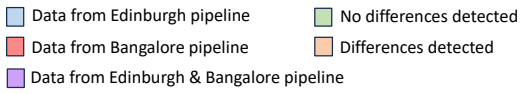
SFARI *Grin2b* heterozygous knockout LE-*Grin2b*^{em1Mcwi}

The GRIN2B gene, located on chromosome 12 in humans, encodes the glutamate (NMDA) receptor subunit epsilon-2 (GluN2B receptor). NMDA receptors are glutamate-gated ion channels that play a key role in neuronal function. Mutations in the GRIN2B gene are associated with neurodevelopmental disorders, typically manifesting as delayed development, intellectual disability, seizures and the development of autisms in humans.
To model this disorder in rats, SFARI commissioned the Medical College of Wisconsin to generate a heterozygous mutant using CRISPR/Cas9 to target the *Grin2b* gene on chromosome 4, resulting in deletion of exon 3, on an outbred Long–Evans background. Homozygous loss of the *Grin2b* gene is lethal. *Grin2b* heterozygous mutant rats (hets) appear healthy, fertile and indistinguishable to WT littermates.

	MALE data	No. Pairs
1	General characterisation	4 pairs
2	Body weight	19 pairs
3	Object Recognition Memory	13 pairs
4	Object Location Memory	13 pairs
5	Marble Interaction	17 pairs
6	Auditory Fear Conditioning	13 pairs
7	Prey Capture	10 pairs
8	One Trial Adult Social Interaction	13 pairs

	FEMALE data	No. Pairs
1	General characterisation	
2	Body weight	22 pairs
3	Object Recognition Memory	experiments ongoing
4	Object Location Memory	experiments ongoing
5	Marble Interaction	13 pairs
6	Auditory Fear Conditioning	14 pairs
7	Prey Capture	10 pairs
8	One Trial Adult Social Interaction	13 pairs



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

1 GENERAL CHARACTERISATION

GLUN2B protein levels in **cortical** homogenate from P0 het rats show a reduction of approximately 50% relative to WT littermates, and no expression in homozygous rats (Fig 1.1).

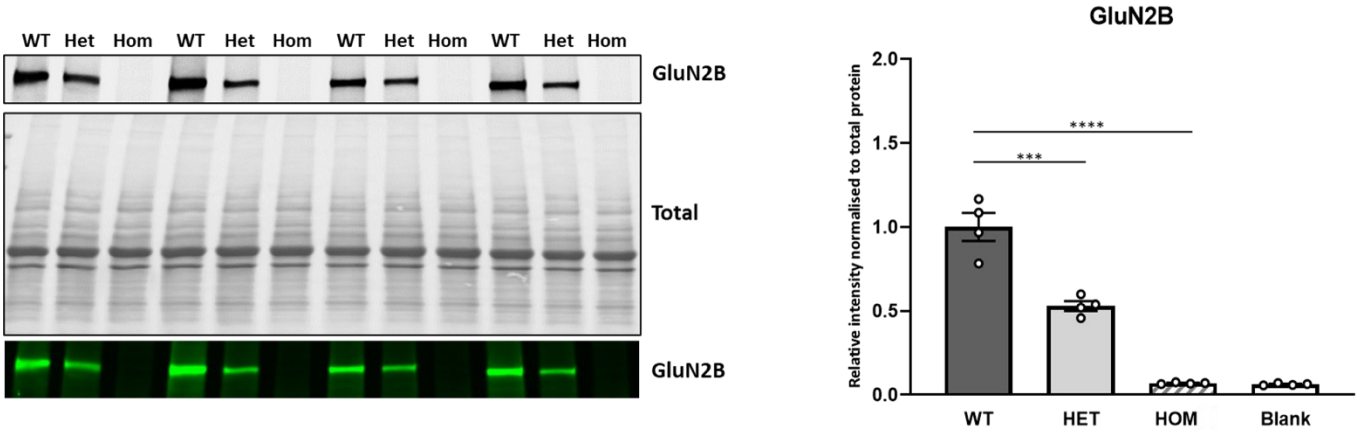


Figure 1.1 GLUN2B protein levels are reduced in cortical samples in het rats. Western blot analysis of GLUN2B protein levels in cortical homogenates from Grin2b Het X Het crossed littermates. *The GLUN2B intensity was normalized to total protein and compared with their WT littermates. Blank in bar graph represents the background hence confirming that there is no expression of GluN2B in Homozygous pups. WT=4, Het=4, Hom=4, One way ANOVA, multiple comparison test, ***p<0.001, ****p<0.0001*

GLUN2B protein levels in **hippocampal** homogenate from adult het rats show a reduction of approximately 50% relative to WT littermates (Fig 1.2).

