Published in final edited form as:
Genes Brain Behav. 2008 August ; 7(6): 629–638. doi:10.1111/j.1601-183X.2008.00399.x.

Exaggerated emotional behavior in mice heterozygous for the sodium channel *Scn8a* ($Na_v1.6$)

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Abstract

The *SCN8A* gene encodes the α-subunit of $Na_v1.6$, a neuronal voltage-gated sodium channel. Mice homozygous for mutations in the *Scn8a* gene exhibit motor impairments. Recently a human family with a heterozygous protein truncation mutation in *SCN8A* has been described. Rather than motor impairment, neuropsychological abnormalities were more common, suggesting a role for *SCN8A* in a more diverse range of behaviors. Here, we characterize mice heterozygous for a null mutation of *Scn8a* (*Scn8a*$^{+/-}$ mice) in a number of behavioral paradigms. We demonstrate that *Scn8a*$^{+/-}$ mice exhibit greater conditioned freezing in the Pavlovian fear conditioning paradigm, but no apparent abnormalities in other learning and memory paradigms including the Morris water maze and conditioned taste avoidance paradigm. Further, we find that *Scn8a*$^{+/-}$ mice exhibit more pronounced avoidance of well-lit, open environments as well as more stress-induced coping behavior. Together, this data suggests that *Scn8a* plays a critical role in emotional behavior in mice. Although the behavioral phenotype observed in the *Scn8a*$^{+/-}$ mice only partially models the abnormalities in the human family, we anticipate that the *Scn8a*$^{+/-}$ mice will serve as a valuable tool for understanding the biological basis of emotion and the human diseases in which abnormal emotional behavior is a primary component.

Introduction

The *SCN8A* gene encodes the α-subunit of $Na_v1.6$, a neuronal voltage-gated sodium channel which is widely expressed in the nervous system (Caldwell *et al.* 2000; Krzemien *et al.* 2000; Tzoumaka *et al.* 2000). $Na_v1.6$ is one of a number of voltage-gated sodium channels that mediate the rising phase of the action potential and also plays a critical role in high-frequency firing in a variety of cell types (Cummins *et al.* 2005; Enomoto *et al.* 2006; Levin *et al.* 2006; Van Wart & Matthews 2006). Several mutations in the *Scn8a* gene have been identified in mice (Burgess *et al.* 1995; Kohrman *et al.* 1996; Meisler *et al.* 2004) and all of these mutations give rise to some form of motor impairment. The most severe of these motor impairments are observed in mice homozygous null for *Scn8a*, which become paralyzed and die by 3 weeks of age. Mice heterozygous for null mutations of *Scn8a* (*Scn8a*$^{+/-}$ mice),

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however, exhibit no obvious motor impairments (Burgess *et al.* 1995). While most attention has been focused upon the motor impairments associated with mutations of *Scn8a* in mice, recent evidence suggests that mutations of the human ortholog result in substantial neuropsychological abnormalities.

A screen of patients with inherited and sporadic ataxia identified a family in which four members were heterozygous for a protein truncation mutation in *SCN8A* (Trudeau *et al.* 2006). The proband in this screen was a 9-year-old boy with marked delay of cognitive, gross motor, and fine motor development as well as a diagnosis of stimulant-responsive attention deficit disorder. The three other heterozygous family members exhibit cognitive impairment and/or neuropsychological abnormalities. The mother has a history of mild cognitive impairment and emotional instability, the maternal aunt has mild cognitive impairment, and her son has been diagnosed with attention deficit hyperactivity disorder and is learning disabled. The segregation of the *SCN8A* mutation with cognitive impairments and neuropsychological abnormalities in this family suggests a role for *SCN8A* in cognition and behavior. Interestingly, none of the mutation-carrying family members, other than the proband, exhibited motor dysfunction. Further suggesting a role for *SCN8A* in complex behaviors and emotional states is a recent report describing a substantial preferential transmission of one allele of a single-nucleotide polymorphism of *SCN8A* in individuals attempting suicide (Wasserman *et al.* 2005).

Motivated by these studies implicating heterozygous mutations of *SCN8A* in impaired cognition and abnormal neuropsychological states, we have carried out a series of behavioral experiments using mice that are heterozygous for a null mutation of *Scn8a* in an attempt to better understand the role of *Scn8a* in brain functions.

Materials and methods

Mice

Experiments were carried out on mice heterozygous for a null mutation, the *Scn8a*^{tg} mutation (Burgess *et al.* 1995), in sodium channel *Scn8a* (*Scn8a*$^{+/-}$ mice) and their wild-type littermates (WT mice). In our laboratory, the *Scn8a*^{tg} mutation has been maintained on a C57BL/6J background by successively crossing offspring carrying the mutation with C57BL/6J wild-type mice purchased from The Jackson Laboratory (Bar Harbor, ME) for more than ten generations. To generate experimental mice, *Scn8a*$^{+/-}$mice were crossed with WT mice. Three groups of mice were studied: group A (12 *Scn8a*$^{+/-}$ and 9 WT), group B (42 *Scn8a*$^{+/-}$ and 28 WT), and group C (15 *Scn8a*$^{+/-}$ and 13 WT). The open field, wire hang, accelerating rotarod, light-dark box, forced-swim test, and shock sensitivity assessment were performed, in the listed order, on group A. Group B was first tested in the Morris water maze, a subset was subsequently evaluated in the Pavlovian fear conditioning paradigm (protocol 2), and a subset of these mice were used in the conditioned taste avoidance paradigm. Group C was first tested in the Morris water maze and then used for Pavlovian fear conditioning (protocol 1). Individual tests in the test batteries were separated at least by one week. Mice were housed in ventilated cages under uniform conditions including a 12h-12h light-dark cycle with lights on at 6 AM, average temperature of 22° C and *ad libitum* food and water. Mice were housed together in groups of 3–5 with same-sex siblings and were tested between 1 PM and 5 PM. Approximately equal numbers of mice of each sex were studied. Experimental groups were not large enough for separate analysis of female and male subjects so mice of the same genotype were pooled for analysis. Because of this, it is possible that sex effects may have been mistaken for genotype effects. That is, genotype effects may have been underestimated or overlooked if they were sex-dependent or if the baseline differences between female and male mice increased the variance in the data. Therefore, ANOVA models were used to test for the sex-dependence of the genotype effects. A three-way repeated measures ANOVA with between-subject factors

for genotype and sex and a repeated measure for training day was applied to Morris water maze, Pavlovian fear conditioning, and accelerating rotarod data; a three-way ANOVA with factors for genotype, sex, and pairing group was used for conditioned taste avoidance; and a two-way ANOVA with factors for genotype and sex was utilized for open field, light-dark box, and forced swim test. These analyses did not reveal any measures in which there was a significant effect of sex or a sex-genotype interaction.

All experiments were conducted according to NIH guidelines for animal care and were approved by the University Committee on Use and Care of Animals of the University of Michigan.

Morris water maze

The Morris water maze (MWM) was performed as previously described (McKinney & Murphy 2006; McKinney *et al.* 2007). The pool was 1.2 meters in diameter and filled with water made opaque with white non-toxic paint. The escape platform consisted of a 10 cm platform submerged 0.5 cm below the surface of the water in the center of one of the quadrants. Water was maintained at 25 ± 2° C. The walls surrounding the pool were adorned with high-contrast posters for use as distal cues. The room was lit by indirect white light (200 lux in center of pool).

For 10 days prior to training, mice were handled for 2–3 minutes once daily. Every training trial began with the mouse on the platform for 15 seconds. The mouse was then placed into the water facing the wall of the pool and allowed to search for the platform. The trial ended either when the mouse climbed onto the platform or when 60 seconds had elapsed. At the end of each trial the mouse was allowed to rest on the platform for 15 seconds. Mice were given 6 trials per day (in blocks of two trials, 1 min inter-trial intervals and 1 hr inter-block intervals) for 5 days, with the starting position chosen pseudo-randomly among 6 start positions. A probe trial was conducted 24 hours after the end of training (on day 6). During the probe trial, the escape platform was removed and mice were placed in the pool at the start location directly opposite of where the platform was previously located and allowed to swim for 60 seconds. Mice were run in the visible-platform version of the MWM 24 hours after the probe trial. The visible-platform version consisted of a single day of training with 6 trials during which the platform was moved to a new location and marked with a distinct local cue. The MWM data presented are from three separate experiments. During the initial MWM experiment it was observed that a number of *Scn8a*$^{+/-}$ mice did not explore the MWM but rather floated passively upon placement in the water. Therefore mice that floated for more than 30 seconds on at least 75% of the trials were eliminated from analysis. In subsequent experiments, if a mouse floated more that 30 seconds on 5 consecutive trials, it was eliminated from the study at that point. All MWM data was acquired with a digital video camera 1.5 meters from the water surface. Images from the digital camera were processed and stored on a Dell Omniplex 270 computer using Actimetrics WaterMaze Software (Actimetrics, Wilmette, IL).

