circling Number | - | Flox | 17.13 | 2.24 | Unpaired two-tailed t-test | < 0.05 | - | - | 3c |
| | | | | Dlx | 23.86 | 2.02 | | | | | |
| Elevated Plus Maze | | Number of entries | Closed | Flox | 23.00 | 3.95 | Unpaired two-tailed t-test | n.s. | - | - | 3d |
| | | | | Dlx | 28.50 | 3.45 | | | | | |
| | | | Open | Flox | 18.57 | 3.43 | Unpaired two-tailed t-test | < 0.05 | - | - | |
| | | | | Dlx | 7.67 | 1.12 | | | | | |
| | | Time in Open Arms (s) | - | Flox | 112.50 | 23.84 | Unpaired two-tailed t-test | < 0.05 | - | - | 3e |
| | | | | Dlx | 58.47 | 3.20 | | | | | |
| 3-Chamber | | Time Spent in Chamber (s) - Social Preference | Empty | Flox | 123.61 | 27.42 | Two-way ANOVA | Interaction, Treatment < 0.001 | Bonferroni's (E v M) | < 0.001 | 3g |
| | | | Center | Flox | 88.87 | 15.97 | | | | | |
| | | | Mouse | Flox | 400.75 | 23.39 | | | | | |
| | | | Empty | Dlx | 254.66 | 18.86 | | | Bonferroni's (E v M) | n.s. | |
| | | | Center | Dlx | 93.69 | 15.67 | | | | | |
| | | | Mouse | Dlx | 277.16 | 31.50 | | | | | |
| Social Interaction | | Time in Quadrant (s) | Empty | Flox | 259.80 | 23.52 | Two-way ANOVA | Treatment < 0.05 | Bonferroni's (E v M) | < 0.05 | 3f |
| | | | Mouse | Flox | 388.81 | 33.62 | | | | | |
| | | | Empty | Dlx | 228.02 | 47.69 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | Dlx | 216.16 | 43.40 | | | | | |
| Contextual Fear Conditioning | | % Freeze | Control | Flox | 24.24 | 2.62 | Two-way ANOVA | Genotype, Time, Interaction < 0.001 | Bonferroni's (Flox v Dlx) | n.s. | 3h |
| | | | | Dlx | 24.43 | 2.71 | | | | | |
| | | | Train | Flox | 75.56 | 7.66 | | | Bonferroni's (Flox v Dlx) | n.s. | |
| | | | | Dlx | 72.50 | 6.48 | | | | | |
| | | | 30 min | Flox | 80.30 | 5.86 | | | Bonferroni's (Flox v Dlx) | < 0.001 | |
| | | | | Dlx | 39.77 | 3.08 | | | | | |
| | | | 24 hr | Flox | 86.87 | 2.45 | | | Bonferroni's (Flox v Dlx) | < 0.001 | |
| | | | | Dlx | 51.70 | 7.73 | | | | | |

## Cre- vs Cre+

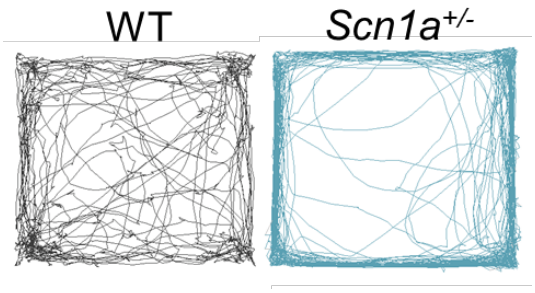
| Test | # of Animals | Measurement | Treatment | Genotype | Average | s.e.m. | Statistical Test | *p* value | *post hoc* Test | *p* value | Figure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Open Field | Cre- = 7<br>Cre+ = 7 | Total Distance Traveled (cm) | - | Cre- | 2566.88 | 125.02 | Unpaired two-tailed t-test | n.s. | - | - | S14a |
| | | | | Cre+ | 2334.79 | 168.35 | | | | | |
| | | Time in Center (s) | - | Cre- | 31.86 | 3.66 | Unpaired two-tailed t-test | n.s. | - | - | S14b |
| | | | | Cre+ | 28.08 | 3.70 | | | | | |
| Circling | | Total Circling Number | - | Cre- | 15.57 | 1.76 | Unpaired two-tailed t-test | n.s. | - | - | S14c |
| | | | | Cre+ | 17.83 | 1.35 | | | | | |
| Social Interaction | | Time in Quadrant (s) | Empty | Cre- | 298.55 | 26.79 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (E v M) | < 0.05 | S14d |
| | | | Mouse | Cre- | 411.11 | 36.68 | | | | | |
| | | | Empty | Cre+ | 274.72 | 32.75 | | | Bonferroni's (E v M) | < 0.01 | |
| | | | Mouse | Cre+ | 453.16 | 30.96 | | | | | |
| Contextual Fear Conditioning | | % Freeze | Control | Cre- | 34.19 | 2.68 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (Flox v Dlx) | n.s. | S14e |
| | | | | Cre+ | 41.69 | 4.87 | | | | | |
| | | | Train | Cre- | 69.91 | 9.04 | | | Bonferroni's (Flox v Dlx) | n.s. | |
| | | | | Cre+ | 57.69 | 5.96 | | | | | |
| | | | 30 min | Cre- | 64.73 | 6.87 | | | Bonferroni's (Flox v Dlx) | n.s. | |
| | | | | Cre+ | 72.91 | 7.85 | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 24 hr | Cre- | 57.38 | 6.74 | | | Bonferroni's (Flox v Dlx) | n.s. | |
| | | | | Cre+ | 69.86 | 3.52 | | | | | |