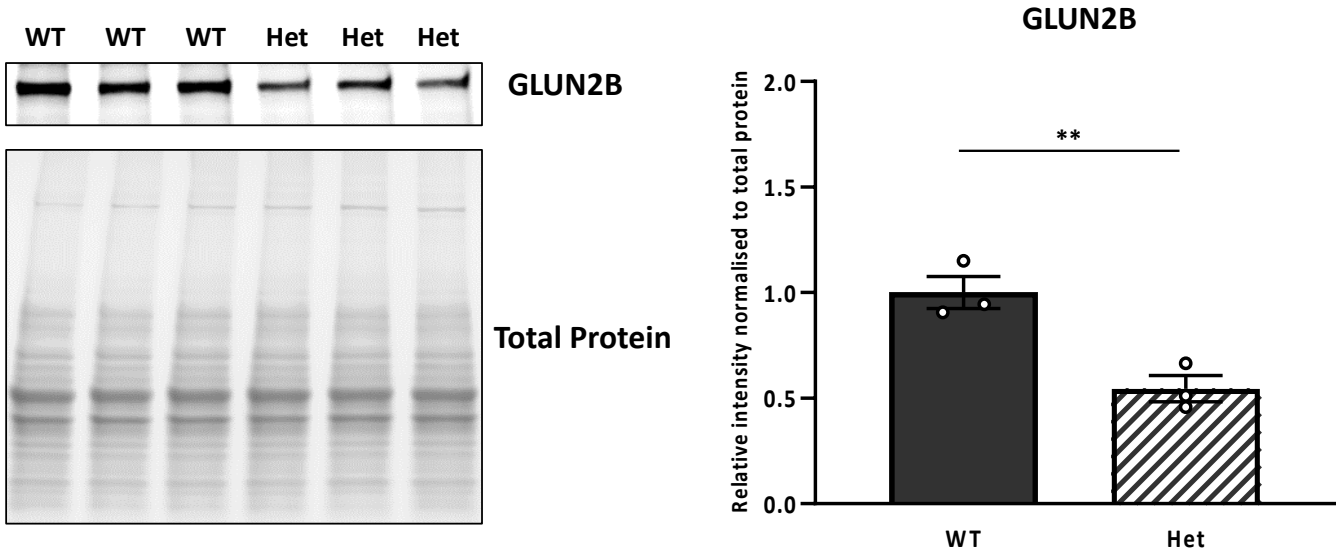


Figure 1.2. GLUN2B protein levels are reduced in hippocampal samples in het rats. Western blot analysis of GLUN2B expression in hippocampus homogenates from adult het and WT littermates. *The GLUN2B intensity was normalized to total protein and compared against WT littermates. WT=3, Het=3, t-test, **p<0.01*