Pavlovian fear conditioning

Pavlovian fear conditioning was performed as previously described (McKinney & Murphy 2006; McKinney *et al.* 2007). The Pavlovian fear conditioning apparatus (Med Associates Inc., St. Albans, VT) consisted of 4 conditioning chambers each with a stainless steel grid floor designed for mice, through which the unconditioned stimulus (US; foot shock) was delivered. The grid floor is over a stainless steel drop-pan, which was lightly cleaned with 95% ethyl alcohol to provide a background odor. The conditioning chambers were arranged in a 2 × 2 configuration on a steel rack in an isolated room lit by adjustable indirect lighting (150 lux at center of chamber) and each chamber was outfitted with an individual video camera. Fear was assessed by measuring freezing behavior. Freezing was defined as the absence of movement

except that associated with respiration and was measured by subjecting the video signal to a sensitive global motion-detection algorithm (Freezeframe and Freezeview software; Actimetrics, Wilmette, IL). Freezing data is presented as per cent freezing which is the amount of time an individual animal spent freezing divided by the duration of the trial and multiplied by 100.

Two conditioning protocols were used. In the first, mice received 3 training trials (1 trial per day) in which a 3 minute baseline was followed by a 30 second tone which co-terminated with a 2 second, 0.70 mA foot shock delivered via the grid floor. Mice were removed from the chambers after an additional 30 seconds. Twenty-four hours after the last training trial (on day 4), context conditioning was assessed by returning mice to the same chambers and assessing freezing during a 5-minute trial in the absence of tone or shock. Cued conditioning was assessed on the following day (day 5). For cued conditioning, the conditioning chambers were re-configured by using white plastic inserts that covered the grid floor and walls to change the appearance and geometry of the chambers (i.e., semi-circular instead of square). In addition the chamber was cleaned with 2% acetic acid (as opposed to 95% ethanol) to provide a novel background odor. After 2 minutes of baseline, freezing was measured in response to a 3 minute tone.

In the second protocol, mice were placed in the chambers and received five 0.7 mA, 2 sec unsignaled foot shocks on day 1. Stimulus-free periods (2 minutes) preceded, separated, and followed the foot shocks. On the day following training (day 2), a subset of the mice of each genotype were returned to the conditioning chambers for a 60 min shock-free session while the remaining mice remained in their home cages to serve as a retention controls. Twenty-four hours later (day 3), all mice were returned to the same chambers for a 5 minute test of context fear.

Shock sensitivity

To measure shock sensitivity, naïve mice were placed individually in a mouse fear conditioning chamber and given 1-second foot shocks of increasing intensity starting at 0.05 mA and increased in 0.05 mA increments. Intensity of foot shock required to first elicit each of the following three responses was measured: flinching (startle or crouching), jumping (at least two paws leaving grid floor), and vocalizing (any auditory response).

Conditioned taste avoidance

Conditioned taste avoidance (CTA) was performed as in Josselyn et al. (2004). Mice were singly-housed in cages with food, but no water for 20 hours before the experiment. During a 5-day habituation period, mice were given access to two drinking bottles filled with water for decreasing periods of time each day (5 hours, 2 hours, 1 hour, 30 minute, 30 minute drinking period). On day 6, mice were presented with a single bottle filled with 2 mM saccharin for 30 minutes. Thirty minutes after the drinking session, mice were injected with 0.15 M LiCl at a dose of 2% body weight. Twenty four hours later (day 7), mice were given a choice test in which they were presented with two bottles for 30 minutes; one containing 2 mM saccharin and the other containing water. Relative bottle location (i.e., left vs. right side) was counterbalanced to control for any location preferences that may have existed. Liquid consumed from each bottle was measured and an avoidance index (AI) calculated as follows: [(grams of water consumed) / (grams of water + grams of saccharin consumed)] × 100.

Home cage activity

Mice were separated from littermates and individually housed in ventilated home cages under familiar conditions (see above) for a 48 hour acclimation period. Following acclimation, lids were removed from the cages and the cages were placed below a ceiling mounted tracking

camera for 2 hours. Total distanced traveled was measured using the video signals from the digital camera sent to a desktop PC and processed on-line using Actimetrics LimeLight software (Actimetrics, Wilmette, IL).

Open field

The open field experiments were conducted as previously described (McKinney & Murphy 2006; McKinney *et al.* 2007). Mice were placed singly in the center of the white acrylic chamber (71 × 71 × 30 cm) lit by indirect white light (200 lux at center of chamber) and allowed to explore for 5 minutes. The open field was divided into an 8 × 8 grid which contained a center zone (53.25 × 53.25 cm) and a peripheral zone (the outer 8.875 cm on all sides). Total distance traveled and distance traveled in center zone was measured using the video signals from digital cameras sent to a desktop PC and processed on-line using Actimetrics LimeLight software (Actimetrics, Wilmette, IL).

Light-dark box

The light-dark box was performed as previously described (McKinney *et al.* 2007). The light-dark box is 46 cm long with two-thirds of the length comprising the light compartment (made of white acrylic) and one-third comprising the dark compartment (made of black acrylic with a lid). Mice were placed in the light compartment under indirect white lighting (200 lux at center of compartment) and their behavior was observed for 10 minutes. Total time spent in the light compartment and the number of light-dark transitions between the two compartments was scored by using Actimetrics LimeLight software (Actimetrics, Wilmette, IL).

Forced swim test

The forced swim apparatus is composed of a Plexiglas cylinder submerged in the Morris water maze pool. The cylinder is 76 cm in height and 25 cm in diameter, the lower half of the cylinder has holes in it to allow free exchange of water between the cylinder and pool. The pool was filled to a height of approximately 46 cm and water temperature was maintained at 25±2° C. Mice were placed individually into the cylinder. A test duration of 6-min was used. All test sessions were recorded by a digital camera positioned directly above the cylinder. Video signals from the digital camera were sent to a desktop PC, stored, and subsequently scored by a trained observer blind to genotype of the mice. Latency to first immobility and duration of immobility during the test period were scored. A mouse was judged to be immobile when making only those movements necessary to keep its head above water.

Wire hang test

The wire hang test was performed as described previously (Levin *et al.* 2006). Mice were placed on a sheet of wire mesh and the sheet was inverted for a maximum of 60 seconds. The latency of the mouse to lose its grip and fall from the sheet to a padded surface was measured.

Rotarod

The rotarod was performed as previously described (McKinney & Murphy 2006; McKinney *et al.* 2007). Mice were placed on the rotating drum (3 cm diameter) of an accelerating rotarod (UGO Basile Accelerating Rotarod) and the time that each mouse was able to walk on top of the drum was measured. The speed of the rotarod accelerated from 4 to 40 rpm over a 5-minute period. Mice were given 1 trial/day for 5 days with a maximum time of 300 seconds (5 minutes). Latency to fall or to first passive rotation was measured.

Statistical Analysis

All data are presented as mean ± SEM. Performance measured during training in the Morris water maze, Pavlovian fear conditioning/extinction and accelerating rotarod were analyzed by repeated-measures ANOVA with a between-subject factor for genotype and a repeated measure for training trial or day. Because three separate Morris watermaze experiments were conducted, a two-way repeated-measures ANOVA with a between-subject factor for experiment number and a repeated measure for training trial or day was performed to assure that there were no differences between experiments before they were combined for additional analysis. Freezing measured 24 hours after extinction training (long-term extinction) was subjected to a two-way ANOVA with genotype and training group (extinction or retention control) as factors. The conditioned taste avoidance data was analyzed using a two-way ANOVA with genotype and pairing group as factors. Two-tailed t-tests between genotypes were used to analyze data from the open field, light-dark box, and forced swim test as well as some of data from the Morris water maze and Pavlovian fear conditioning (as noted in the text). Results were considered significantly different when $P < 0.05$.

