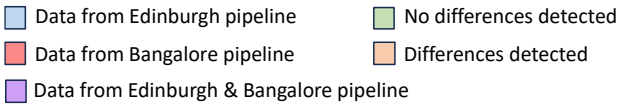
SFARI *Scn2a* heterozygous knockout
LE-*Scn2a*^{em1Mcwi}

The SCN2A gene, located on chromosome 2 in humans, encodes the neuronal sodium voltage gated ion channel alpha 2.1 (NaV1.2). Sodium gated ion channels are critical for neuronal function and mutations in the SCN2A gene that lead to a loss of expression, changes in structure, or expression patterns of the NaV1.2 ion channel are a leading cause of autism spectrum disorder (ASD), intellectual disability (ID), infantile seizures, movement disorders and encephalopathy in humans.
To model this disorder in rats, SFARI commissioned the Medical College of Wisconsin to generate a heterozygous mutant rats using CRISPR/SpCas9 to target the *Scn2a* gene on chromosome 3 on an outbred Long–Evans background. The resulting mutation is net 4-bp deletion in exon 5 comprising a 10-bp deletion (shown in lower case: CTACGGGATccctggaattGGTTGGATTTCACAGTCATT) and a 6-bp insertion (TTCACT), which causes inactivation of one copy of the gene. Homozygous loss of the *Scn2a* gene is lethal. *Scn2a* heterozygous mutant rats (hets) appear healthy, fertile and indistinguishable from wild-type (WT) littermates.

MALE data		No. pairs
1	General characterisation	3 pairs
2	Body weight	31 pairs
3	Object Recognition Memory	8 pairs
4	Marble Interaction	17 pairs
5	Auditory Fear Conditioning	13 pairs
6	Prey Capture	12 pairs
7	One Trial Adult Social Interaction	8 pairs

FEMALE data		No. pairs
1	General characterisation	
2	Body weight	21 pairs
3	Object Recognition Memory	8 pairs
4	Marble Interaction	12 pairs
5	Auditory Fear Conditioning	13 pairs
6	Prey Capture	11 pairs
7	One Trial Adult Social Interaction	15 pairs



Other ongoing tasks: Neonatal Reflexes, Object Location Memory, Water Maze, Active Place Avoidance, Juvenile Paired Play, Tactile reactivity (Hairy back and hind paw)