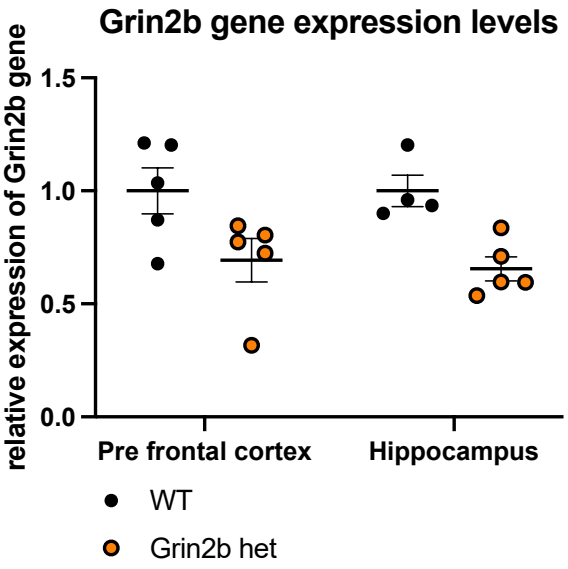


Figure 1.3 Grin2b RNA levels are reduced in het rats. Expression levels (mean ± SEM) of the Grin2b gene *measured using RNA sequencing, levels are expressed relative to the mean level in WT samples, in prefrontal cortex and hippocampal brain tissue samples.*

2	BODY WEIGHT

Male and female *Grin2b* het and wild-type (WT) littermates do not differ in body weight at 12 wks, (Fig. 2) or across life (data not shown). As typically seen, males weigh more than females.

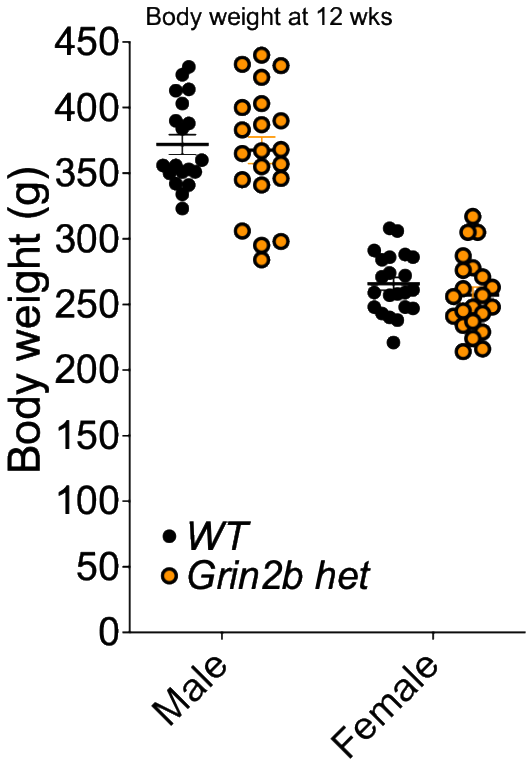


Figure 2. Body weight is unaltered in Grin2b het rats. Body weight (g) ± SEM at 12wks of age. *Male and female rats on a weight control diet from 6+ wks to maintain at 85-95% free feeding weight. N = 19 WT male, 22 WT female, 21 het male, 22 het female*

3 OBJECT RECOGNITION MEMORY

Male data

Memory was assessed in the short (5 min intertrial interval) and long-term (24 hr inter trial interval) object recognition (OR) memory task. *Grin2b* het rats spent less time than WT rats exploring the objects during the test trial (Fig. 5A). Both WT and *Grin2b* het rats showed preference for the novel object in the short (Fig. 5B, C) and long-term tasks (data available upon request). This result was confirmed in the Edinburgh pipeline.