Results

A subset of *Scn8a*$^{+/-}$ mice float excessively in the Morris water maze, complicating the assessment of spatial learning and memory

Forty-two *Scn8a*$^{+/-}$mice and twenty-eight WT mice were tested in the MWM, however, sixteen failed to complete the task due to excessive floating reminiscent of stress-induced immobility that is observed in the related forced swim test, a common test for screening anti-depressant efficacy (Jacobson & Cryan 2007; Porsolt *et al.* 1977). Excessive floating was observed in fourteen *Scn8a*$^{+/-}$mice (33% of those tested) and two WT mice (7% of those tested). For mice in the first of the three MWM experiments (15 *Scn8a*$^{+/-}$mice and 13 WT mice), total floating across the five days of training was summed and compared between genotypes. *Scn8a*$^{+/-}$mice exhibited significantly more total floating than WT mice (347.5 ± 110.2 seconds for *Scn8a*$^{+/-}$mice and 40.0 ± 10.6 seconds for WT mice; $t_{(1,26)} = 2.6$, $p < 0.05$; data not shown). Complete floating data was not collected for the remaining two MWM experiments as mice were eliminated once they reached criteria for exclusion (see methods). Among the mice remaining after eliminating those that exhibited excessive floating, MWM performance did not differ between genotypes [*Scn8a*$^{+/-}$mice (N = 28) and WT mice (N = 26)]. As acquisition training progressed, mice of both genotypes exhibited significant decreases in the latency required to find the platform ($F_{(4,208)} = 65.95$, $p < 0.0001$; Figure 1A), but there was no difference between genotypes ($F_{(1,52)} = 2.26$, $p > 0.05$) or significant interaction between training day and genotype ($F_{(4,208)} = 0.61$, $p > 0.05$). During the probe trial (Figure 1B), both *Scn8a*$^{+/-}$and WT mice spent significantly more time in the quadrant where the platform was previously located than would be expected by random searching ($t_{(1,27)} = 8.06$ $p < 0.0001$ and $t_{(1,25)} = 8.59$, $p < 0.0001$, respectively using a single group t-test with a hypothesized mean of 25% which would be chance performance). However, there was no significant difference in the amount of time that *Scn8a*$^{+/-}$mice spent in the training quadrant compared to their WT littermates ($t_{(1,52)} = 1.52$; $p > 0.05$). In the visible-platform version of the MWM, when the platform is marked with a distinct proximal cue (a flag), both groups found the platform with similar average latencies across the 6 trials ($t_{(1,52)} = 0.67$, $p > 0.05$; Figure 1C).

Scn8a$^{+/-}$ mice exhibit more freezing during Pavlovian fear conditioning

Prior to the first tone-shock pairing on day 1 using the first protocol, neither *Scn8a*$^{+/-}$(N = 15) nor WT (N = 13) mice exhibited significant freezing (Figure 2A). As training progressed, both genotypes exhibited significant increases in freezing to context ($F_{(2,52)} = 102.92$; $p < 0.0001$). *Scn8a*$^{+/-}$mice consistently displayed significantly more freezing than the WT mice ($F_{(1,26)} = 11.70$; $p < 0.01$; Figure 2A). Exposure to the context alone (in the absence of tone) on day 4

also produced significantly more freezing in *Scn8a*$^{+/-}$mice ($F_{(1,26)} = 5.9$; $p < 0.05$; Figure 2B). On the day after the context test, cued conditioning was assessed in a reconfigured context. Interestingly, *Scn8a*$^{+/-}$mice exhibited significantly more freezing upon being placed in the reconfigured context and prior to the tone than WT mice; that is the Scn8a +/− mice exhibited greater generalization of freezing to the new context. (15 ± 3% vs. 7± 1%, respectively, t = 2.6; $p < 0.05$).Both *Scn8a*$^{+/-}$and WT mice froze significantly more after the tone presentation than at baseline ($F_{(1,26)} = 26.1$; $p < 0.0001$; Figure 2C), however, *Scn8a*$^{+/-}$mice froze more than WT mice ($F_{(1,26)} = 65$; $p < 0.05$; Figure 2C), but there was no genotype-tone interaction ($F_{(1,26)} = 1.43$; $p > 0.05$; Figure 2C).

Neither *Scn8a*$^{+/-}$(N = 31) nor WT (N = 24) mice froze significantly prior to the first shock of the second conditioning protocol (Figure 3A). During the second training protocol, both genotypes exhibited significant increases in freezing as training progressed ($F_{(5,53)} = 298.14$; $p < 0.0001$; Figure 3A), however, *Scn8a*$^{+/-}$mice, consistently froze more than WT mice during the 2 minutes between and following shocks ($F_{(1,53)} = 9.93$; $p < 0.05$; Figure 3A). Twenty-four hours after conditioning (on day 2), extinction training was performed on a subset of mice (N = 21 for *Scn8a*$^{+/-}$mice and N = 16 for WT mice) by returning them to the conditioning chambers for one hour. Both genotypes exhibited a significant decrease in freezing across the hour suggesting that extinction training was successful for both *Scn8a*$^{+/-}$and WT mice ($F_{(5, 175)} = 11.40$; $p < 0.0001$; Figure 3B). Although the *Scn8a*$^{+/-}$mice froze more than WT mice throughout the one-hour session ($F_{(1,35)} = 5.00$; $p < 0.05$), there was no training-genotype interaction ($F_{(5, 175)} = 0.71$; $p > 0.05$) suggesting that the rates of extinction were not different between the two groups. Twenty-four hours after extinction training (on day 3), all mice were returned to the conditioning chambers to assess long-term extinction. There was a significant effect of genotype ($F_{(1,51)} = 12.46$; $p < 0.001$) and group (extinction group vs. retention controls; $F_{(1,51)} = 82.17$; $p < 0.0001$), but no genotype-group interaction ($F_{(1,51)} = 0.03$; $p > 0.001$) on day 3 freezing.

Finally, in a separate group of mice the minimum shock intensity required to elicit flinching (*Scn8a*$^{+/-}$mice = 0.19 mA and WT mice = 0.17 mA; $t_{(1,19)} = 1.19$), jumping (*Scn8a*$^{+/-}$mice = 0.25 mA and WT mice = 0.22 mA; $t_{(1,19)} = 0.54$), and vocalization (*Scn8a*$^{+/-}$mice = 0.30 mA and WT mice = 0.28 mA; $t_{(1,19)} = 1.80$) was found not to be different between *Scn8a*$^{+/-}$(N= 12) and WT mice (N = 9).

Scn8a$^{+/-}$ mice perform as well as WT mice in the conditioned taste avoidance paradigm

Twenty-four hours following a saccharin-lithium chloride pairing, both *Scn8a*$^{+/-}$mice (N = 7) and WT mice (N =5) displayed significant avoidance of saccharin (avoidance indices = [(grams of water consumed) / (grams of water + grams of saccharin consumed)] × 100; 77.76 ± 3.06 and 79.20 ± 3.36; Figure 4), respectively. While there was an effect of pairing group (saccharin-lithium chloride vs. saccharin-saline pairing) on avoidance index 24 hours after pairing ($F_{(1,20)} = 81.44$; $p < 0.0001$; Figure 4), there was no effect of genotype ($F_{(1,20)} = 1.13$; $p > 0.05$; Figure 4) or pairing group-genotype interaction ($F_{(1,20)} = 0.55$; $p > 0.05$; Figure 4). These data suggest that S*cn8a*$^{+/-}$mice learn to avoid saccharin following a saccharin-lithium chloride pairing as well as WT mice. S*cn8a*$^{+/-}$mice and WT mice that received a saccharin-saline pairing did not display an avoidance of saccharin 24 hours later (avoidance indices of 33.72 ± 2.97 and 41.86 ± 8.31, respectively; Figure 4) suggesting that the aversive nature of the injection does not induce avoidance of saccharin and that S*cn8a*$^{+/-}$mice do no differ from WT mice with respect to their reaction to saccharin presentation.