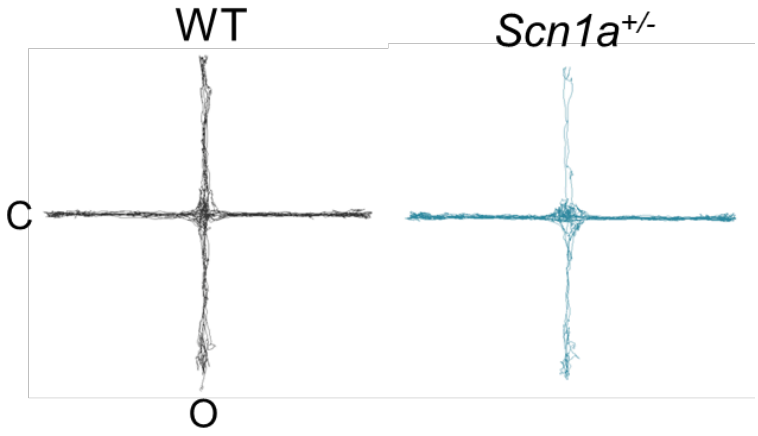
## Drug Study

| Test | # of Animals | Measurement | Treatment | Genotype | Average | s.e.m. | Statistical Test | *p* value | *post hoc* Test | *p* value | Figure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Open Field | Control: WT = 10 *Scn1a*$^{+/-}$= 12 Clonazepam: WT = 10 *Scn1a*$^{+/-}$= 10 Clearance: WT = 6 *Scn1a*$^{+/-}$= 10 | Total Distance Traveled (cm) | Pre | *Scn1a*$^{+/-}$ | 5010.84 | 466.80 | One-way ANOVA | n.s. | Tukey's (CON vs CLN) | n.s. | S21a |
| | | | CLZ | *Scn1a*$^{+/-}$ | 4365.65 | 662.08 | | | | | |
| | | | Post | *Scn1a*$^{+/-}$ | 4020.30 | 538.58 | | | | | |
| | | Time in Center (s) | Pre | *Scn1a*$^{+/-}$ | 12.90 | 3.22 | One-way ANOVA | n.s. | Tukey's (CON vs CLN) | n.s. | S21b |
| | | | CLZ | *Scn1a*$^{+/-}$ | 9.19 | 3.11 | | | | | |
| | | | Post | *Scn1a*$^{+/-}$ | 13.19 | 2.68 | | | | | |
| Elevated Plus Maze | *Scn1a*$^{+/-}$= 10 | Number of entries-Closed arms | Pre | *Scn1a*$^{+/-}$ | 23.90 | 3.42 | Unpaired two-tailed t-test | n.s. | - | - | S21c |
| | | | CLZ | *Scn1a*$^{+/-}$ | 25.40 | 4.32 | | | | | |
| | | Number of entries-Open arms | Post | *Scn1a*$^{+/-}$ | 5.30 | 0.94 | Unpaired two-tailed t-test | n.s. | - | - | |
| | | | Pre | *Scn1a*$^{+/-}$ | 5.40 | 0.99 | | | | | |
| | | Time in Open Arms (s) | CLZ | *Scn1a*$^{+/-}$ | 31.43 | 7.95 | Unpaired two-tailed t-test | n.s. | - | - | S21d |
| | | | Post | *Scn1a*$^{+/-}$ | 30.90 | 9.71 | | | | | |
| Drug Sedation | WT = 10 *Scn1a*$^{+/-}$= 12 | Total Distance Traveled (cm) | Control | WT | 3111.42 | 293.98 | One-way ANOVA | < 0.001 | Tukey's (Comparsion with Control) | - | 5a |
| | | | 0.03125 mg/kg | WT | 2813.12 | 239.79 | | | | n.s. | |
| | | | 0.0625 mg/kg | WT | 2245.56 | 160.21 | | | | n.s. | |
| | | | 0.125 mg/kg | WT | 1793.83 | 286.58 | | | | < 0.01 | |
| | | | 0.25 mg/kg | WT | 1465.91 | 317.25 | | | | < 0.001 | |
| | | | 0.5 mg/kg | WT | 1152.48 | 159.92 | | | | < 0.001 | |
| | | | Control | *Scn1a*$^{+/-}$ | 5010.84 | 466.80 | One-way ANOVA | < 0.001 | Tukey's (Comparsion with Control) | - | |
| | | | 0.03125 mg/kg | *Scn1a*$^{+/-}$ | 4751.20 | 549.39 | | | | n.s. | |
| | | | 0.0625 mg/kg | *Scn1a*$^{+/-}$ | 4365.65 | 662.08 | | | | n.s. | |
| | | | 0.125 mg/kg | *Scn1a*$^{+/-}$ | 2016.20 | 661.96 | | | | < 0.01 | |
| | | | 0.25 mg/kg | *Scn1a*$^{+/-}$ | 875.28 | 397.19 | | | | < 0.001 | |
| | | | 0.5 mg/kg | *Scn1a*$^{+/-}$ | 522.47 | 131.33 | | | | < 0.001 | |
| 3-Chamber | Pre: WT = 10 *Scn1a*$^{+/-}$= 12 CLZ: WT = 8 *Scn1a*$^{+/-}$= 10 Post: WT = 8 *Scn1a*$^{+/-}$= 10 | Time in Chamber (s) - Pre | Empty | WT | 168.62 | 14.37 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (E v M) | < 0.01 | S23b |
| | | | Center | WT | 131.08 | 15.68 | | | | | |
| | | | Mouse | WT | 298.13 | 21.63 | | | | | |
| | | Time in Chamber (s) - CLZ | Empty | WT | 111.09 | 28.26 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Center | WT | 116.88 | 28.84 | | | | | |
| | | | Mouse | WT | 391.27 | 31.42 | | | | | |
| | | Time in Chamber (s) - Post | Empty | WT | 130.59 | 26.50 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Center | WT | 85.83 | 30.14 | | | | | |
| | | | Mouse | WT | 397.23 | 54.35 | | | | | |
| | | Time in Chamber (s) - Pre | Empty | Scn1a+/- | 255.18 | 22.54 | Two-way ANOVA | Interaction , Treatment < 0.001 | Bonferroni's (E v M) | n.s. | S23d |
| | | | Center | Scn1a+/- | 100.26 | 8.18 | | | | | |
| | | | Mouse | Scn1a+/- | 249.64 | 24.79 | | | | | |
| | | Time in Chamber (s) - CLZ | Empty | Scn1a+/- | 143.70 | 25.79 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Center | Scn1a+/- | 74.82 | 15.95 | | | | | |
| | | | Mouse | Scn1a+/- | 392.36 | 31.04 | | | | | |
| | | Time in Chamber (s) - Post | Empty | Scn1a+/- | 333.50 | 32.10 | | | Bonferroni's (E v M) | < 0.01 | |
| | | | Center | Scn1a+/- | 76.28 | 16.20 | | | | | |
| | | | Mouse | Scn1a+/- | 209.96 | 32.20 | | | | | |
| | | Time in Close Interaction (s) - Pre | Empty | WT | 57.44 | 7.13 | Two-way ANOVA | Interaction < 0.05 Treatment < 0.001 | Bonferroni's (E v M) | n.s. | S23a |
| | | | Mouse | WT | 149.15 | 20.82 | | | | | |
| | | Time in Close Interaction (s) - CLZ | Empty | WT | 60.31 | 19.76 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | WT | 227.38 | 31.62 | | | | | |
| | | Time in Close Interaction (s) - Post | Empty | WT | 51.89 | 14.59 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | WT | 285.45 | 49.85 | | | | | |
| | | Time in Close Interaction (s) - Pre | Empty | *Scn1a*$^{+/-}$ | 120.26 | 10.22 | Two-way ANOVA | Interaction < 0.01 | Bonferroni's (E v M) | n.s. | S23c |
| | | | Mouse | *Scn1a*$^{+/-}$ | 126.90 | 21.29 | | | | | |
| | | Time in Close Interaction (s) - CLZ | Empty | *Scn1a*$^{+/-}$ | 90.62 | 16.81 | | | Bonferroni's (E v M) | < 0.01 | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 223.62 | 43.17 | | | | | |