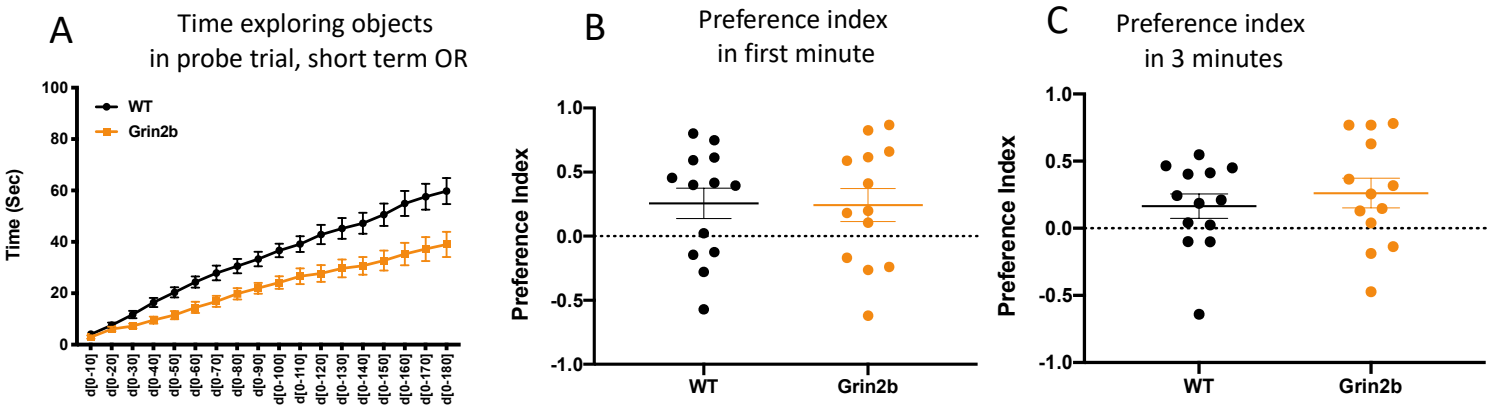


Figure 3. No impairment in long or short term object recognition memory in het rats. Object recognition performance measures (mean ± SEM) in the short term (5 min interval) task. *A) Accumulated time exploring the objects during the probe trial. B) preference index for the novel object in the first minute of the probe trial (one sample t-test WT: P =0.055, het P = 0.085), and in the C) full 3 mins of the probe trial one sample t-test: WT P =0.093, het P = 0.035). N = 13 per group. Data from Bangalore Pipeline, same result found in Edinburgh Pipeline.*

4 OBJECT LOCATION MEMORY

Long-term object location (OLM; 1 hr intertrial interval) memory was impaired in *Grin2b* het rats relative to WT littermates (Fig. 4 B, C). *Grin2b* het rats also spent less time investigating the objects in the test trial than WT rats (Fig. 4A).

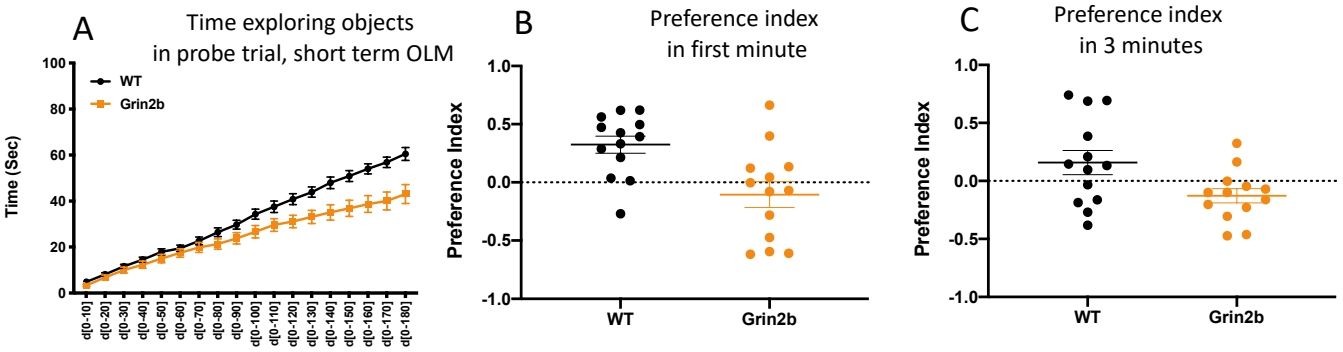


Figure 4. Impaired object location memory after 1 hr inter trial interval in Grin2b het rats. Object location performance measures (mean ± SEM) in the short- term task (1 hour inter trial interval). *A) Accumulated time exploring the objects in the probe trial, B) preference index for the moved object in the first minute of the probe trial (one sample t-test WT: P = 0.001, het P = 0.362) and in the C) full 3 mins of the probe trial (one sample t-test WT: P = 0.15, het P = 0.061). N = 13 per group. Data from Bangalore Pipeline, data collection ongoing in Edinburgh.*

5	MARBLE INTERACTION

To investigate the impact of a reduction in GRIN2B levels on interest in novel objects, we tested the rats in the marble interaction paradigm (Fig. 5A). There were no differences between *Grin2b* het and WT rats of either sex in the time that the rats spent manipulating the marbles with their forepaws during the 20-minute trial (Fig. 5B; 2-way ANOVA: all main effects ns).