Avoidance of well-lit, open areas and expression of stressed-induced coping are more pronounced in S*cn8a*$^{+/-}$ mice than WT mice

Locomotor activity in a familiar home cage did not differ between *Scn8a*$^{+/-}$(N = 11) and WT (N = 8) mice (10447.1 ± 1219.5 cm and 10837.9 ± 1010.7 cm, respectively; $t_{(1,17)} = 0.23$; $p > 0.05$) . However, differences were observed when locomotion and exploration were evaluated in novel environments as illustrated in the open-field test and light-dark box. Data from the open field experiments are presented in Figur60e 5. Total distance traveled in the open-field test (Figure 5A) did not differ significantly between *Scn8a*$^{+/-}$mice (N=12) and WT mice (N = 9; $t_{(19)} = 1.24$; $p = 0.21$). Both *Scn8a*$^{+/-}$and WT mice avoided the center zone of the open field as demonstrated by the fact that both genotypes traveled a significantly smaller percentage of total distance traveled in the center zone than would be expected if mice were exploring randomly (i.e., 56.25 % distance; *Scn8a*$^{+/-}$mice = 27.8 ± 8.2% distance; $t_{(11)} = 12.02$; $p < 0.0001$; and WT = 44.6 ± 10.7% distance; $t_{(8)} = 3.28$; $p < 0.05$; Figure 5B). *Scn8a*$^{+/-}$ mice, however, traveled a significantly smaller percentage of total distance on average in the center zone of the open field when compared to WT mice ($t_{(1,19)} = 4.1$, $p < 0.001$; Figure 5B). It seems unlikely that the behavior observed in the *Scn8a*$^{+/-}$mice is due to a motivational deficit as there was no difference between *Scn8a*$^{+/-}$and WT mice in terms of the latency to enter the perimeter for the first time ($t_{(19)} = 1.4$, $p > 0.05$; data not shown) nor did we observe any difference in the latency to renter the center portion of the open field after the first trip to the perimeter ($t_{(19)} = 1.6$, $p > 0.05$; data not shown).

Data from the light-dark box are represented in Figure 6. Both *Scn8a*$^{+/-}$(N = 12) and WT mice (N = 9) avoided the light side of the light-dark box as demonstrated by the fact that both genotypes spent less time in the light side than would be expected if mice were exploring randomly (i.e., 400 seconds; $t_{(11)} = 23.4$, $p < 0.0001$ for *Scn8a*$^{+/-}$mice and $t_{(8)} = 12.9$, $p < 0.0001$ for WT mice; Figure 6A). *Scn8a*$^{+/-}$mice, however, spent less time exploring the light side than WT mice ($t_{(1,19)} = 2.53$, $p < 0.05$; Figure 6A). Additionally, the number of times *Scn8a*$^{+/-}$mice transitioned between the two sides of the light-dark box was significantly smaller than that for WT mice ($t_{(1,19)} = 2.53$, $p < 0.05$; Figure 6B). It is important to note that this decrease in exploratory activity is unlikely due to impaired locomotor activity or lack of motivation as S*cn8a*$^{+/-}$mice did not differ from WT mice with respect to the amount of time it takes to make their first entry into the dark side ($t_{(1,19)} = 0.59$, $p > 0.05$: Figure 6C). Similarly, there was no significant difference in the duration that the two groups spent in the dark side of the light-dark box during their first visit ($t_{(1,19)} = 1.46$, $p > 0.05$; data not shown).

Data from the FST are illustrated in Figure 7. After placement in the forced swim apparatus, latency to the first bout of immobility was much shorter in S*cn8a*$^{+/-}$mice (N = 12) than in WT mice (N = 9; $t_{(1,19)} = 2.59$, $p < 0.05$; Figure 7A). Also, S*cn8a*$^{+/-}$mice spent more total time immobile during the 6-minute session than WT mice ($t_{(1,19)} = 2.28$, $p < 0.05$; Figure 7B).

No major deficits in neuromuscular strength or motor function are observed in *Scn8a*$^{+/-}$ mice

Neuromuscular strength as measured using the wire hang test (Figure 8A) was unimpaired in *Scn8a*$^{+/-}$mice as latency to fall from an inverted piece of wire mesh did not differ between *Scn8a*$^{+/-}$mice (N=12) and WT mice (N=9; $t_{(19)} = 0.89$; $p > 0.05$). The accelerating rotarod was used to assess overall balance and motor coordination (Figure 8B). Both genotypes improved performance across training days as reflected in the significant effect of training day ($F_{4,76} = 14.06$, $p < 0.05$). While there appears to be a trend towards a difference on training days 2–5, there was no statistically significant effect of genotype ($F_{1,19} = 4.36$; $p = 0.06$) or training day-genotype interaction ($F_{4,76} = 1.75$; $p = 0.15$).

Discussion

The principle findings of present study are (1) that S*cn8a*$^{+/-}$mice freeze more than WT mice in response to both cued and contextual stimuli in the Pavlovian fear conditioning paradigm and (2) avoidance of well-lit, open environments as well as expression of stress-induced coping are more pronounced in S*cn8a*$^{+/-}$mice. In aggregate, these findings suggest a role for S*cn8a* in emotional behavior. In the Pavlovian fear conditioning paradigm, S*cn8a*$^{+/-}$mice continue freezing more than WT mice even after extensive context extinction training. This behavior does not seem to reflect globally enhanced cognitive abilities, as *Scn8a*$^{+/-}$mice do not perform differently than WT mice in either the Morris water maze or conditioned taste avoidance paradigm. Further arguing that *Scn8a*$^{+/-}$mice and WT mice do not differ in their cognitive abilities is the observation that *Scn8a*$^{+/-}$mice exhibit similar degrees of within-session and long-term extinction of Pavlovian conditioned fear. Although our experiments suggest that learning and memory abilities do not differ between *Scn8a*$^{+/-}$and WT mice, the possibility remains that *Scn8a*$^{+/-}$mice exhibit a very specific enhancement in learning ability that only manifests in acquisition and/or consolidation of Pavlovian conditioned fear. Finally, it should be noted that because a significant number of the *Scn8a*$^{+/-}$mice exhibited floating behavior during the water maze experiments our conclusion that the remaining mice were not impaired in this task is likely based on a biased sample and therefore may be subject to alternate interpretations.

Our fear conditioning experiments revealed that *Scn8a*$^{+/-}$mice had abnormal performance specifically in a learning paradigm with a strong emotional component; therefore we hypothesized that this behavior might actually reflect the tendency of *Scn8a*$^{+/-}$mice to react more strongly to stressors (i.e. exhibit greater emotionality). Emotionality in rodents was first described by Hall (Hall 1934, 1936a, 1936b) and later expanded upon by Broadhurst (Broadhurst 1957) and Gray (Gray 1973). Here, we use the term emotionality to refer to “a psychological trait that moderates an organism’s response to stress (Willis-Owen & Flint 2007).” In rodents, the principles of avoidance, autonomic activation, behavioral inhibition, and immobility in aversive environments have been used to design a number of behavioral paradigms to assess emotionality. Three of these paradigms, the open-field test, light-dark box, and forced swim test, were used to test the hypothesis that deletion of *Scn8a* leads to greater emotionality. The open-field test and light-dark box pit the innate desire of mice to explore novel environments against their innate fear of well-lit, open environments. The degree to which a mouse avoids the center of the open field or light side of the light-dark box has often been described as an index of anxiety-like behavior (Bourin & Hascoet 2003; Choleris *et al.* 2001; Treit & Fundytus 1988). In both the open-field test and light-dark box, the *Scn8a*$^{+/-}$mice exhibited behavior consistent with greater emotionality. The Porsolt forced swim test is based on the observation that when rodents are placed in an inescapable cylinder filled with water they initially exhibit escape-oriented movements but ultimately develop an immobile posture. Immobility in the FST is thought to be strategy for coping with the stress of the task (Holmes 2003) and is decreased by antidepressants (Porsolt *et al.* 1977). When exposed to the forced swim test, *Scn8a*$^{+/-}$mice rapidly became immobile and spent significantly more time in an immobile state when compared to their WT littermates. The tendency of *Scn8a*$^{+/-}$mice to more readily exhibit this coping strategy was first observed in the MWM: a task with obvious similarities to the FST. Passive floating (immobility) in the MWM was significantly more common in *Scn8a*$^{+/-}$mice than WT mice and many *Scn8a*$^{+/-}$mice had to be eliminated from analysis. The effect of *Scn8a* on this behavior may be augmented through an interaction with the C57BL/6 genetic background, a genetic background that has been demonstrated to reliably exhibit high levels of immobility in the FST (Jacobson & Cryan 2007). Taken collectively these experiments suggest that *Scn8a*$^{+/-}$mice do indeed exhibit greater emotionality than WT mice which likely explains the exaggerated conditioned freezing observed in *Scn8a*$^{+/-}$ mice. Consistent with these results are previous studies suggesting that emotionality and conditioned

fear in rodents are mediated by some of the same genes (Aguilar *et al.* 2002; Ponder *et al.* 2007). Further, it is interesting to note that enhanced emotionality in humans is correlated with both anxiety and depression disorders (Willis-Owen & Flint 2007) and humans with anxiety disorders exhibit greater fear learning than those without (Lissek *et al.* 2005).