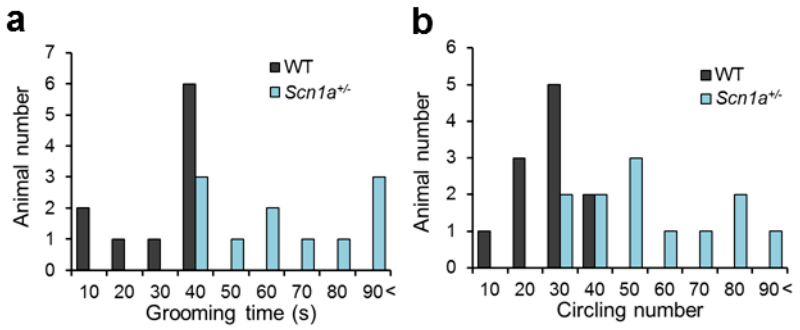
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Time in Close Interaction (s) - Post | Empty | *Scn1a*$^{+/-}$ | 161.17 | 40.52 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 107.50 | 32.04 | | | | | |
| | | Interaction Ratio (Mouse/Object) | Control | WT | 2.85 | 0.49 | Two-way ANOVA | Treatment < 0.05 | Bonferroni's | n.s. | 5c |
| | | | Clonazepam | WT | 4.11 | 0.90 | | | | | |
| | | | Cleared | WT | 5.03 | 1.37 | | | | | |
| | | Interaction Ratio (Mouse/Object) | Control | *Scn1a*$^{+/-}$ | 1.16 | 0.23 | | | Bonferroni's | < 0.01 | |
| | | | Clonazepam | *Scn1a*$^{+/-}$ | 6.03 | 2.37 | | | | | |
| | | | Cleared | *Scn1a*$^{+/-}$ | 1.05 | 0.39 | | | | | |
| Social Interaction | Control: WT = 10 *Scn1a*$^{+/-}$= 11 Clonazepam: WT = 10 *Scn1a*$^{+/-}$= 10 Clearance: WT = 6 *Scn1a*$^{+/-}$= 10 | Time Spent in Quadrant (s) - Pre | Empty | WT | 315.93 | 20.54 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (E v M) | < 0.05 | S17b |
| | | | Mouse | WT | 415.37 | 16.14 | | | | | |
| | | Time Spent in Quadrant (s) - CLZ | Empty | WT | 305.87 | 38.15 | | | Bonferroni's (E v M) | < 0.01 | |
| | | | Mouse | WT | 439.41 | 23.95 | | | | | |
| | | Time Spent in Quadrant (s) - Post | Empty | *WT* | 236.21 | 35.31 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | WT | 400.87 | 23.73 | | | | | |
| | | Time Spent in Quadrant (s) - Pre | Empty | *Scn1a*$^{+/-}$ | 340.32 | 20.22 | Two-way ANOVA | Interaction < 0.05 Treatment < 0.01 | Bonferroni's (E v M) | n.s. | S22d |
| | | | Mouse | *Scn1a*$^{+/-}$ | 349.47 | 15.24 | | | | | |
| | | Time Spent in Quadrant (s) - CLZ | Empty | *Scn1a*$^{+/-}$ | 274.33 | 39.27 | | | Bonferroni's (E v M) | < 0.05 | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 405.12 | 35.02 | | | | | |
| | | Time Spent in Quadrant (s) - Post | Empty | *Scn1a*$^{+/-}$ | 271.73 | 49.76 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 276.02 | 43.98 | | | | | |
| | | Time Spent in Quadrant (s) - Pre | Empty | WT | 132.07 | 14.70 | Two-way ANOVA | Treatment < 0.001 | Bonferroni's (E v M) | < 0.001 | S22a |
| | | | Mouse | WT | 274.45 | 16.82 | | | | | |
| | | Time Spent in Quadrant (s) - CLZ | Empty | WT | 105.70 | 14.88 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | WT | 235.46 | 20.99 | | | | | |
| | | Time Spent in Quadrant (s) - Post | Empty | WT | 115.62 | 14.53 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | WT | 256.60 | 23.86 | | | | | |
| | | Time Spent in Quadrant (s) - Pre | Empty | *Scn1a*$^{+/-}$ | 190.61 | 24.27 | Two-way ANOVA | Interaction , Treatment < 0.001 | Bonferroni's (E v M) | n.s. | S22c |
| | | | Mouse | *Scn1a*$^{+/-}$ | 183.33 | 12.62 | | | | | |
| | | Time Spent in Quadrant (s) - CLZ | Empty | *Scn1a*$^{+/-}$ | 96.36 | 115.62 | | | Bonferroni's (E v M) | < 0.001 | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 218.51 | 256.60 | | | | | |
| | | Time Spent in Quadrant (s) - Post | Empty | *Scn1a*$^{+/-}$ | 105.47 | 19.29 | | | Bonferroni's (E v M) | n.s. | |
| | | | Mouse | *Scn1a*$^{+/-}$ | 85.71 | 25.45 | | | | | |
| | | Interaction Ratio (Mouse/Object) | Pre | WT | 2.25 | 0.24 | Two-way ANOVA | Treatment < 0.01 Genotype < 0.05 | Bonferroni's | n.s. | 5b |
| | | | CLZ | WT | 2.61 | 0.33 | | | | | |
| | | | Post | WT | 2.32 | 0.25 | | | | | |
| | | Interaction Ratio (Mouse/Object) | Pre | *Scn1a*$^{+/-}$ | 1.06 | 0.12 | | | Bonferroni's | < 0.001 | |
| | | | CLZ | *Scn1a*$^{+/-}$ | 3.05 | 0.81 | | | | | |
| | | | Post | *Scn1a*$^{+/-}$ | 0.84 | 0.23 | | | | | |
| Contextual Fear Conditioning | WT = 7 *Scn1a*$^{+/-}$= 6 | % Freeze - Control | Control | WT | 11.21 | 4.45 | Two-way ANOVA | Time < 0.001 | Bonferroni's (CON v CLN) | n.s. | 5d |
| | | | Clonazepam | WT | 29.93 | 8.32 | | | | | |
| | | % Freeze - Train | Control | WT | 61.64 | 15.40 | | | Bonferroni's (CON v CLN) | n.s. | |
| | | | Clonazepam | WT | 62.50 | 6.73 | | | | | |
| | | % Freeze - 30 min | Control | WT | 73.87 | 9.65 | | | Bonferroni's (CON v CLN) | n.s. | |
| | | | Clonazepam | WT | 89.00 | 4.92 | | | | | |
| | | % Freeze - 24 hr | Control | WT | 78.82 | 8.42 | | | Bonferroni's (CON v CLN) | n.s. | |
| | | | Clonazepam | WT | 76.88 | 5.45 | | | | | |
| | | % Freeze - Control | Control | *Scn1a*$^{+/-}$ | 21.81 | 7.60 | Two-way ANOVA | Interaction , Time, Treatment < 0.05 | Bonferroni's (CON v CLN) | n.s. | 5e |
| | | | Clonazepam | *Scn1a*$^{+/-}$ | 24.90 | 11.95 | | | | | |
| | | % Freeze - Train | Control | *Scn1a*$^{+/-}$ | 55.92 | 10.95 | | | Bonferroni's (CON v CLN) | n.s. | |
| | | | Clonazepam | *Scn1a*$^{+/-}$ | 45.74 | 11.51 | | | | | |
| | | % Freeze - 30 min | Control | *Scn1a*$^{+/-}$ | 23.10 | 5.53 | | | Bonferroni's (CON v CLN) | < 0.01 | |
| | | | Clonazepam | *Scn1a*$^{+/-}$ | 56.75 | 5.67 | | | | | |
| | | % Freeze - 24 hr | Control | *Scn1a*$^{+/-}$ | 14.28 | 4.36 | | | Bonferroni's (CON v CLN) | < 0.01 | |
| | | | Clonazepam | *Scn1a*$^{+/-}$ | 55.18 | 11.38 | | | | | |



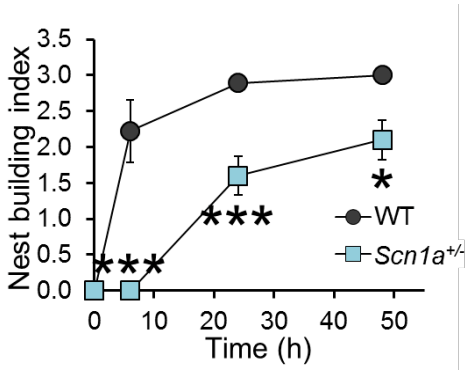

**Supplementary Figure 1. Representative tracks for open field test.** WT and *Scn1a*$^{+/-}$ mice were allowed to explore novel open field arena (38 cm x 42 cm) for 10 min. *Scn1a*$^{+/-}$ mice explore much more in the boundary of the arena, and explore much less in the center (15 cm x 15 cm square imaginary area) compared with WT mice.