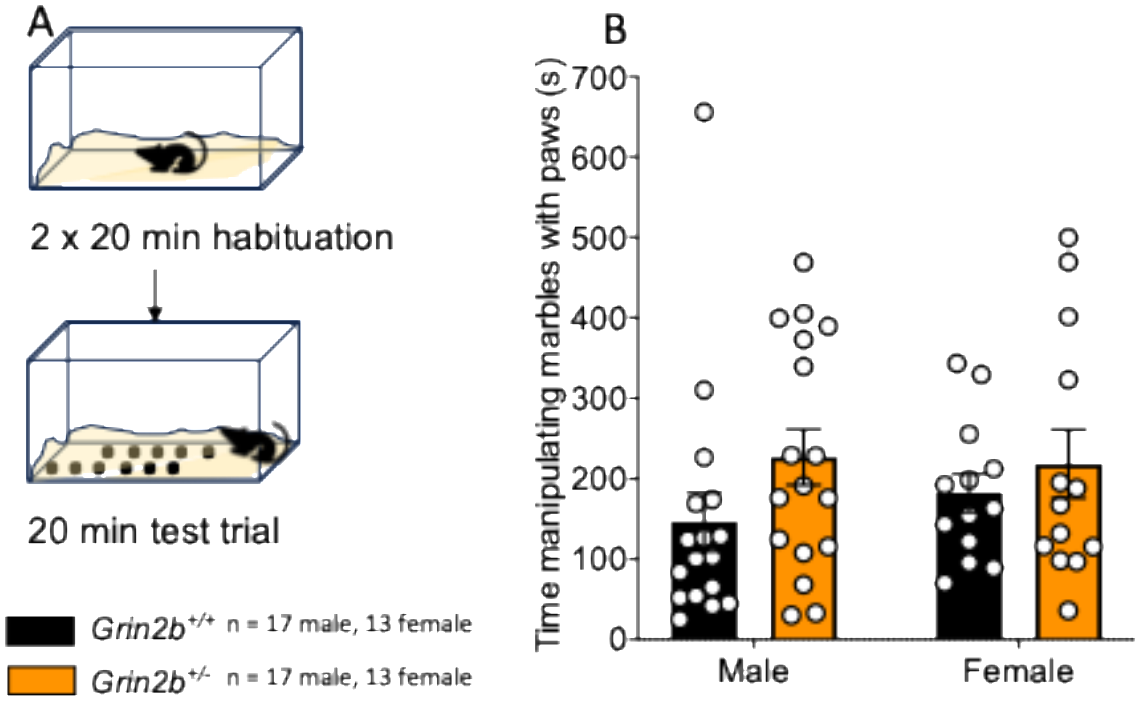


Figure 5. Grin2b het rats manipulate marbles same amount of time as WT rats. *A) Task Schematic of the two habituation sessions and the test trial of the marble interaction task on three separate days. B) Time (s) mean ± SEM rats spent manipulating marbles with their forepaws out of the 20-min test trial. Data from Edinburgh pipeline*

6 AUDITORY FEAR CONDITIONING

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 *Grin2b* het and WT rats (Fig. 6A).

All groups learned the association between tone and foot shock with freezing levels increasing over tones during conditioning trials (Fig. 6B, F; 2-way RM-ANOVA: tone: $F_{2.16,110.0} = 180.6$, $P < 0.0001$). *Grin2b* het males had lower freezing to tone 3 than WT rats (2-way RM-ANOVA: tone*genotype: $F_{3,153} = 180.6$, $P < 0.0001$; tone*sex: $F_{3,153} = 2.9$, $P = 0.036$; Fig. 6B).

All groups demonstrated recall of the conditioned response 24hr after training, on recall D1 (Fig. 6C, G; 2-way RM-ANOVA: tone: $F_{7.2,368.1} = P < 0.0001$). Male *Grin2b* het rats displayed significantly lower freezing to the first tone, relative to WT rats (Fig. 6C).