1	GENERAL CHARACTERISATION

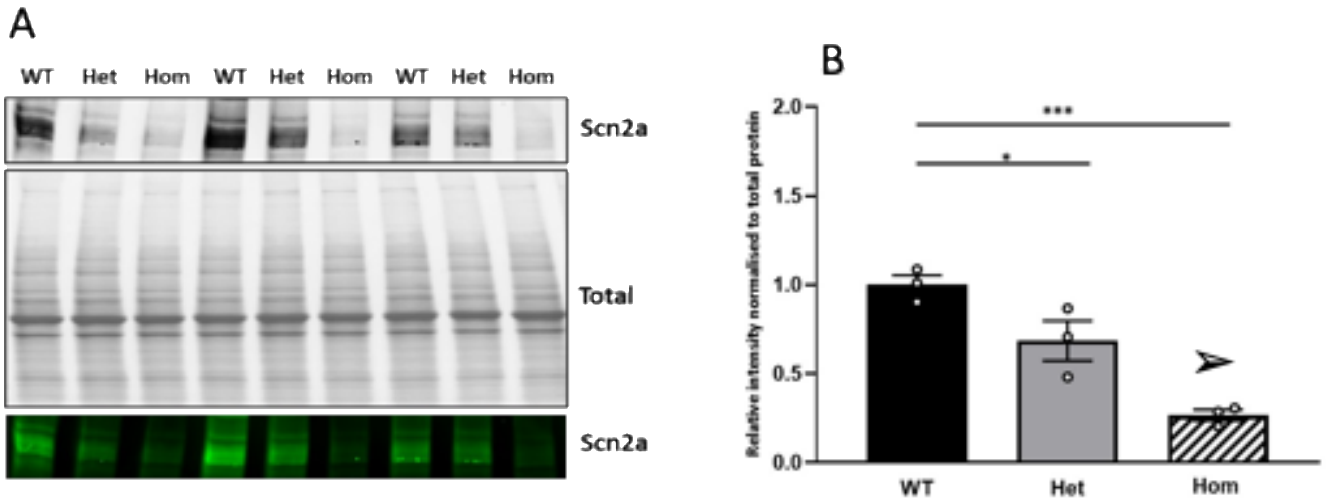


Figure 1.1 SCN2a protein levels are reduced in het rats. Western blot analysis of SCN2A protein levels in cortical homogenates from Scn2a Het X Het crossed p0 littermates. *The SCN2A intensity was normalized with total protein and compared with their WT and Hom littermates. Green blot image is the same as in grey. WT=3, Het=3 and Hom =3, one-way ANOVA, multiple-comparison test, * p<0.05, *** p<0.001.*

➢ *Antibody used in this experiment: #ab300113, a pan SCN antibody that may have cross reactivity with other SCN proteins, thus the remaining bands in the hom may be due to cross reactivity of the antibody to other SCNs.*

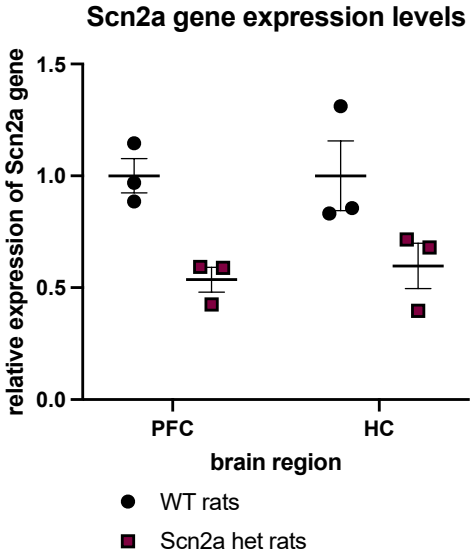


Figure 1.2. Scn2a gene expression levels are reduced in het rats. Expression levels (mean ±SEM) of the Scn2a gene *measured using RNA sequencing, levels are expressed relative to the mean level in WT samples, in prefrontal cortex (PFC) and hippocampal (HC) samples.*

2 BODY WEIGHT

Scn2a het and wild-type (WT) littermates do not differ in body weight across life, data from male and female pups (Fig. 2A) and from adult rats at 10 weeks of age (Fig. 2B).