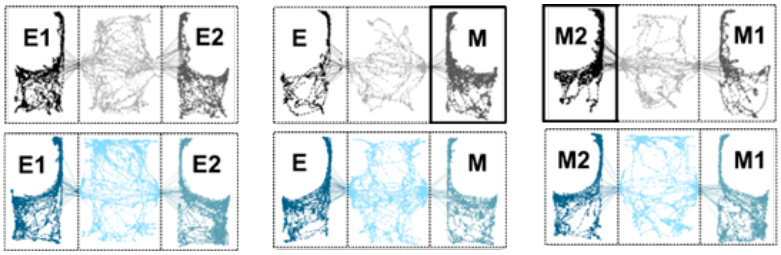

**Supplementary Figure 2. Representative tracks for elevated plus maze.** WT and *Scn1a*$^{+/-}$ mice were allowed to explore an elevated plus maze for 10 min. *Scn1a*$^{+/-}$ mice spend more time in the closed arm (c), and less time in the open arm (o) compared with WT mice.



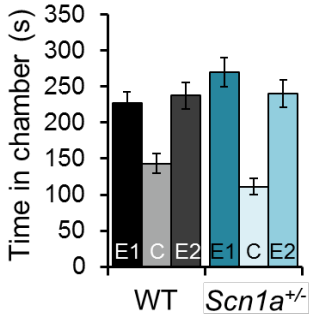

**Supplementary Figure 3. Histogram of repetitive behaviors. a**, Grooming behavior data of WT and *Scn1a*$^{+/-}$ mice (Fig. 1e) is replotted as a histogram. **b**, Circling behavior data of WT and *Scn1a*$^{+/-}$ mice (Fig. 1f) is replotted as a histogram. In both cases, all individual *Scn1a*$^{+/-}$ mice have longer grooming times and more circling than the mean for WT mice (WT: 28 s for grooming; 22 circles).



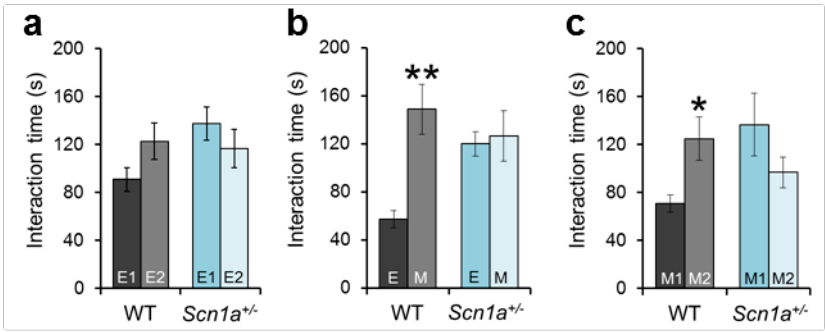

**Supplementary Figure 4. Nest building test.** *Scn1a*$^{+/-}$ mice have a decreased nest-building index compared with WT mice. All values are means ± s.e.m. from 9 – 10 mice per genotype. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



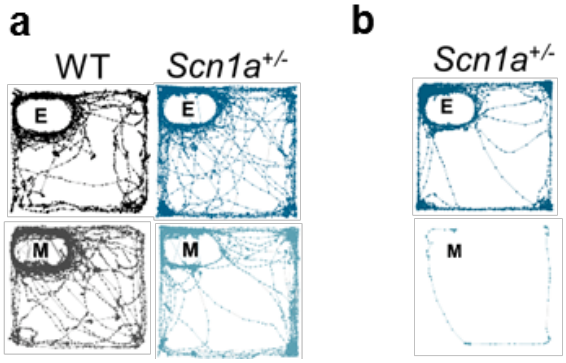

**Supplementary Figure 5. Representative tracks for the 3-chamber test.** Representative video-tracks of mice for each three-chamber experiment, which measures the amount of time that the mouse spends with the chamber with an empty cage (E: Empty), in the center change (C: Center) or in the chamber with either a stranger or familiar mouse (M: Mouse). Top row, WT mice; bottom row, *Scn1a*$^{+/-}$ mice.



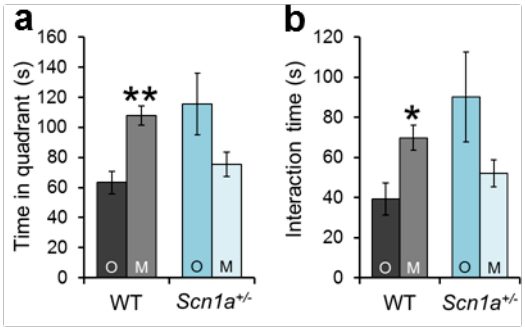

**Supplementary Figure 6. Three-chamber test for social interaction.** Both WT and *Scn1a*$^{+/-}$ mice have no preference for either side of three-chamber unit in the habituation period. E, Empty. C, Center. All data shown are means ± s.e.m. from 10 - 12 mice per genotype.



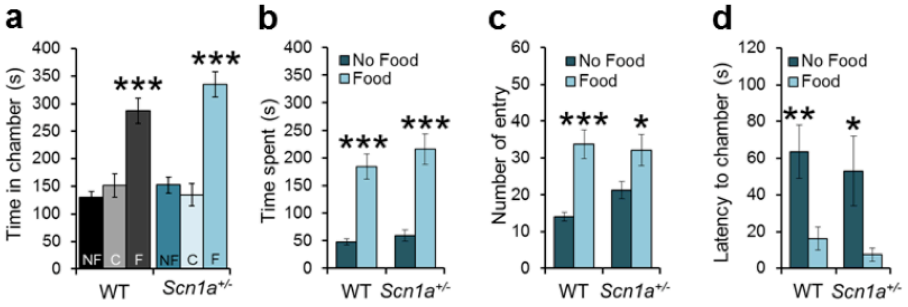

**Supplementary Figure 7. Social interaction in the three-chamber test.** **a**, Both WT and *Scn1a*$^{+/-}$ mice had no preference for either side of three-chamber unit in the habituation period. **b**, In the three-chamber test, *Scn1a*$^{+/-}$ mice had no preference for the stranger mouse, whereas WT mice interacted a longer time with the stranger mouse than with the empty cage. **c**, *Scn1a*$^{+/-}$ mice had no preference for novel mouse, compared with familiar mouse, whereas WT mice spent a longer time with novel mouse. E, Empty. M, Mouse. All data shown are means ± s.e.m. from 10 - 12 mice per genotype. $^{**}P < 0.01$, $^{***}P < 0.001$.



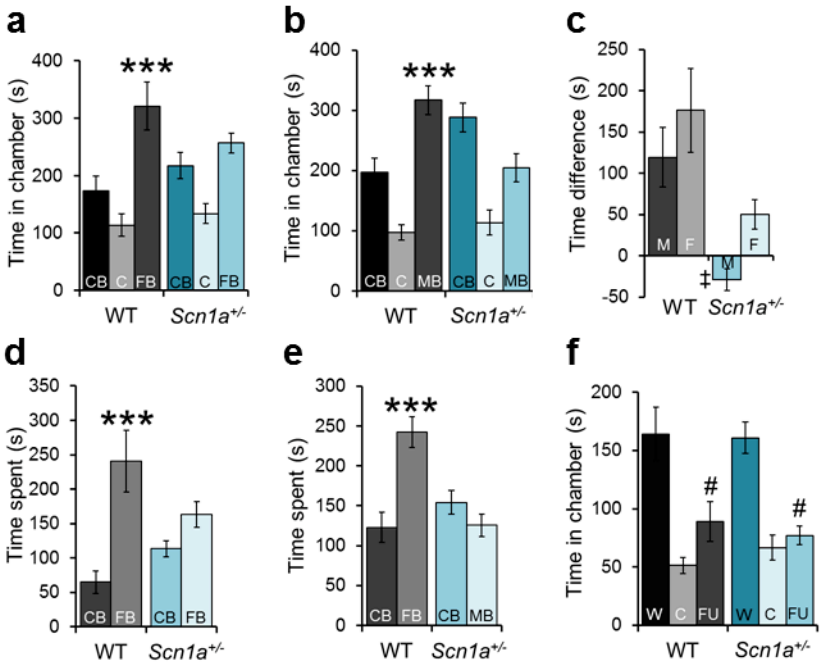

**Supplementary Figure 8. Representative tracks in the social interaction test.** **a**, Representative video-tracks of mice in the social interaction test. Left column, WT mice; right column, *Scn1a*$^{+/-}$ mice. Top row, empty cage is placed (E); bottom row, mouse is placed in the cage (M). **b**, Tracks from a *Scn1a*$^{+/-}$ mouse that displayed immobilization behavior when a stranger mouse is encountered. This example shows an unusually long single period of immobilization (bottom).