Because of the association of *Scn8a* mutations with motor impairment in mice and the fact that many of the tasks used in the present study measure locomotion as an index of emotionality, one alternate explanation is that the abnormal emotional behavior observed in the *Scn8a*$^{+/-}$mice was the result of a non-specific motor impairment. This seems unlikely. First, we did not observe any difference in baseline freezing prior to delivery of the foot shock suggesting similar levels of ambulation in the Pavlovian fear conditioning experiments. Second, we did not observe any difference in the total distance traveled in the open field or home cage. Additionally, *Scn8a*$^{+/-}$mice performed identically to their WT littermates on both the hidden and visible versions of the Morris water maze. Finally, when neuromuscular strength and motor coordination were measured directly, we found no significant difference between *Scn8a*$^{+/-}$and WT mice on the wire hang test and the accelerating rotarod. Because the performance of *Scn8a*$^{+/-}$mice is identical to WT mice on the early trials but trends towards a difference on later trials this may reflect a subtle impairment in sensorimotor learning in *Scn8a*$^{+/-}$mice (Lalonde *et al.* 1995). However, in light of previous reports suggesting that anxious mice exhibit similar motor impairments (Lepicard *et al.* 2003; Lepicard *et al.* 2000; Metz *et al.* 2005) we are reluctant to make this claim.

Alternatively, many of the results presented here might be explained by a greater passivity or globally impaired motivation in *Scn8a*$^{+/-}$mice. This too seems unlikely given that *Scn8a*$^{+/-}$mice exhibit decreased exploratory behavior in novel, but not familiar, environments. In addition, *Scn8a*$^{+/-}$and WT mice had similar latencies to leave the brightly lit side of the light dark box and the duration of their first visit to the darken compartment were not significantly different. In the open field, we found no difference between *Scn8a*$^{+/-}$and WT mice in terms of the latency to enter the perimeter for the first time nor did we observe any difference in the latency to renter the center portion of the open field after the first trip to the perimeter.

The impetus for the present study arose from earlier work on a human family segregating a null allele of *SCN8A*. Heterozygous individuals exhibit a variety of abnormalities including cognitive impairment, emotional instability, and attention deficit disorder. Like most murine models of human disease, *Scn8a*$^{+/-}$mice exhibit some, but not all, of the abnormalities observed in the human family. The proband exhibited significant motor impairment and ataxia, but the motor function in *Scn8a*$^{+/-}$mice appears to be normal. Cognitive impairment was present in each heterozygous family members but *Scn8a*$^{+/-}$mice did not display cognitive impairment in the Morris water maze or conditioned taste avoidance paradigm. In the Pavlovian fear conditioning paradigm, open field, light-dark box, and forced swim test, *Scn8a*$^{+/-}$mice appeared to be more emotionally reactive that WT mice.

Previous screens for mutations in *SCN8A* in human populations, including the one that prompted this study, focused on populations with motor disorders. The data presented here together with data from the human family (Trudeau *et al.* 2006) suggest a larger role for *SCN8A* in emotional behavior. An alternate strategy for identifying human families with mutations in *SCN8A* may be to screen populations with depression, anxiety disorders, and other emotional abnormalities. Because enhanced emotionality correlates with depression and anxiety in humans, understanding the mechanism responsible for increased emotionality in S*cn8a*$^{+/-}$mice will likely provide valuable insight into the biological basis of common human psychiatric disorders.

Acknowledgments

This work was supported by grants from The National Institute of General Medical Sciences 5T32GM008322 (to BCM), National Institute of Neurological Disorders and Stroke NS34509 (to MHM).

References

Aguilar R, Gil L, Flint J, Gray JA, Dawson GR, Driscoll P, Gimenez-Llort L, Escorihuela RM, Fernandez-Teruel A, Tobena A. Learned fear, emotional reactivity and fear of heights: a factor analytic map from a large F2 intercross of Roman rat strains. Brain Res Bull 2002;57:17–26. [PubMed: 11827733]

Bourin M, Hascoet M. The mouse light/dark box test. Eur J Pharmacol 2003;463:55–65. [PubMed: 12600702]

Broadhurst PL. Determinants of Emotionality in the Rat .1. Situational Factors. Br J Psychol 1957;48:1–12. [PubMed: 13413179]

Burgess DL, Kohrman DC, Galt J, Plummer NW, Jones JM, Spear B, Meisler MH. Mutation of a new sodium channel gene, *Scn8a*, in the mouse mutant 'motor endplate disease'. Nat Genet 1995;10:461–465. [PubMed: 7670495]

Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR. Sodium channel $Na_V1.6$ is localized at nodes of ranvier, dendrites, and synapses. Proc Natl Acad Sci U S A 2000;97:5616–5620. [PubMed: 10779552]

Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25:235–260. [PubMed: 11378179]

Cummins TR, Dib-Hajj SD, Herzog RI, Waxman SG. $Na_V1.6$ channels generate resurgent sodium currents in spinal sensory neurons. FEBS Lett 2005;579:2166–2170. [PubMed: 15811336]

Enomoto A, Han JM, Hsiao C-F, Wu N, Chandler SH. Participation of sodium currents in burst generation and control of membrane excitability in mesencephalic trigeminal neurons. J Neurosci 2006;26:3412–3422. [PubMed: 16571748]

Gray, JA. The psychology of fear and stress. New York: McGraw-Hill; 1973.

Hall CS. Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J Comp Psychol 1934;18:385–403.

Hall CS. Emotional behavior in the rat. II. The relationship between need and emotionality. J Comp Psychol 1936a;22:61–68.

Hall CS. Emotional behavior in the rat. III. The relationship between emotionality and ambulatory activity. J Comp Psychol 1936b;22:345–352.

Holmes PV. Rodent models of depression: reexamining validity without anthropomorphic inference. Crit Rev Neurobiol 2003;15:143–174. [PubMed: 14977368]

Jacobson L, Cryan J. Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. Behav Genet 2007;37:171–213. [PubMed: 17029009]

Josselyn SA, Kida S, Silva AJ. Inducible repression of CREB function disrupts amygdala-dependent memory. Neurobiol Learn Mem 2004;82:159–163. [PubMed: 15341801]

Kohrman DC, Smith MR, Goldin AL, Harris J, Meisler MH. A missense mutation in the sodium channel Scn8a Is responsible for cerebellar ataxia in the mouse mutant jolting. J Neurosci 1996;16:5993–5999. [PubMed: 8815882]

Krzemien DM, Schaller KL, Levinson SR, Caldwell JH. Immunolocalization of sodium channel isoform NaCh6 in the nervous system. J Comp Neurol 2000;420:70–83. [PubMed: 10745220]

Lalonde R, Bensoula AN, Filali M. Rotorod sensorimotor learning in cerebellar mutant mice. Neurosci Res 1995;22:423–426. [PubMed: 7478307]

Lepicard EM, Venault P, Negroni J, Perez-Diaz F, Joubert C, Nosten-Bertrand M, Berthoz A, Chapouthier G. Posture and balance responses to a sensory challenge are related to anxiety in mice. Psychiatry Res 2003;118:273–284. [PubMed: 12834821]

Lepicard EM, Venault P, Perez-Diaz F, Joubert C, Berthoz A, Chapouthier G. Balance control and posture differences in the anxious BALB/cByJ mice compared to the non anxious C57BL/6J mice. Behav Brain Res 2000;117:185–195. [PubMed: 11099772]

Levin SI, Khaliq ZM, Aman TK, Grieco TM, Kearney JA, Raman IM, Meisler MH. Impaired motor function in mice with cell-specific knockout of sodium channel Scn8a ($Na_V1.6$) in cerebellar purkinje neurons and granule cells. J Neurophysiol 2006;96:785–793. [PubMed: 16687615]

Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS. Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther 2005;43:1391–1424. [PubMed: 15885654]

McKinney BC, Murphy GG. The L-type voltage-gated calcium channel $Ca_V1.3$ mediates consolidation, but not extinction, of contextually-conditioned fear in mice. Learn Mem 2006;13:584–589. [PubMed: 17015855]

McKinney BC, Schneider JS, Schafer GL, Lowing JL, Mohan S, Zhao MX, Heng MY, Albin RL, Seasholtz AF, Akil H, Murphy GG. Decreased locomotor activity in mice expressing tTA under control of the CaMKIIα promoter. Genes Brain Behav. 2007 doi:10.1111/j.1601-1183X.2007.00339.x.