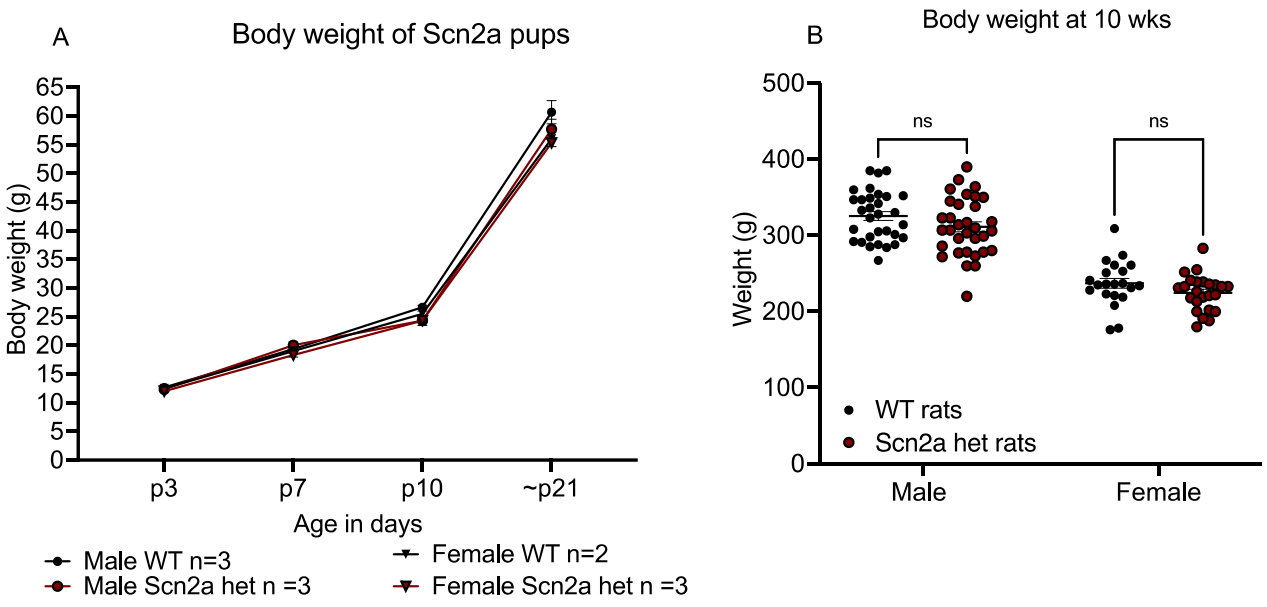


Figure 2. Body weight unaltered in Scn2a het rats*. *Body weight (g) mean ± SEM *A) from postnatal day (P) 3 until approx. P21 and B) at 10 wks of age in male and female rats on a weight control diet to maintain at 85-95% free feeding weight. N = 31 WT male, 21 WT female, 33 het male, 24 het female*

3	OBJECT RECOGNITION MEMORY

Memory was assessed in the short (5 min intertrial interval, ITI) and long-term (24 hr ITI) object recognition (OR) memory task (Fig. 3A). Object exploration levels for sample and test trials are shown in Fig. 3 B and C respectively. Group mean discrimination indices (DI) in the test trial did not significantly differ amongst groups (2-way ANOVA: all main effects ns). And the DI for all groups was significantly above chance (one sample t-test against the null hypothesis that DI = 0; all groups: $t_7 > 3.3$, $P < 0.01$; Fig. 3E) showing that all groups are preferentially exploring the novel object, demonstrating good object recognition memory in the short-term test in both WT and *Scn2a* het rats (5 min ITI; Fig. 3 D-E).

Object exploration in the test trial and sample trial of the long-term object recognition task shown in fig. 3 F-G. Group mean discrimination indices (DI) in the test trial did not significantly differ amongst groups (2-way ANOVA: all main effects ns). And the DI for the WT male and female and het female rats were significantly above chance (one sample t-test against the null hypothesis that DI = 0; all groups: $t_7 > 3.0$, $P < 0.02$; Fig. 3I) and there was a trend for the het male to be above chance ($t_7 = 2.2$, $P = 0.6$; Fig. 3I), suggesting intact long term object recognition memory in WT and *Scn2a* het rats (Fig. 3H-I).