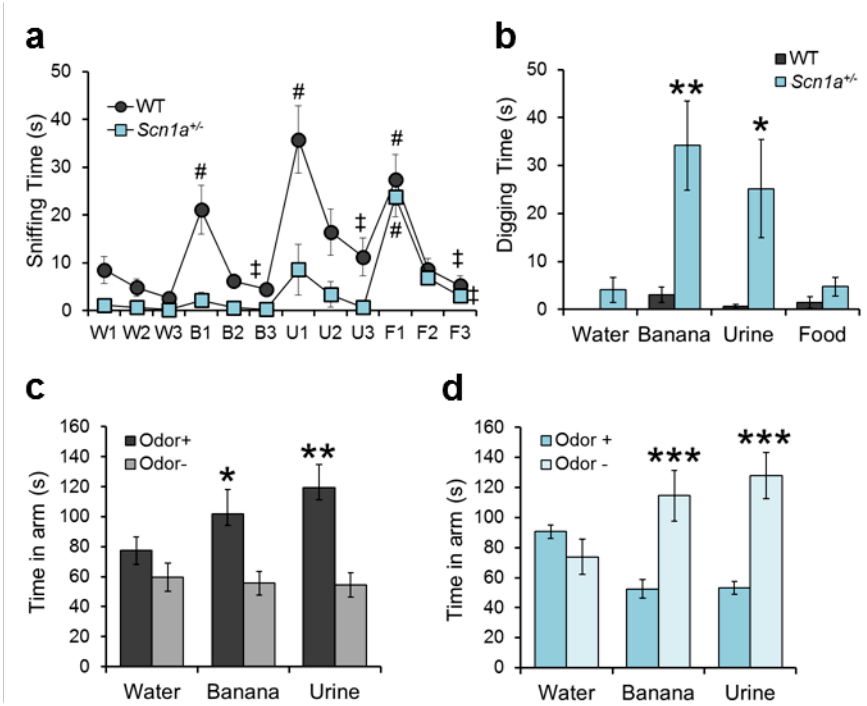

**Supplementary Figure 9. Social-preference test. a**, **b**, When presented both an object and a stranger mouse simultaneously, only WT mice preferred to stay in the quadrant where the caged stranger mouse was placed (**a**), and preferred to interact more with the caged stranger mouse (**b**). *Scn1a*$^{+/-}$ mice had no preference for the stranger mouse, instead they display a tendency toward avoidance of the stranger mouse ($p$ = 0.09). O, Object. M, Mouse. All data shown are means ± s.e.m. from 7 mice per genotype. *$P$ < 0.05, **$P$ < 0.01.



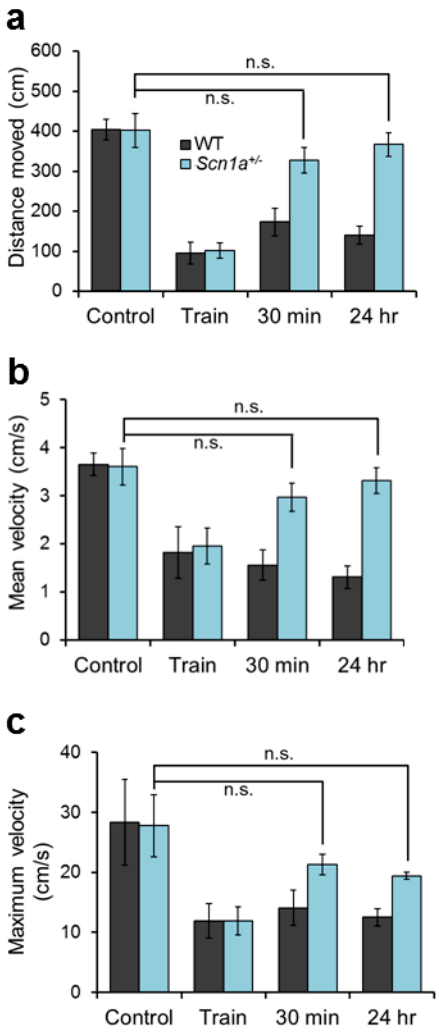

**Supplementary Figure 10. Olfactory discrimination test using the three-chamber experimental paradigm.** A 3-chamber test was used to test the olfactory sensing in WT and *Scn1a*$^{+/-}$ mice. **a**, Both WT and *Scn1a*$^{+/-}$ mice spent more time in a chamber, where the olfactory cue (food odor) was located. **b**, Both WT and *Scn1a*$^{+/-}$ mice preferred to interact with the food olfactory cue. **c**, Both WT and *Scn1a*$^{+/-}$ mice entered the olfactory cue chamber more frequently. **d**, Both WT and *Scn1a*$^{+/-}$ mice spent less time finding the olfactory cue chamber than the other chamber. NF, No Food odor. C, Center. F, Food odor. All data shown are means ± s.e.m. from 9 mice per genotype. ***$P$ < 0.001.



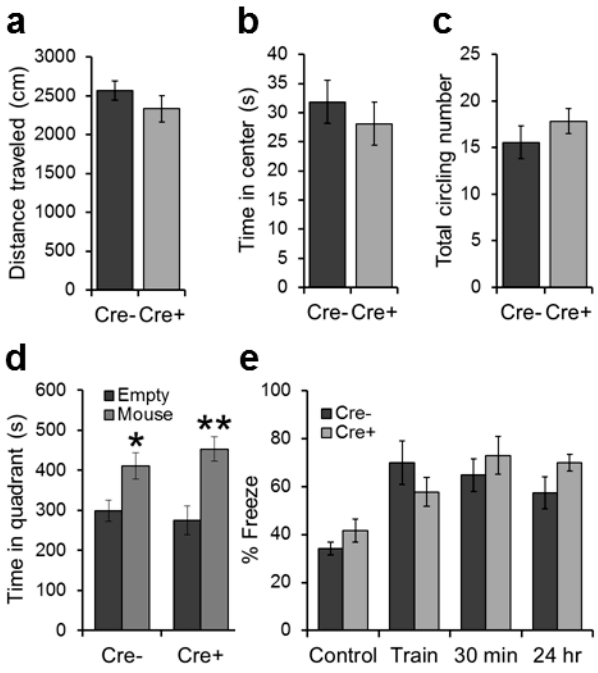

**Supplementary Figure 11. Olfactory preference test using social cues.** **a**, **b**, WT mice spent more time in the chamber, where female bedding (**a**) or male bedding (**b**) was located. In contrast, *Scn1a*$^{+/-}$ mice displayed no difference in time spent between clean bedding and female bedding (**a**), and spent less time in the male bedding area compared with the clean bedding area (**a**). **c**, Close interaction of mice with social olfactory cues was calculated by subtracting the interaction time with a neutral cue from the interaction time with a social cue. Whereas WT mice spent more time with both male and female social cues, *Scn1a*$^{+/-}$ mice avoided interacting with the male social cue. **d, e**, Whereas WT mice spent more time in the chamber with male bedding (**d**) or female bedding (**e**) than in the chamber with clean bedding, *Scn1a*$^{+/-}$ mice had no preference for either chamber. **f**, Both WT mice and *Scn1a*$^{+/-}$ mice displayed strong avoidance to fox urine, which had never been exposed before to the test mice. ‡, 95% Confidence Interval does not include zero. CB, Clean Bedding. C, Center. FB, Female Bedding. MB, Male Bedding. M, Male. F, Female. W, Water. FU, Fox Urine. All data shown are means ± s.e.m. from 9 mice per genotype. *$P$ < 0.05, **$P$ < 0.01, $^{\#}$,***$P$ < 0.001.