Extinction indices show that all groups showed similar (good) levels of extinction learning of the tone foot shock association in recall on D1 (Fig. 6 D, H).

Extinction memory, during recall on D2 did not differ between the groups, all groups showed low levels of freezing to the tone (Fig. 6 E, I).

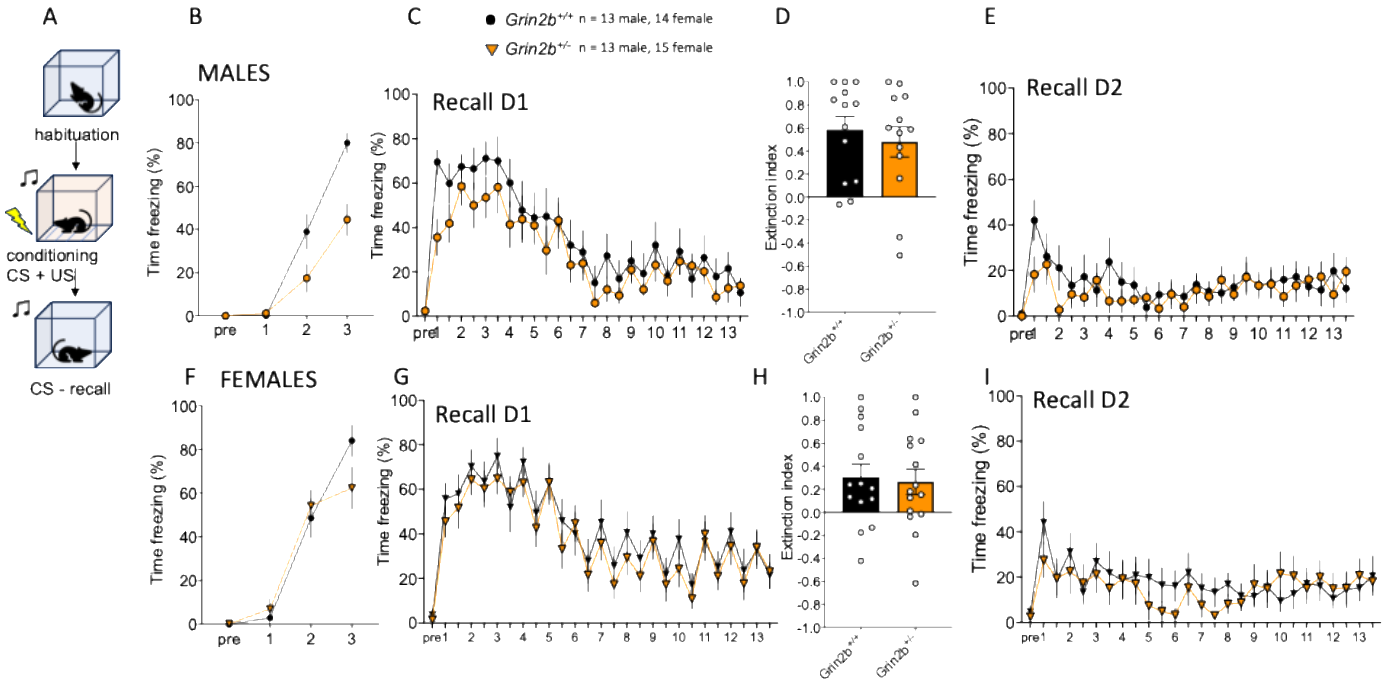


Figure 6. Male Grin2b het rats show reduced freezing in auditory fear conditioning and recall trials. Auditory fear conditioning parameters: *A) Task schematic of the 3 stages of the task: habituation, conditioning in which the conditional stimuli (auditory tone) and the unconditional stimulus (foot 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.*

7	PREY CAPTURE

We investigated the impact of a reduction of GRIN2B levels on performance in the prey capture task in which rats hunt a cricket in an arena (four trials per day for 5 days; Fig. 7A). Hunting forms part of the natural behavioural repertoire of a rat, and 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 *Grin2b* het rat are less likely than WT to catch prey in all trials (Fig7 B, C). However, in the trials in which rats catch successfully, the latencies to catch appear similar between WT and *Grin2b* het rats (Fig. 7D, E) regardless of sex.