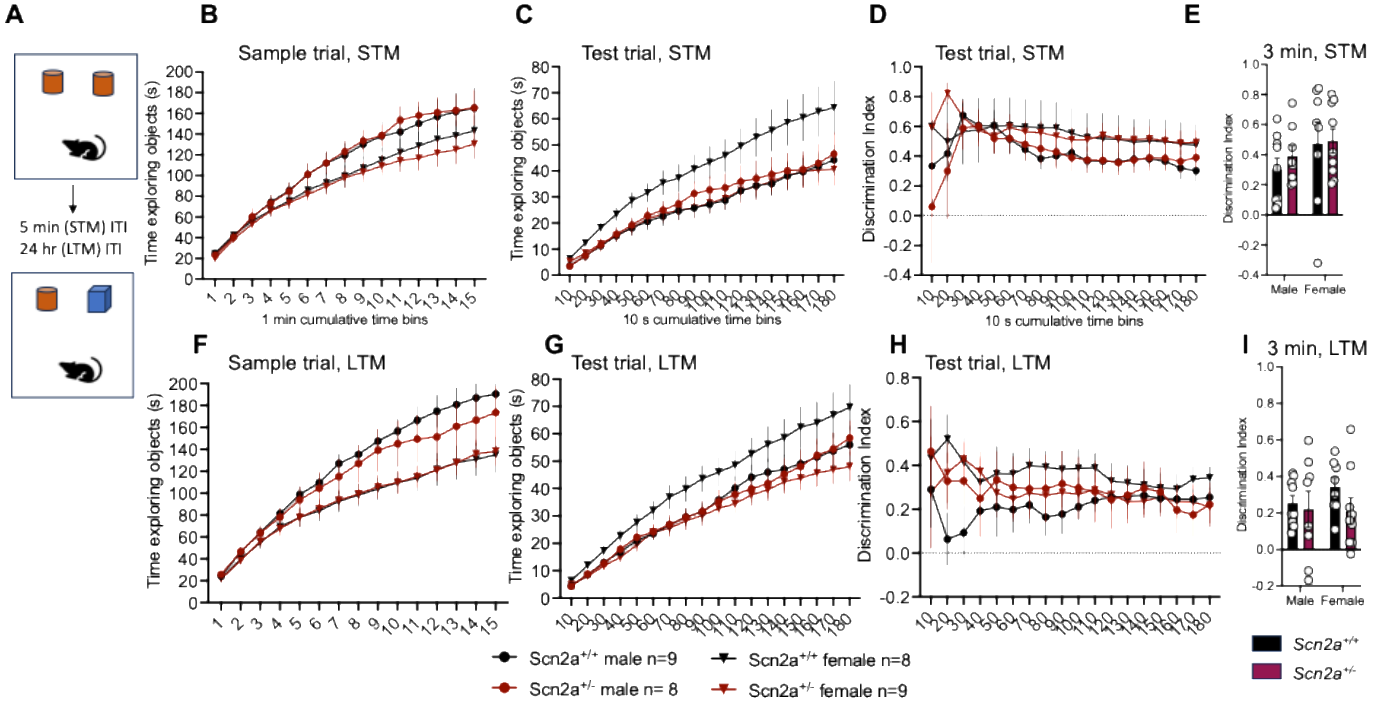


Figure 3. No impairment in long- or short-term object recognition memory in het rats. Object recognition; performance measures (mean ± SEM) in the short term (5 min interval) task and long term (24 hr) memory task. *A) Task Schematic of the short-term (5 min interval) and long-term (24 hr interval) memory tasks. Rats received the sample trial (top) and the test trial (bottom). B) Duration of object exploration during the sample trial in the short-term memory task. C) Duration of object exploration during the test trial in the short-term memory task. D) Preference index for the novel object during the 3 min test trial in the short-term memory task over 10s cumulative time bins. E) Overall preference index for the novel object during the 3 min test trial in the short-term memory task. F) Duration of object exploration during the sample trial in the long-term memory task. G) Duration of object exploration during the test trial in the long-term memory task. H) Preference index for the novel object during the 3 min test trial in the long-term memory task over 10s cumulative time bins. I) Overall preference index for the novel object during the 3 min test trial in the long-term memory task. Edinburgh pipeline. Statistical analysis will be performed when data collection is complete.*

4	MARBLE INTERACTION

To investigate the impact of a reduction in SCN2A levels on interest in novel objects, we tested the rats in the marble interaction paradigm (Fig. 4A). The impact of genotype was dependent on sex, with female *Scn2a* het rats showing reduced levels of marble exploration during the trial compared to female WT rats, there was no difference in marble exploration levels between the genotypes in male rats (Fig. 4B; 2-way ANOVA; sex*genotype; $F_{1,57}$= 5.3, P = 0.025, P< 0.05: post hoc Fisher's LSD). Female WT rats explored the marbles more than male WT rats.