Meisler MH, Plummer NW, Burgess DL, Buchner DA, Sprunger LK. Allelic mutations of the sodium channel SCN8A reveal multiple cellular and physiological functions. Genetica 2004;122:37–45. [PubMed: 15619959]

Metz GA, Jadavji NM, Smith LK. Modulation of motor function by stress: a novel concept of the effects of stress and corticosterone on behavior. Eur J Neurosci 2005;22:1190–1200. [PubMed: 16176362]

Ponder CA, Kliethermes CL, Drew MR, Muller J, Das K, Risbrough VB, Crabbe JC, Gilliam TC, Palmer AA. Selection for contextual fear conditioning affects anxiety-like behaviors and gene expression. Genes Brain Behav 2007;6:736–749. [PubMed: 17309658]

Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–732. [PubMed: 559941]

Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacology Biochemistry and Behavior 1988;31:959–962.

Trudeau MM, Dalton JC, Day JW, Ranum LPW, Meisler MH. Heterozygosity for a protein truncation mutation of sodium channel *SCN8A* in a patient with cerebellar atrophy, ataxia, and mental retardation. J Med Genet 2006;43:527–530. [PubMed: 16236810]

Tzoumaka E, Tischler AC, Sangameswaran L, Eglen RM, Hunter JC, Novakovic SD. Differential distribution of the tetrodotoxin-sensitive rPN4/NaCh6/Scn8a sodium channel in the nervous system. J Neurosci Res 2000;60:37–44. [PubMed: 10723066]

Van Wart A, Matthews G. Impaired firing and cell-specific compensation in neurons lacking $Na_V1.6$ sodium channels. J Neurosci 2006;26:7172–7180. [PubMed: 16822974]

Wasserman D, Geijer T, Rozanov V, Wasserman J. Suicide attempt and basic mechanisms in neural conduction: Relationships to the *SCN8A* and *VAMP4* genes. Am J Med Genet 2005;133B:116–119. [PubMed: 15635639]

Willis-Owen SAG, Flint J. Identifying the genetic determinants of emotionality in humans; insights from rodents. Neurosci Biobehav Rev 2007;31:115–124. [PubMed: 17010437]

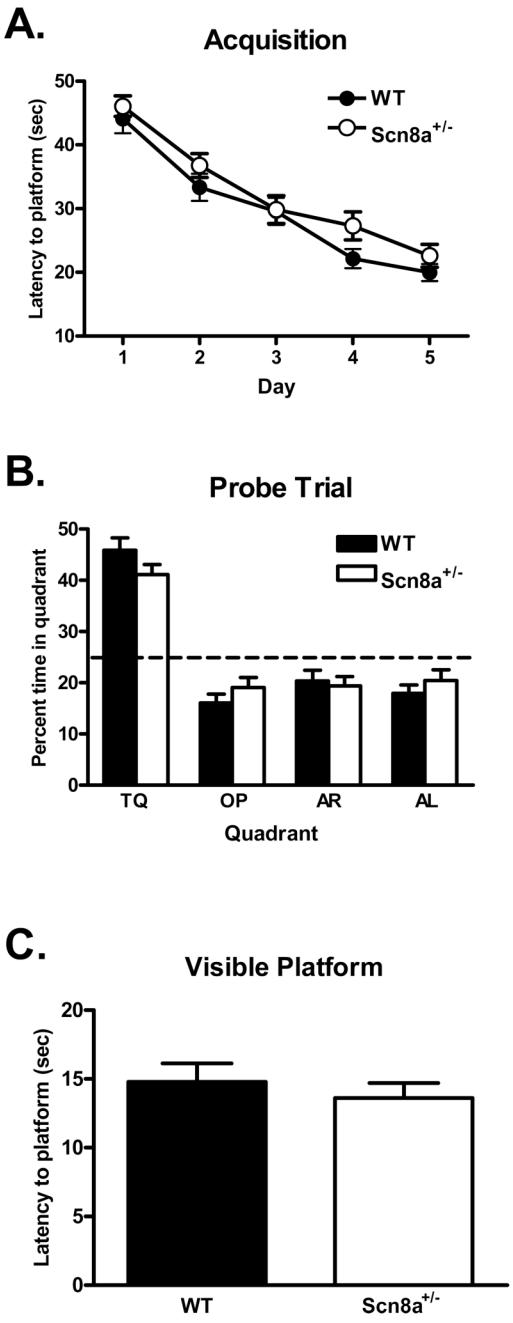

Figure 1.

Among mice remaining after those exhibiting excessive floating were eliminated from analysis, there was no difference in performance between *Scn8a*$^{+/-}$ *and* WT mice in the Morris water maze. **(A)** The time to reach the hidden platform during training was not significantly different *Scn8a*$^{+/-}$ mice (N = 28) when compared WT mice (N = 26). **(B)** A 60 second probe trial completed 24 hours after the last training trial (day 6) reveals that both *Scn8a*$^{+/-}$ mice and WT mice spend a significant amount of time during the trial searching in the quadrant where the platform was previously located (TQ; training quadrant) but there was no significant difference between the genotypes. The dashed line (25%) represents random performance. (AR, AL, OP abbreviated for Adjacent Right, Adjacent Left & Opposite, respectively). **(C)** Average latency

to platform for *Scn8a*$^{+/-}$ mice during the visible-platform version of the Morris water maze was not significantly different when compared with WT mice. All data are presented as mean ± S.E.M.

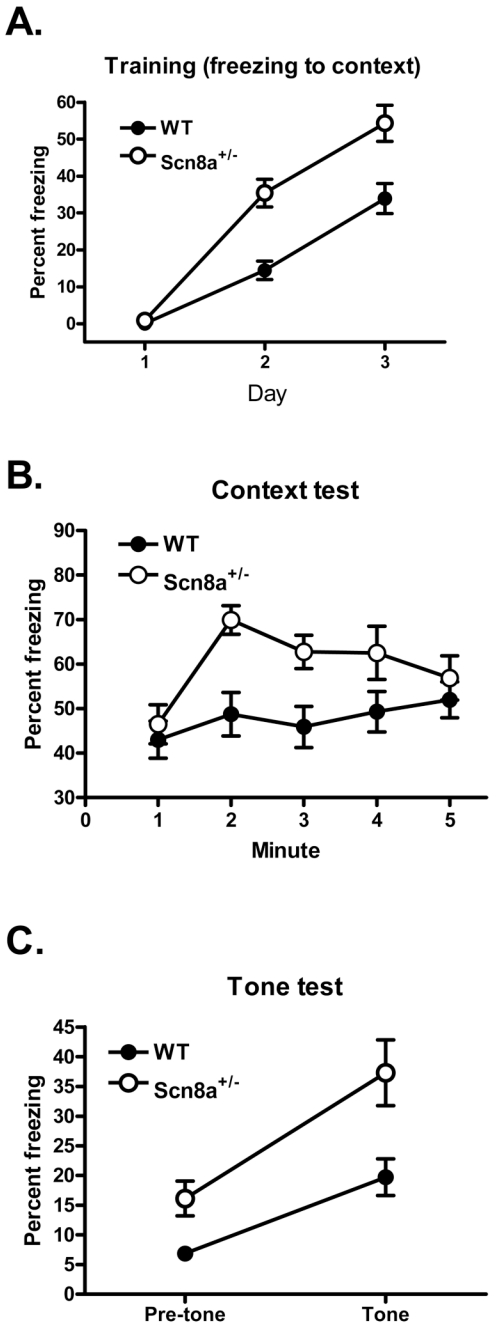

Figure 2.

Scn8a$^{+/-}$ mice exhibit greater freezing to training context and tone than WT mice. **(A)** As training progressed, both *Scn8a*$^{+/-}$ mice (N = 15) and WT mice (N = 13) exhibited increases in freezing to training context with *Scn8a*$^{+/-}$ mice freezing significantly more than WT mice. **(B)** *Scn8a*$^{+/-}$ mice exhibited greater freezing to context upon 5 minute exposure to training context than WT mice. **(C)** *Scn8a*$^{+/-}$ mice exhibited greater generalization of fear to the reconfigured context and greater freezing to tone than WT mice. All data are presented as mean ± S.E.M.