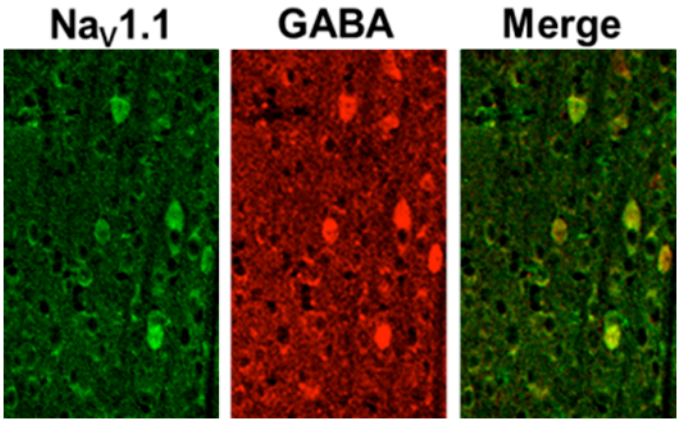

**Supplementary Figure 12. Olfactory habituation/dishabituation and olfactory choice tests. a**, Whereas WT mice exhibited significant habituation and dishabituation to banana flavor (B), male mouse urine (U), and finely ground food pellet (F), *Scn1a*$^{+/-}$ mice exhibited no significant habituation and dishabituation to the odor stimuli, but exhibited strong habituation and dishabituation behavior to ground food pellet (F). **b**, Whereas WT mice displayed no digging behavior during odor presentation, *Scn1a*$^{+/-}$ mice displayed substantially increased digging behavior when banana flavor and male urine were presented indicating that these odors are aversive to *Scn1a*$^{+/-}$ mice. **c**, **d**, Y-maze olfactory choice test. Whereas WT mice displayed strong preference to the odor-containing arm (**c**), *Scn1a*$^{+/-}$ mice displayed strong avoidance to the odor-containing arm (**d**). All data shown are means ± s.e.m. from 7 mice per genotype. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $^{\#}P < 0.05$ for dishabituation; $^{\ddagger}P < 0.05$ for habituation.



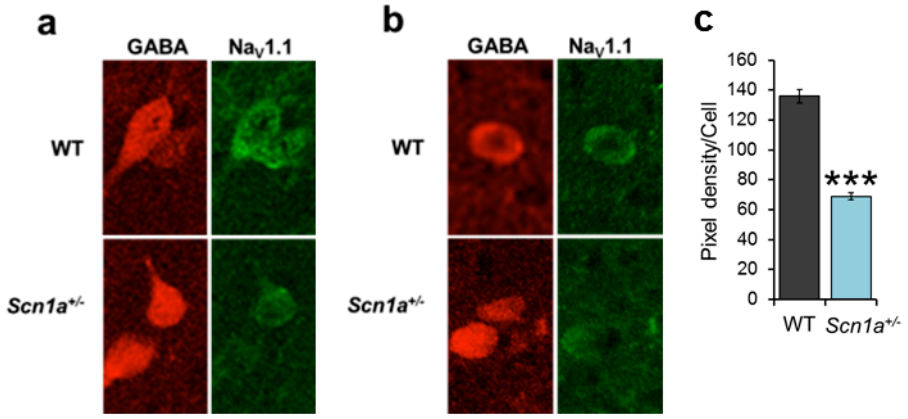

**Supplementary Figure 13. Behavioral parameters during contextual fear conditioning.** Behavioral parameters, such as total distance moved (**a**), mean velocity (**b**), and maximum velocity (**c**) were measured during contextual fear conditioning (Fig. 3c). *Scn1a*$^{+/-}$ mice display substantially increased activities during both test sessions when compared with wildtype littermates. These increased activities are simply a reflection of the decreased freezing behaviors. However, the increased activity during testing is not greater than the activity during control session in *Scn1a*$^{+/-}$ mice, which indicates that *Scn1a*$^{+/-}$ mice display no panic-fleeing responses, and therefore do not perceive the shock chamber as a fearful context. All data shown are means ± s.e.m from 9 mice per genotype. n.s., Not Significant ($P > 0.05$).



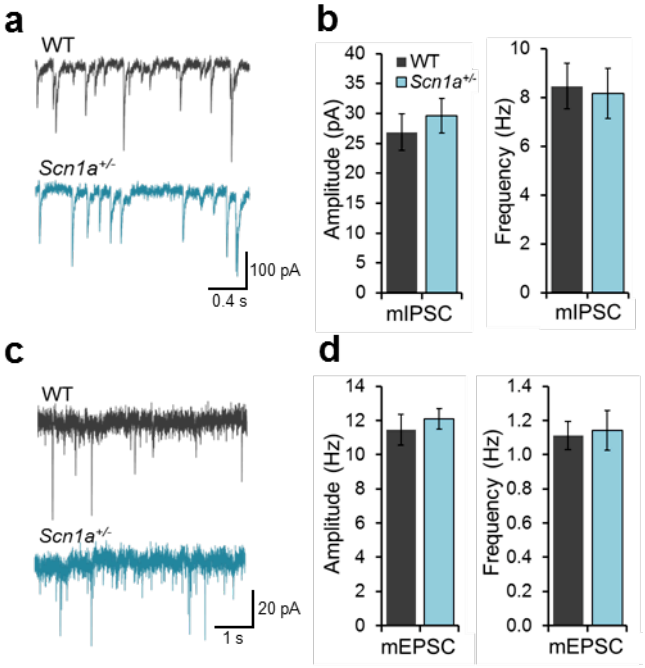

**Supplementary Figure 14. No behavioral phenotypes in the Dlx1/2-I12b-Cre transgenic mice.** Open field (**a - c**), social preference tests (**d**), and contextual fear conditioning (**e**) were performed to test the effects of Cre transgene expression in the mice's behaviors. The data show that Dlx1/2-I12b-Cre transgenic mice display no autism-related phenotypes or impaired context-dependent fear memory, which are observed in the Dlx-Cre$^{+}$ *Scn1a*$^{f/+}$ mice. All data shown are means ± s.e.m. from 7 mice per genotype.



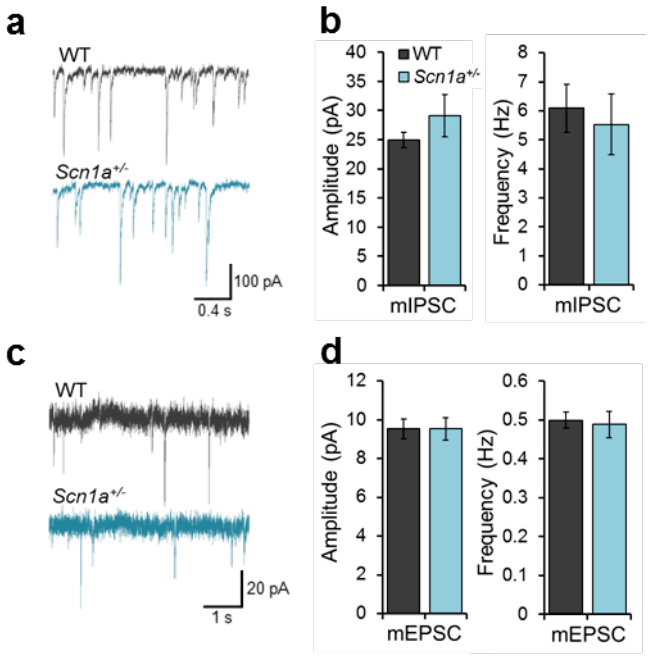

**Supplementary Figure 15. Co-expression of Na$_V$1.1 and GABA in the prefrontal cortex.** Co-immunolabeling of Na$_V$1.1 and GABA revealed co-expression of Na$_V$1.1 and GABA in the deep layer of prefrontal cortex in WT mice.