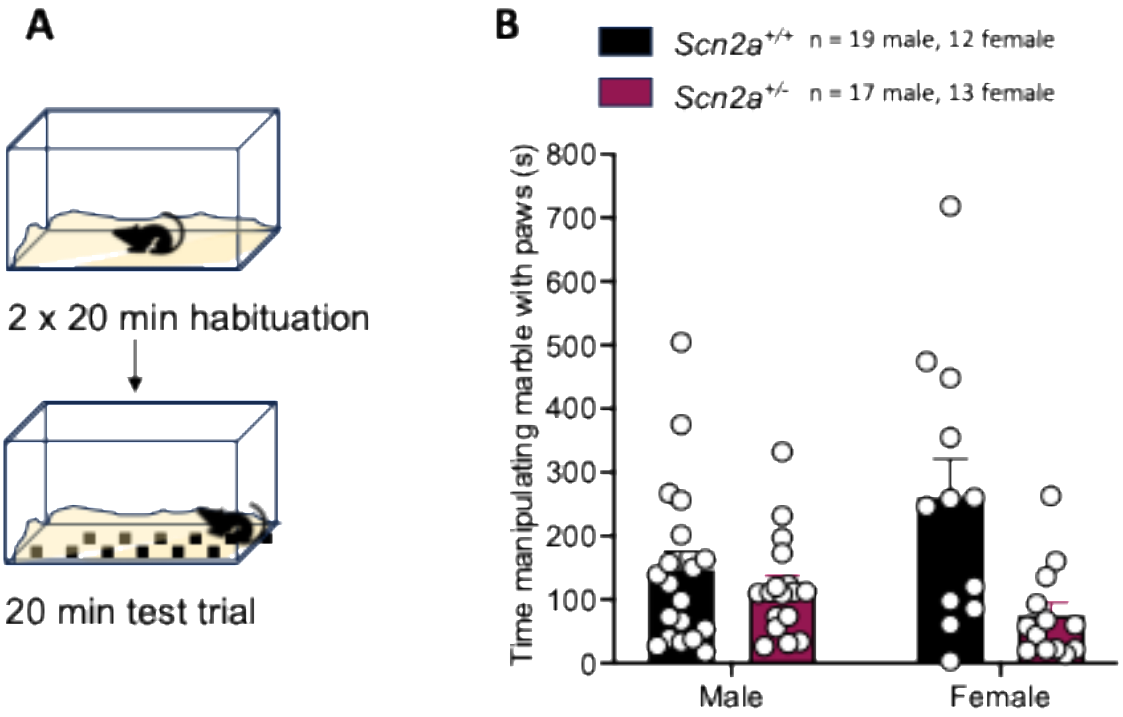


Figure 4. Scn2a het rats manipulate marbles less that WT. The marble interaction paradigm: A) *Task Schematic of the two habituation sessions and the test trial of the marble interaction task on three separate days.* ***B)*** *Time (s) mean ± SEM rats spent manipulating the marbles with their forepaws during the 20 minute trial.*

5 AUDITORY FEAR CONDITIONING

To investigate the effect of a reduction in SCN2A levels on cognitive function and adaptive behaviours, we used a classical auditory fear conditioning paradigm to assess acquisition, recall and subsequent extinction of the association between a neutral stimulus (a tone) and a mildly unpleasant stimulus (foot shock) in in WT and *Scn2a* het male and female rats (Fig. 5A).

Scn2a het rats, regardless of sex, learned the association between tone and foot shock as well as WT rats during the conditioning trials, (Fig. 5B, F; 2-way RM ANOVA: main effect of tone: $F_{2.0,91.4} = 74.5$, $P < 0.0001$; main effect of genotype: ns, analysis performed on male and female data together, data plotted separately for clarity).

During recall trials, 24hr after conditioning, both WT and *Scn2a* het rats displayed high levels of freezing, suggesting intact recall of the conditioned response (fig. 5C, G, 2-way ANOVA: main effect of tone: $F_{8.2, 373.2} = 18.1$, $P < 0.0001$). However, during extinction trials (tones 5 onwards), unlike WT rats, *Scn2a* het rats continued to display high levels of freezing (Fig. 5B, 2-way ANOVA: genotype*tone: $F_{13,585} = 1.7$, $P = 0.055$).

Scn2a het rats displayed reduced extinction indices, compared to WT rat, suggesting impaired extinction learning in *Scn2a* het rats (Fig. 5D, H).

Freezing during recall the following day, during extinction memory trials, was still higher in *Scn2a* het rats compared to WT rats (Fig 5. E, I).