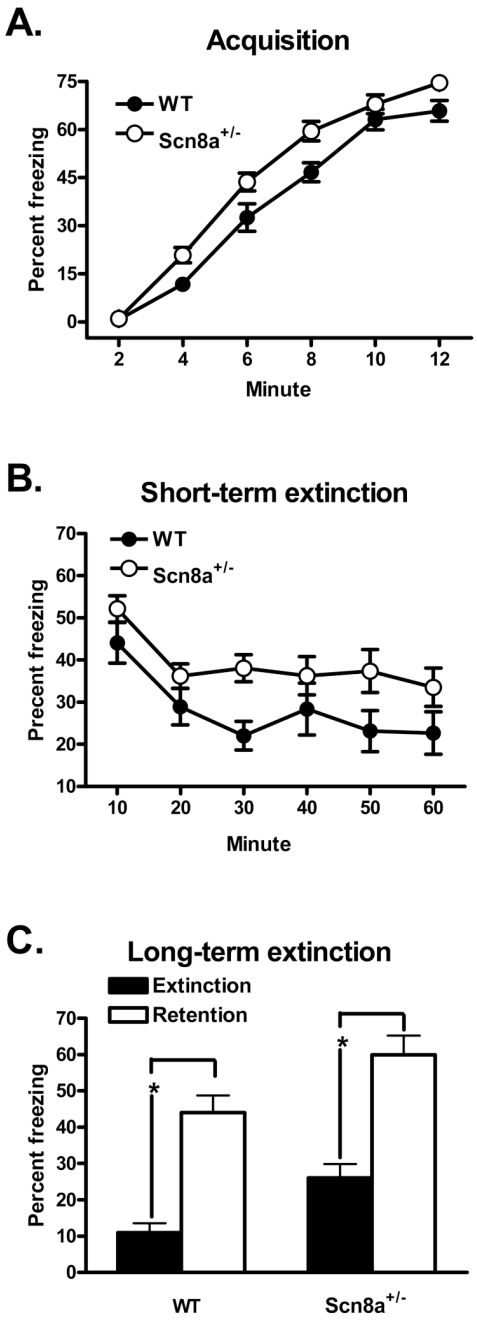

Figure 3.

Scn8a$^{+/-}$ mice extinguish contextually-conditioned fear as well as WT mice. **(A)** Both *Scn8a*$^{+/-}$ mice (N = 31) and WT mice (N = 24) exhibited increases in freezing as training progressed with *Scn8a*$^{+/-}$ mice consistently freezing more than WT mice in the intervals following and between shocks. **(B)** *Scn8a*$^{+/-}$ mice (N = 21) exhibited more freezing than WT mice (N = 16) across a one-hour exposure to the conditioning chambers, but there was no genotype-training interaction. **(C)** Twenty-four hours after extinction training, mice were re-exposed to the conditioning chambers. *Scn8a*$^{+/-}$ mice froze significantly more than WT mice and retention control mice froze more than mice in the extinction group, but there was no

interaction between genotype or group. All data are presented as mean ± S.E.M. (*) $p < 0.05$ two-way ANOVA; training group factor.

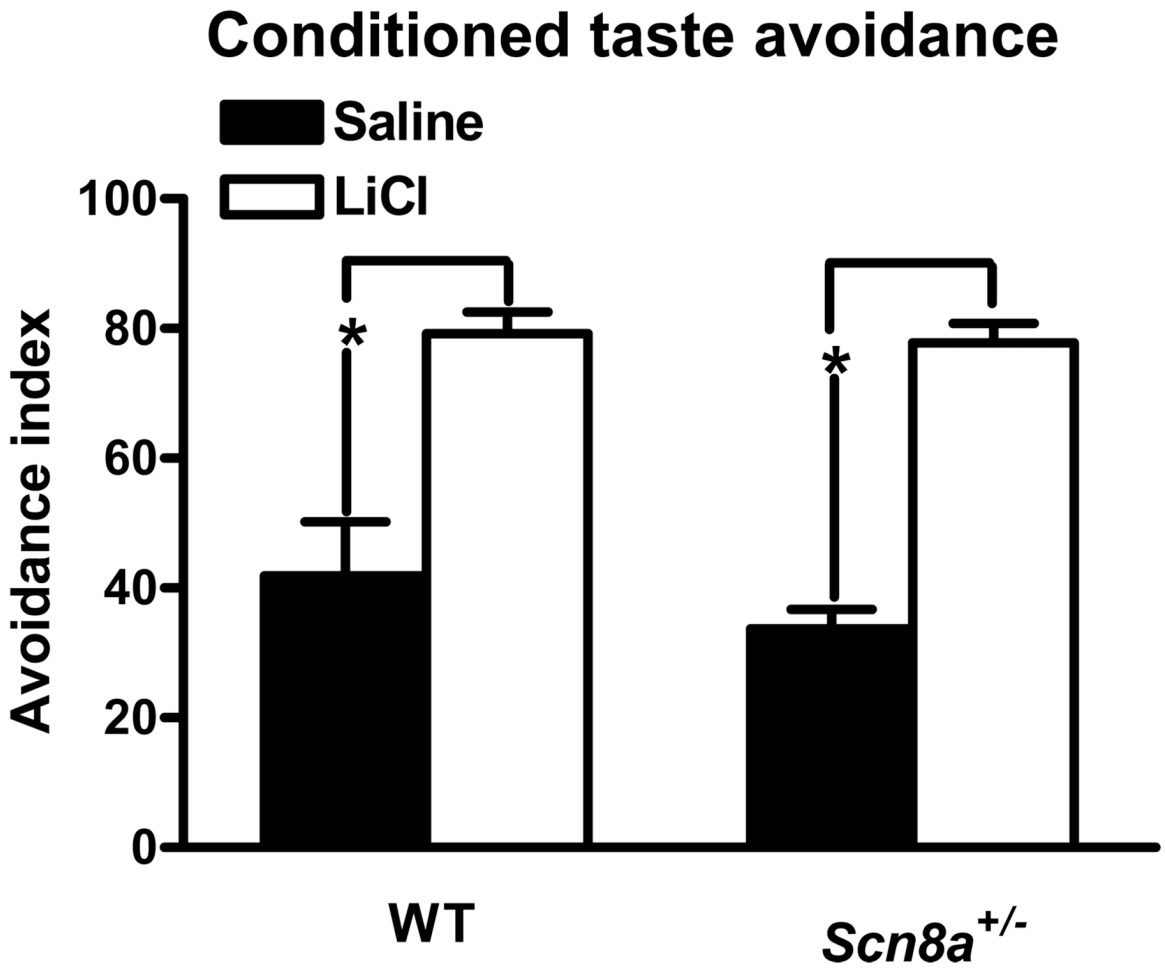

Figure 4.
Scn8a$^{+/-}$ mice (N = 7) and WT (N = 5) mice learn to avoid saccharin following a saccharin-lithium chloride pairing equally well. All data are presented as mean ± S.E.M. (*) $p < 0.05$; two-way ANOVA treatment group factor.

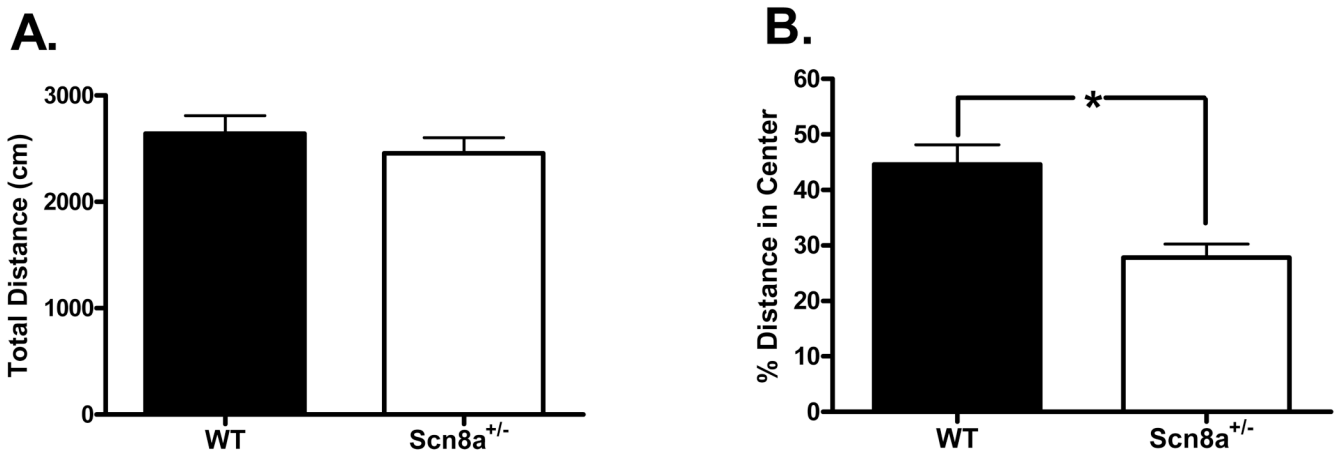

Figure 5.