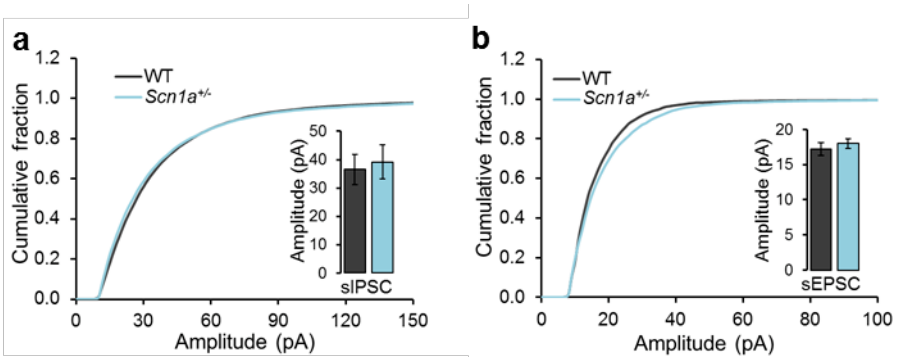

**Supplementary Figure 16. Reduced expression of Na$_V$1.1 channels in *Scn1a*$^{+/-}$ GABAergic interneurons. a**, **b**, Co-immunolabeling of Na$_V$1.1 and GABA revealed reduced expression of Na$_V$1.1 and GABA in the hippocampal CA1 region (**a**), and in the deep layer of prefrontal cortex (**b**) in *Scn1a*$^{+/-}$ mice. **c**, Average pixel density per cell was significantly decreased in the *Scn1a*$^{+/-}$ GABAergic neurons in the deep layer of prefrontal cortex. No reduction in the total number of GABAergic neurons per microscopic field was observed: hippocampal CA1 (n= 19.6 ± 0.4 for WT, n= 20.2 ± 0.4 for *Scn1a*$^{+/-}$; p = 0.41, Student's t-test); in prefrontal cortex (n= 34.7 ± 2.6 for WT, n= 34.6 ± 2.3 for *Scn1a*$^{+/-}$; p = 0.98, Student's t-test). All data shown are means ± s.e.m. ***$P < 0.001$.



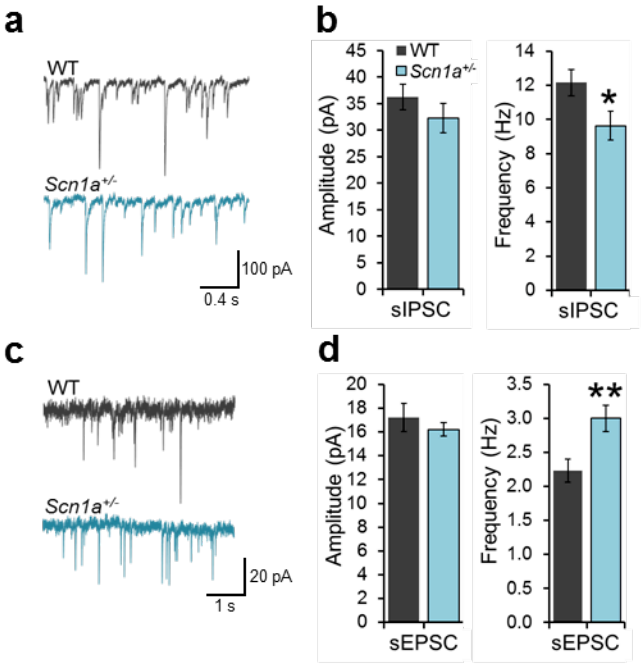

**Supplementary Figure 17. Intact synaptic functions in hippocampal GABAergic interneurons**. **a**, example traces of miniature IPSCs from WT and *Scn1a*$^{+/-}$ mice hippocampal CA1 region. **b**, the amplitude and the frequency of miniature IPSCs were unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices. **c**, example traces of miniature EPSCs from WT and *Scn1a*$^{+/-}$ mice hippocampal CA1 region. **d**, the amplitude and the frequency of miniature EPSCs were unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices. All data shown are means ± s.e.m. from 9 -11 recordings per genotype.



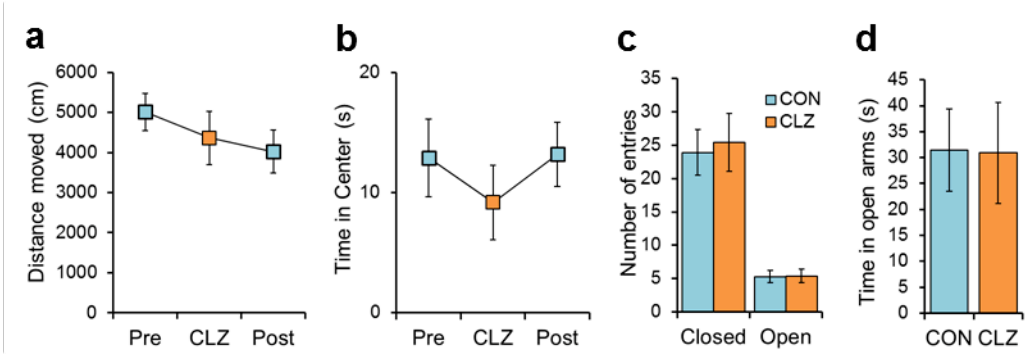

**Supplementary Figure 18. Intact synaptic functions in prefrontal cortex GABAergic interneurons**. **a**, example traces of miniature IPSCs from WT and *Scn1a*$^{+/-}$ mice prefrontal cortex. **b**, the amplitude and the frequency of miniature IPSCs were unchanged in *Scn1a*$^{+/-}$ prefrontal cortex slices when compared to WT slices. **c**, example traces of miniatures EPSCs from WT and *Scn1a*$^{+/-}$ mice prefrontal cortex. **d**, the amplitude and the frequency of miniature EPSCs were unchanged in *Scn1a*$^{+/-}$ prefrontal cortex slices when compared to WT slices. All data shown are means ± s.e.m. from 11 recordings per genotype.