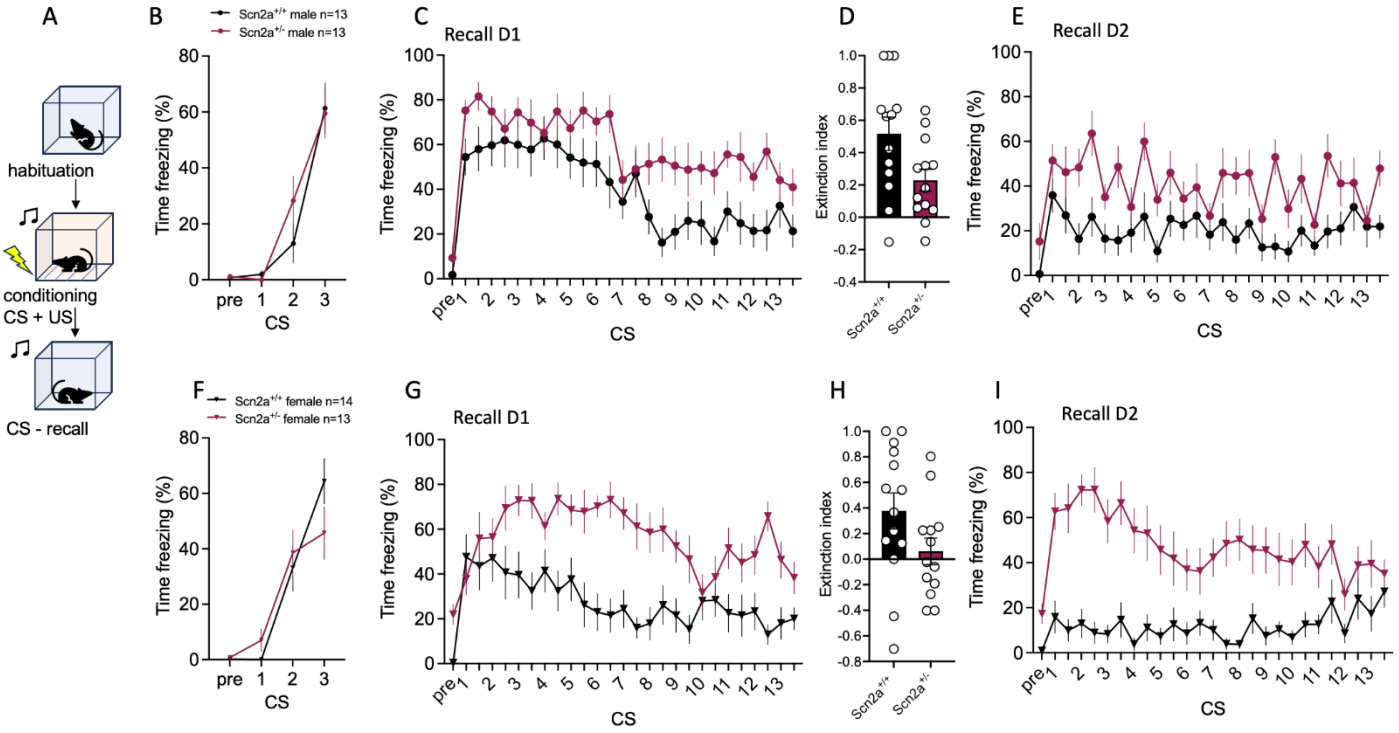


Figure 5. Scn2a het rats show impaired extinction learning and extinction memory in auditory fear conditioning. Auditory fear conditioning parameters: *A) Task schematic of the 3 stages of the task: habituation, conditioning in which the conditional stimuli (auditory tone) and the unconditional stimulus (foot shock) were paired, and recall, where the conditioned stimulus was presented. B) % time freezing (mean ± SEM), in response to the tone during conditioning trials before the onset of the shock ('pre') and during the tones 1-3. C) % time freezing during recall day one (24 hrs after the conditioning stage, freezing to tone and inter tone interval are shown). D) Extinction index from recall D1 (calculated using: [% freezing during tone 1-3] – [% freezing during tone 11-13] / [% freezing tone 1-3 + % freezing tone 11-13]. E) % time freezing 48 hrs after the conditioning stage, to show extinction memory. F – I) same data for females. Data from Edinburgh pipeline. Analyses were performed on male and female data together and only freezing to tone (not during inter tone interval) but data plotted in full and separated by sex for clarity.*

6	PREY CAPTURE

We investigated the impact of a reduction of SCN2A protein levels on performance of rats in the prey capture task in which rats hunt a cricket in an arena (four trials per day for 5 days; Fig 6A). Hunting forms part of the natural behavioural repertoire of a rat, therefore this ecologically appropriate task taps into several behavioural domains (e.g. reward, motivation, motor coordination, visual perception). Data analysis on male and female data will be performed when data collection is complete, but preliminary analyses show that *Scn2a* het rat are as likely as WT to catch prey and that time to catch the prey in male and female *Scn2a* het rats is comparable to WT rats.