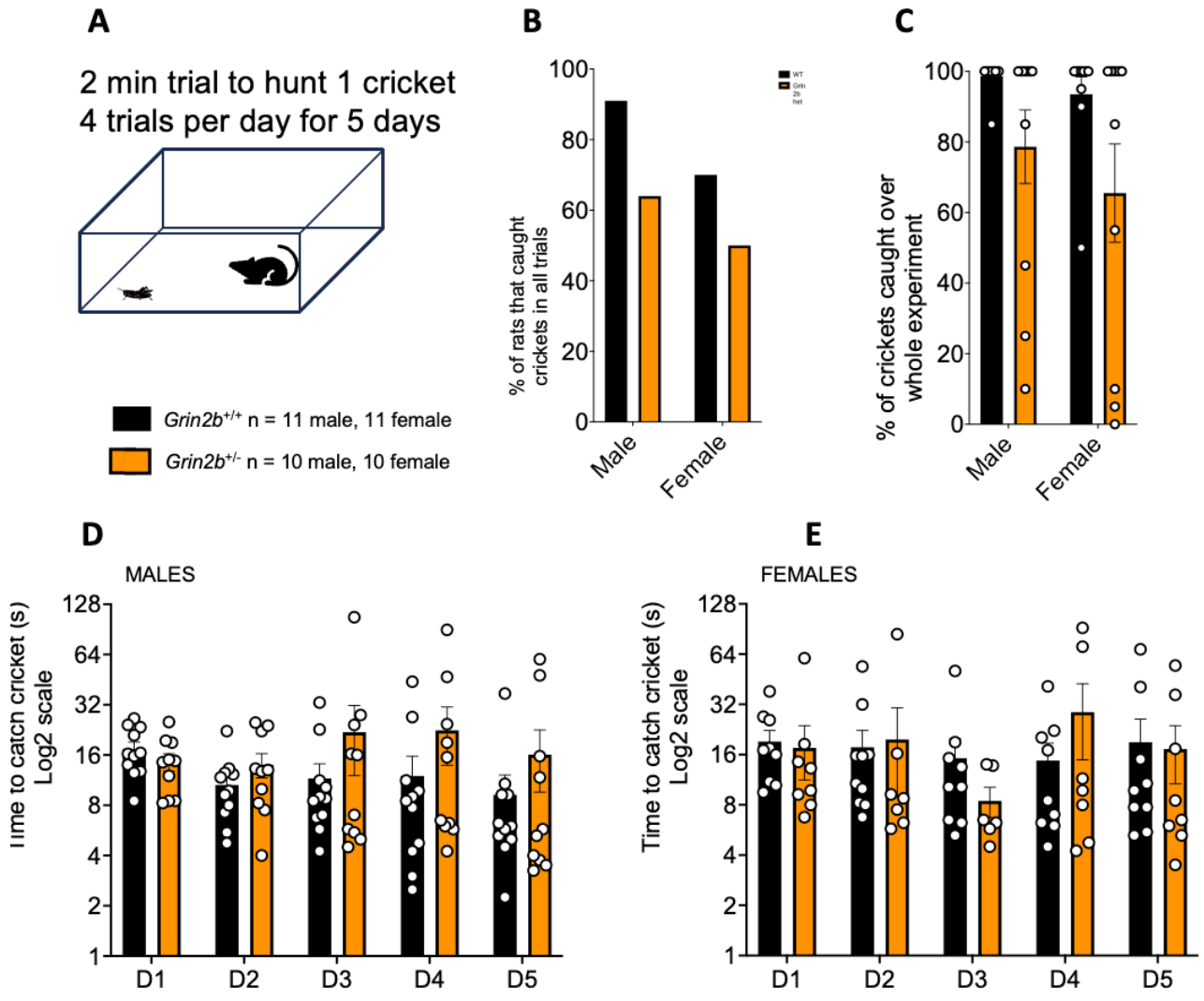


Figure 7. Grin2b het rats are less likely to catch prey, but in the trials in which they catch, they do so as quickly as WT rats. Prey capture performance (mean ± SEM). A) *schematic of the paradigm and sample sizes.* ***B)*** *% of rats that caught crickets in all trials.* ***C)*** *% of crickets that each rat caught over the whole 20 trials.* ***D)*** *mean time to catch cricket for each rat in the four trials on each day in males and* ***E)*** *females on log2 scale. If rats did not catch, the trial was not included. Data from Edinburgh Pipeline.*