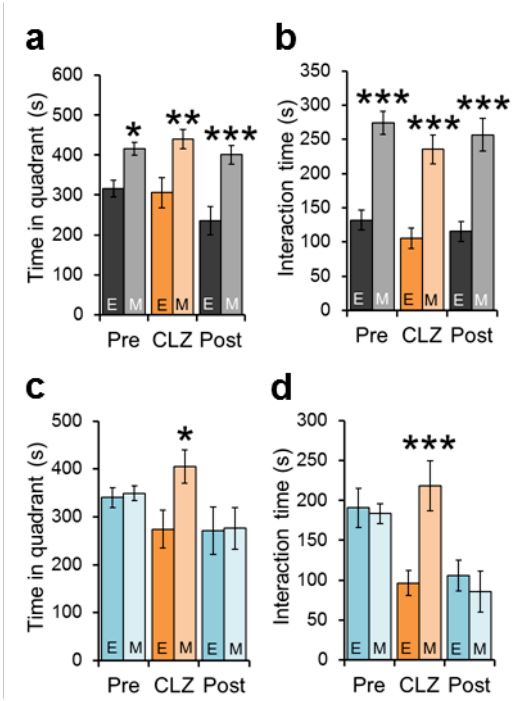

**Supplementary Figure 19. GABAergic neurotransmission in *Scn1a*$^{+/-}$ hippocampal GABAergic interneurons.** **a**, Cumulative plot and average values (inset) of spontaneous IPSC amplitude. The amplitude of spontaneous IPSCs was unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices. **b**, Cumulative plot and average values (inset) of spontaneous EPSC amplitude. The amplitude of spontaneous EPSCs was unchanged in *Scn1a*$^{+/-}$ hippocampal CA1 slices when compared to WT slices. All data shown are means ± s.e.m. from 15 - 19 recordings per genotype.



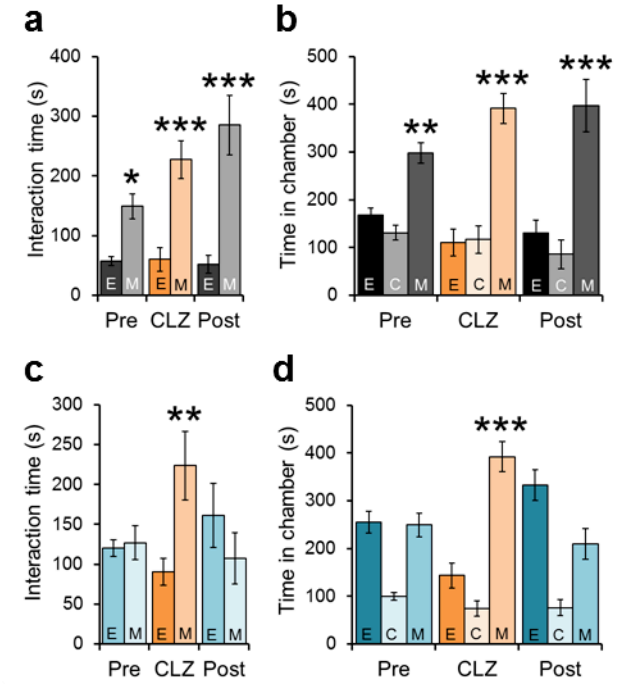

**Supplementary Figure 20. Deficit of spontaneous GABAergic neurotransmission in *Scn1a*$^{+/-}$ prefrontal cortex GABAergic interneurons. a,** example traces of spontaneous IPSCs from WT and *Scn1a*$^{+/-}$ mice prefrontal cortex. **b**, the frequency of spontaneous IPSCs was decreased, but the amplitude of spontaneous IPSCs was unchanged in *Scn1a*$^{+/-}$ prefrontal cortex slices when compared to WT slices. **c**, example traces of spontaneous EPSC from WT and *Scn1a*$^{+/-}$ mice prefrontal cortex region. **d**, the frequency of spontaneous EPSCs was increased, but the amplitude of spontaneous EPSCs was unchanged in *Scn1a*$^{+/-}$ prefrontal cortex slices when compared to WT slices. All data shown are means ± s.e.m. from 14 - 16 recordings per genotype. **P* < 0.05, ***P* < 0.01.



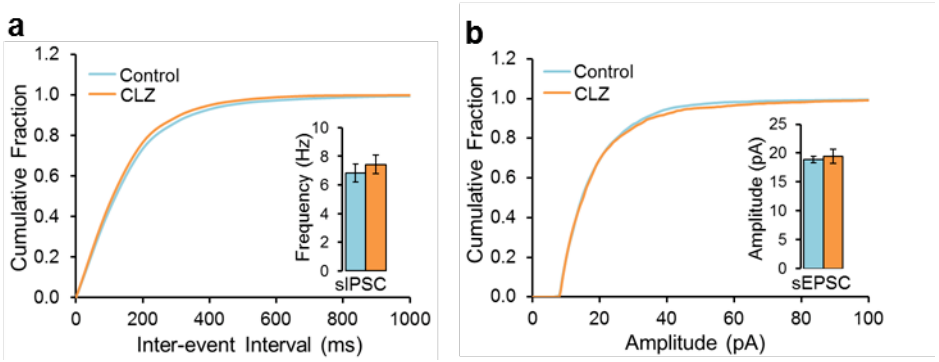

**Supplementary Figure 21. No sedative or anxiolytic effect of low-dose clonazepam *Scn1a*$^{+/-}$ mice. a**, In the open field test, the locomotor activity of *Scn1a*$^{+/-}$ mice was not changed by 0.0625 mg/kg clonazepam (CLZ) treatment. **b**, an anxiolytic effect was not observed in *Scn1a*$^{+/-}$ mice by 0.0625 mg/kg CLZ treatment. **c**, **d**, An elevated plus maze test was performed to further test the anxiolytic effect of 0.0625 mg/kg CLZ on *Scn1a*$^{+/-}$ mice. Low-dose clonazepam did not elicit anxiolytic effects on *Scn1a*$^{+/-}$ mice. Pre, Pre-clonazepam treatment. CLZ, Clonazepam. Post, Post-clonazepam treatment. CON, Control. All data shown are means ± s.e.m. from 10 - 12 mice per genotype. There are no significant effects of CLZ treatment ($P > 0.05$).




**Supplementary Figure 22. Recovery of social interaction deficits by treatment with low-dose clonazepam**. **a**, **b**, In the social interaction test, the social interaction preference in WT mice was not changed by 0.0625 mg/kg CLZ injection, measured by time spent in the quadrant (**a**), or by close interaction time (**b**). **c**, **d**, *Scn1a*$^{+/-}$ mice showed completely recovered social interaction behaviors after a 0.0625 mg/kg CLZ injection, measured by time spent in the quadrant (**c**), or by close interaction time (**d**), and the CLN effect completely disappeared after a 1 week period of drug clearance in the same mice. E, Empty cage. M, Mouse. Pre, Pre-clonazepam treatment. CLZ, Clonazepam. Post, Post-clonazepam treatment. All data shown are means ± s.e.m. from 10 - 11 mice per genotype. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.




**Supplementary Figure 23. Recovery of social preference deficits by treatment with low-dose clonazepam. a**, **b**, In the three-chamber test, the social interaction preference in WT mice was not changed by 0.0625 mg/kg CLZ injection, measured by time spent in close interaction (**a**), or by time spent in the chamber (**b**). **c**, **d**, *Scn1a*$^{+/-}$ mice showed completely recovered social interaction behaviors after a 0.0625 mg/kg CLZ injection, measured by time spent in the quadrant (**c**), or by time spent in the chamber (**d**), and the CLZ effect completely disappeared after a 1-week period of CLZ injection in the same mice. E, Empty cage. C, Center. M, Mouse. Pre, Pre-clonazepam treatment. CLZ, Clonazepam. Post, Post-clonazepam treatment. All data shown are means ± s.e.m. from 10 - 12 mice per genotype. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.




**Supplementary Figure 24. Effects of low-dose clonazepam treatment on GABAergic neurotransmission. a**, Cumulative plot and average values (insets) of spontaneous IPSC frequency. The frequency of spontaneous IPSCs was unchanged by 10 μM CLZ in *Scn1a*$^{+/-}$ hippocampal CA1 slices. **b**, Cumulative plot and average values (insets) of spontaneous EPSC amplitude. The amplitude of spontaneous EPSC was unchanged by 10 μM CLZ in *Scn1a*$^{+/-}$ hippocampal CA1 slices. All data shown are means ± s.e.m. from 15 - 20 recordings per treatment group.