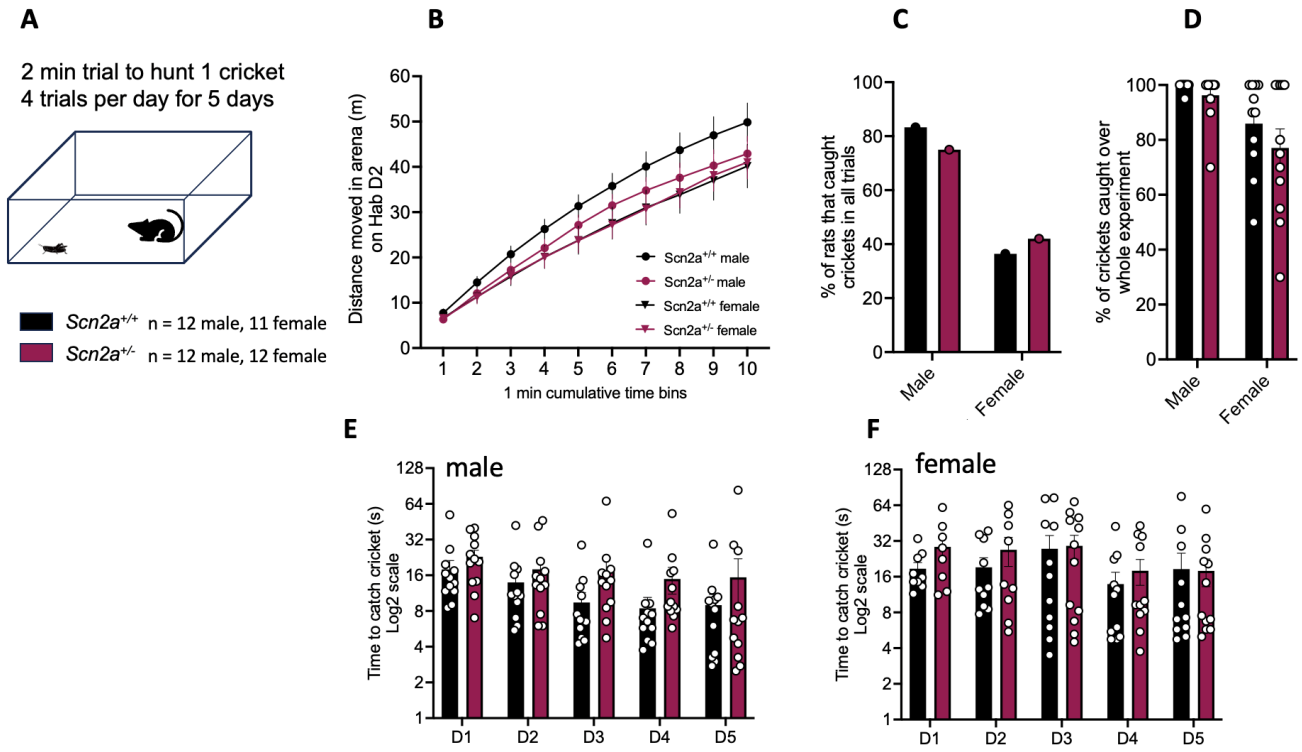


Figure 6. Performance in prey capture task is unaltered in Scn2a het rats. Prey capture: performance (mean ± SEM). A) *Schematic of task and sample sizes.* ***B)*** *Distance (m) moved in the arena, during the 10 min habituation on D2, during which no hunting takes place, in 1-min cumulative time bins, tracked using ANYmaze software.* ***C)*** *% of rats that caught crickets in all trials.* ***D)*** *% of crickets that each rat caught over the whole 20 trials.* ***E)*** *mean time catch cricket for each rat in the four trials on each day in males and* ***F)*** *females on a log2 scale. If rats did not catch, the trial was not included. Data from Edinburgh Pipeline.*

7	ONE TRIAL ADULT SOCIAL INTERACTION

To investigate the impact of reduced levels of SCN2A on social behaviour, we exposed WT and *Scn2a* het male and female rats to an unfamiliar same sex conspecific for 10 minutes in an arena and recorded how much the test rat investigated the unfamiliar rat (e.g. sniffed, followed closely, groomed, pinned etc. Fig. 7A).