8	ONE TRIAL ADULT SOCIAL INTERACTION

To investigate the impact of reduced levels of GRIN2B on social behaviour, we exposed WT and *Grin2b* het 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; Fig. 8A).

Both male and female *Grin2b* het rats spent the same amount of time as WT socially investigating an unfamiliar conspecific in this test (Fig. 8B, 2-way ANOVA: all main effects ns).

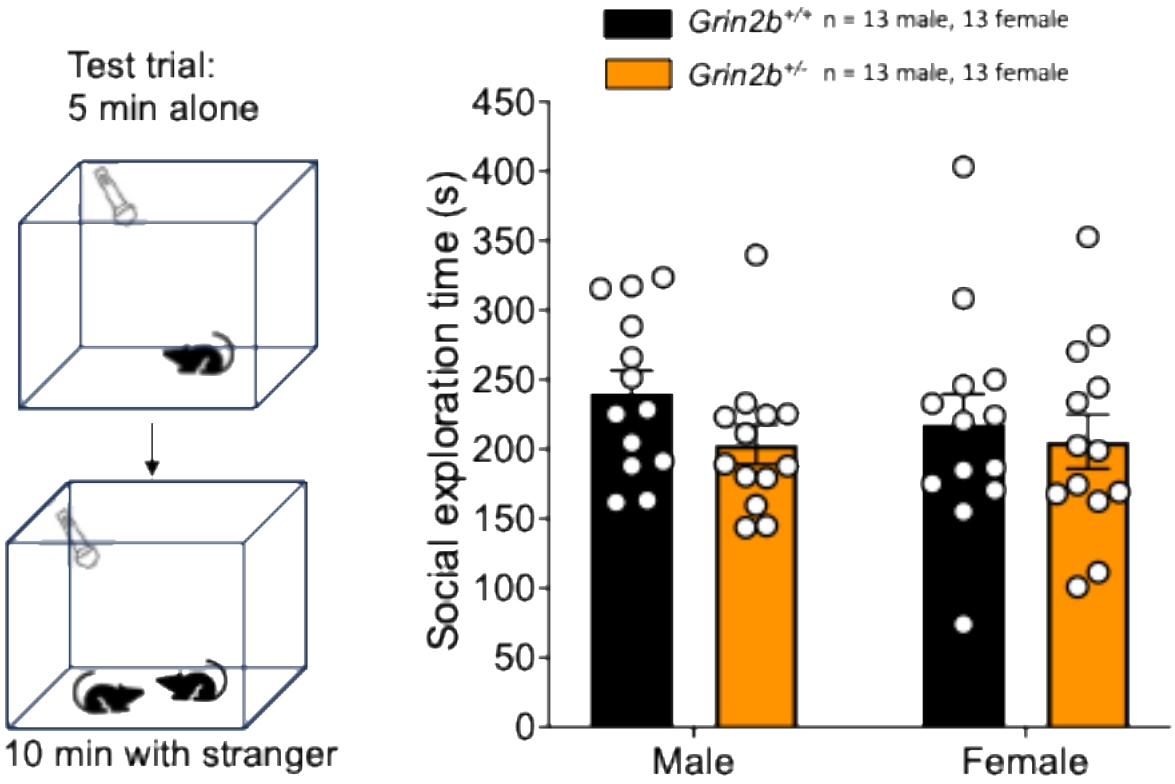


Figure 8. No difference in time spent engaged in social exploration of a novel rat between Grin2b het and WT rats. Time (mean ± SEM) spent socially investigating an unfamiliar rat in the 1 trial social interaction paradigm. *N = 13 male and female pairs. Data from Edinburgh Pipeline.*