Scn8a$^{+/-}$ mice exhibit more pronounced avoidance of the center zone of the open field. **(A)** Total distance traveled by *Scn8a*$^{+/-}$ mice (N = 12) was not significantly different when compared to WT mice (N = 9). **(B)** *Scn8a*$^{+/-}$ mice, however, on average traveled less distance in the center of the open field when compared to WT mice. All data are presented as mean ± S.E.M. (*) $p < 0.05$; unpaired t-test.

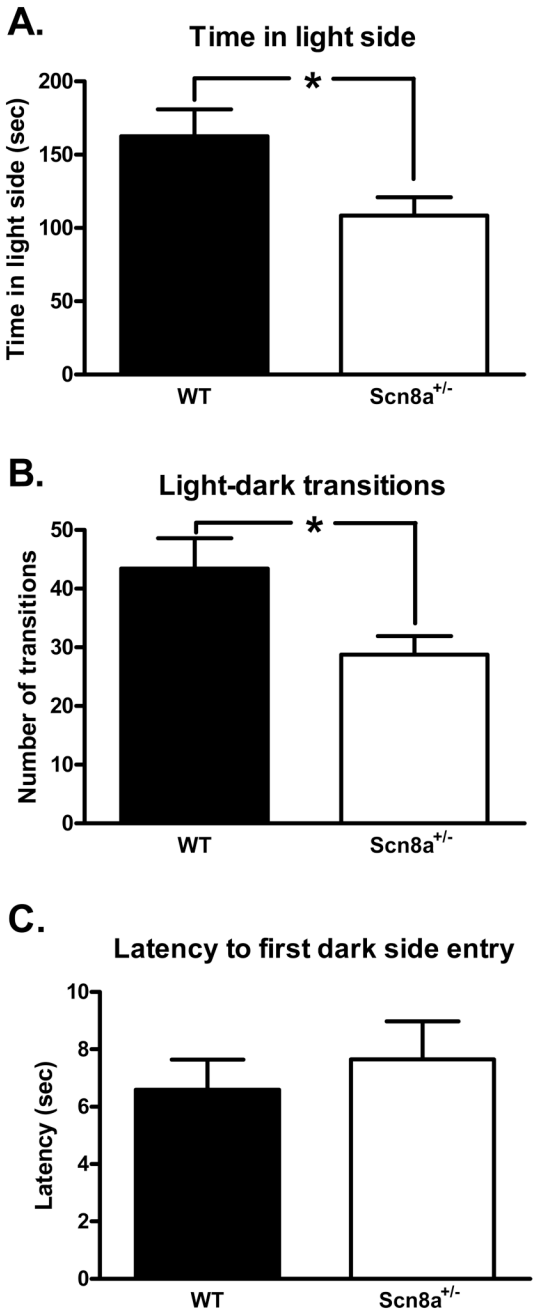

Figure 6.

Scn8a$^{+/-}$ mice exhibit more pronounced avoidance of the light side of the light-dark box than WT mice. **(A)** *Scn8a*$^{+/-}$ mice (N = 12) spent less time exploring the aversive light side of the light-dark box than WT mice (N = 9). **(B)** *Scn8a*$^{+/-}$ mice made significantly fewer transitions between sides of the light-dark box than WT mice. **(C)** Upon initial placement in the light side of the light-dark box, latency to first entry into the dark side did not differ between *Scn8a*$^{+/-}$ and WT mice. All data are presented as mean ± S.E.M. (*) $p < 0.05$; unpaired t-test.

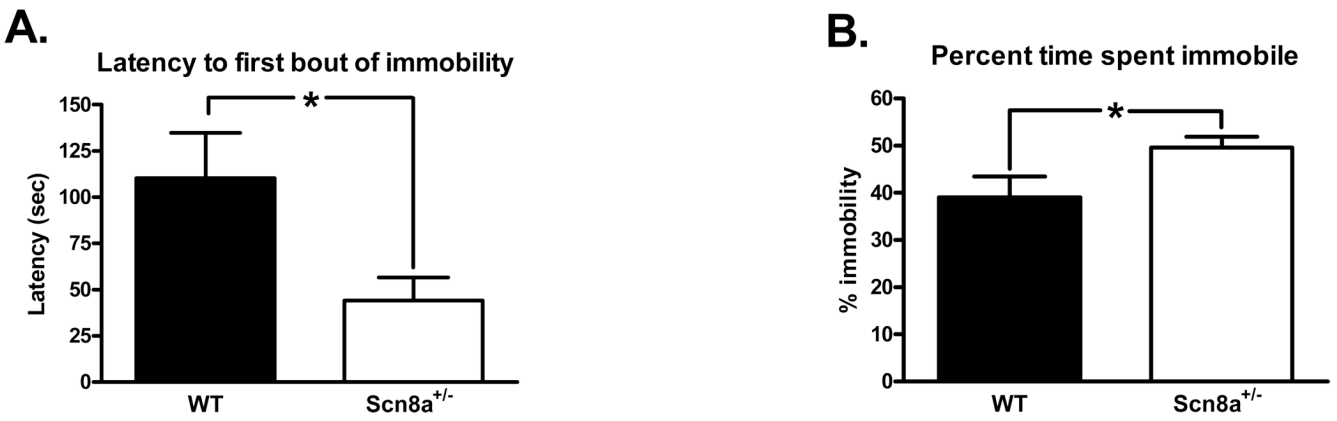

Figure 7.

Scn8a$^{+/-}$ mice exhibit increased immobility in the forced swim test. **(A)** Latency to first bout of immobility was significantly shorter in *Scn8a*$^{+/-}$ mice (N = 12) than WT mice (N = 9). **(B)** *Scn8a*$^{+/-}$ mice spent significantly more total time immobile than WT mice. All data are presented as mean ± S.E.M. (*) $p < 0.05$; unpaired t-test.

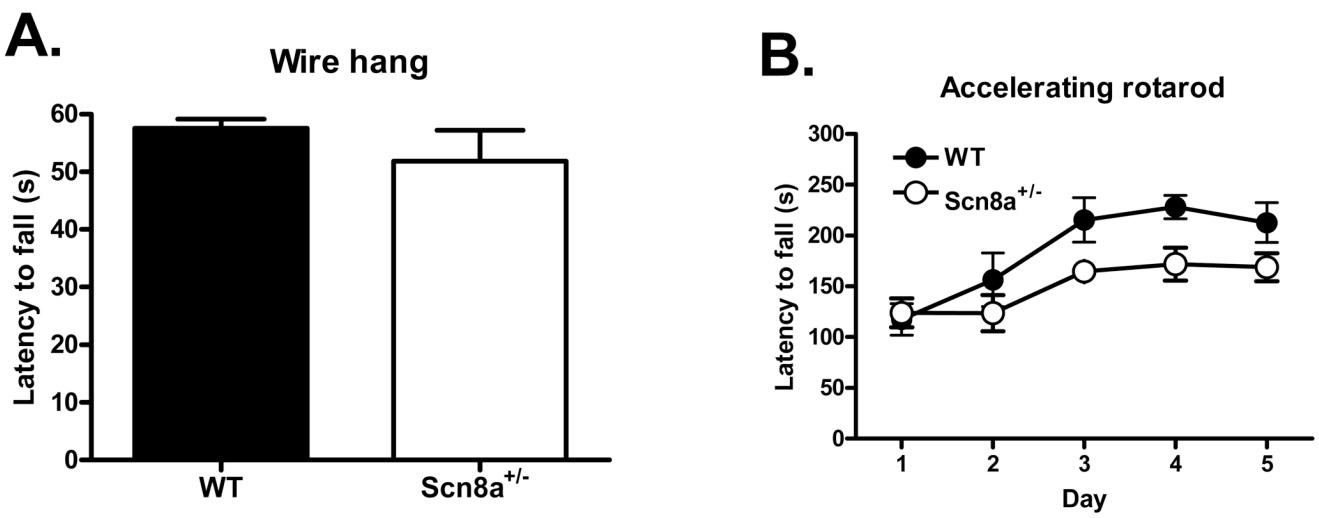

Figure 8.

Motor performance is unimpaired in *Scn8a*$^{+/-}$ mice. **(A)** Neuromuscular strength as assessed by latency to fall from an inverted wire mesh did not differ between *Scn8a*$^{+/-}$ mice (N = 12) and their WT littermates (N = 9). **(B)** Motor coordination and balance as assessed by latency to fall from an accelerating rotarod did not differ between *Scn8a*$^{+/-}$ mice and their WT littermates. All data are presented as mean ± S.E.M.