There was no difference between *Scn2a* het and WT rats in the amount of time they spent socially investigating an unfamiliar conspecific in this test (Fig. 7B, 2-way ANOVA: all main effects and interaction term ns).

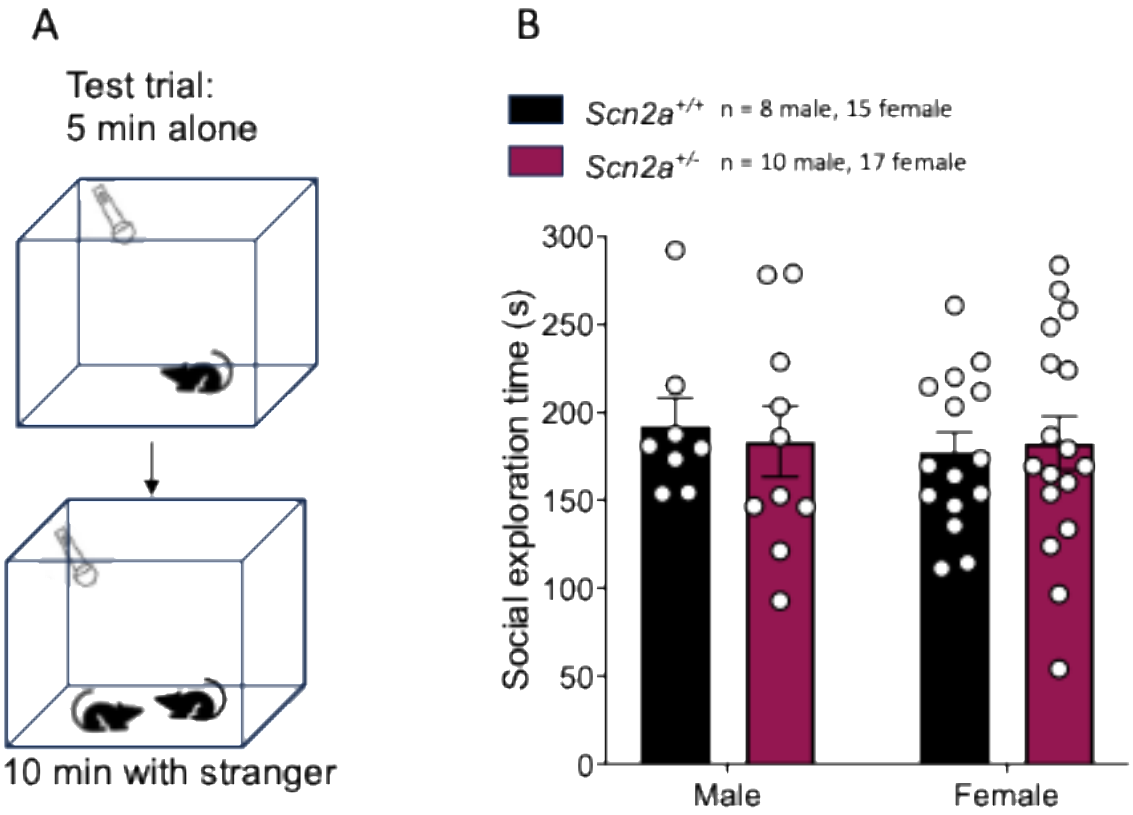


Figure 7. Time spent investigating a novel rat was not altered in Scn2a het rats. Time (mean ± SEM) spent socially investigating an unfamiliar rat in the 1 trial social interaction paradigm*. Data from Edinburgh Pipeline.*