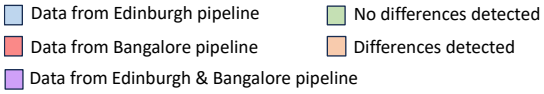
SFARI *Arid1b* heterozygous knockout
LE-*Arid1b*^{em1Mcwi}

The ARID1B (AT-Rich Interaction Domain 1B) gene, located on chromosome 6 in humans, encodes a protein that is a component of the SWI/SNF (SWItch/Sucrose Non-Fermentable) subfamily of ATP-dependent chromatin remodelling complexes, which play a role in transcriptional regulation of certain genes by chromatin remodelling. Mutations in this gene are associated with the development of intellectual disability, autisms, motor impairments and seizures in humans.
To model this disorder in rats, SFARI commissioned the generation of a heterozygous mutant using CRISPR/Cas9 to target the Arid1b gene on chromosome 1, resulting in deletion of exon4, on an outbred Long–Evans background from the Medical College of Wisconsin. Het males were bred with WT females and vice versa to generate litters. Arid1b heterozygous mutant rats (hets) appear healthy and fertile. Homozygous loss of the Arid1b gene is lethal.

	MALE data	No. pairs
1	General characterisation	3 pairs
2	Body weight	38 pairs
3	Object Recognition Memory	14 pairs
4	Object Location Memory	14 pairs
5	Marble Interaction	15 pairs
6	Water Maze	12 pairs
7	Auditory Fear Conditioning	17 pairs
8	Prey Capture	15 pairs
9	One Trial Adult Social Interaction	11 pairs

	FEMALE data	No. pairs
1	General characterisation	
2	Body weight	28 pairs
3	Object Recognition Memory	10 pairs
4	Object Location Memory	scoring ongoing
5	Marble Interaction	16 pairs
6	Water Maze	11 pairs
7	Auditory Fear Conditioning	19 pairs
8	Prey Capture	10 pairs
9	One Trial Adult Social Interaction	11 pairs



Other ongoing tasks in the pipeline: Neonatal Reflexes; Active Place Avoidance, Juvenile Paired Play, Tactile Reactivity: hairy back tape and hind paw tape

1 GENERAL CHARACTERISATION

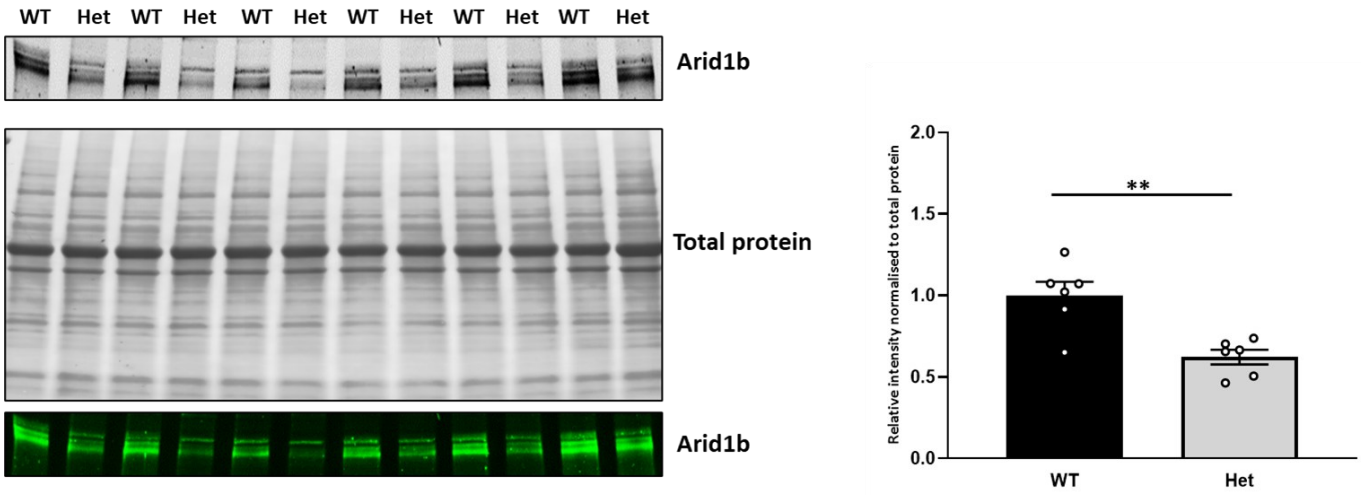


Figure 1.1 ARID1B protein levels are reduced in het rats. Western blot analysis of ARID1B expression in cortical homogenates from Arid1b Het X Het crossed P0 littermates*. The ARID1B intensity was normalized with total protein and compared with their WT littermates. Green blot image is the same as in grey. WT=6 and Het=6, t-test ** p<0.01*

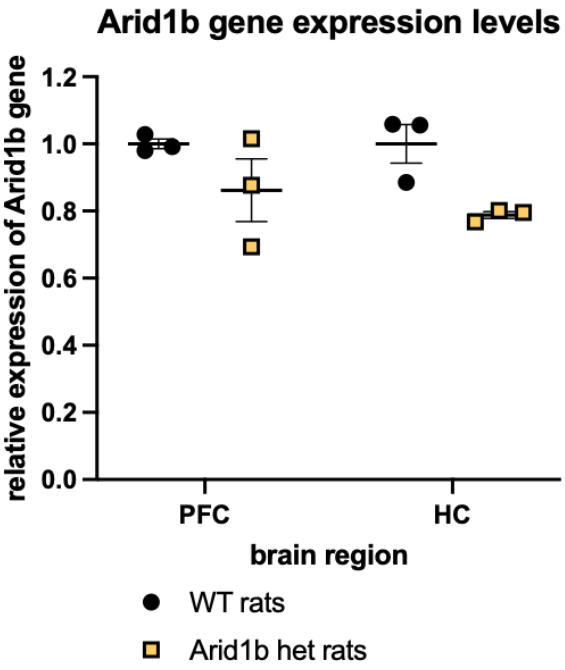


Figure 1.2. Arid1b mRNA levels are reduced to approximately 80% in het relative to WT rats. Expression levels (mean ± SEM) of the Arid1b gene *measured using bulk RNA sequencing, levels are expressed relative to the mean level in WT samples, in prefrontal cortex (PFC) and hippocampal (HC) brain tissue samples. Protein levels are reduced to approximately 50% of WT levels suggesting that not all transgenic RNA goes through nonsense mediated decay but are unable to make functional protein.*

2 BODY WEIGHT

Body weight was lower in *Arid1b* het male and female rats compared to wildtype (WT) littermates across life (Fig. 2A; pup body weights; 2-way ANOVA, main effect of genotype: $F_{1,37}$= 30.7, P < 0.0001, 2B; adult body weights at 10 wks of age 2-way ANOVA, main effect of genotype: $F_{1,129}$= 34.5, P < 0.0001).

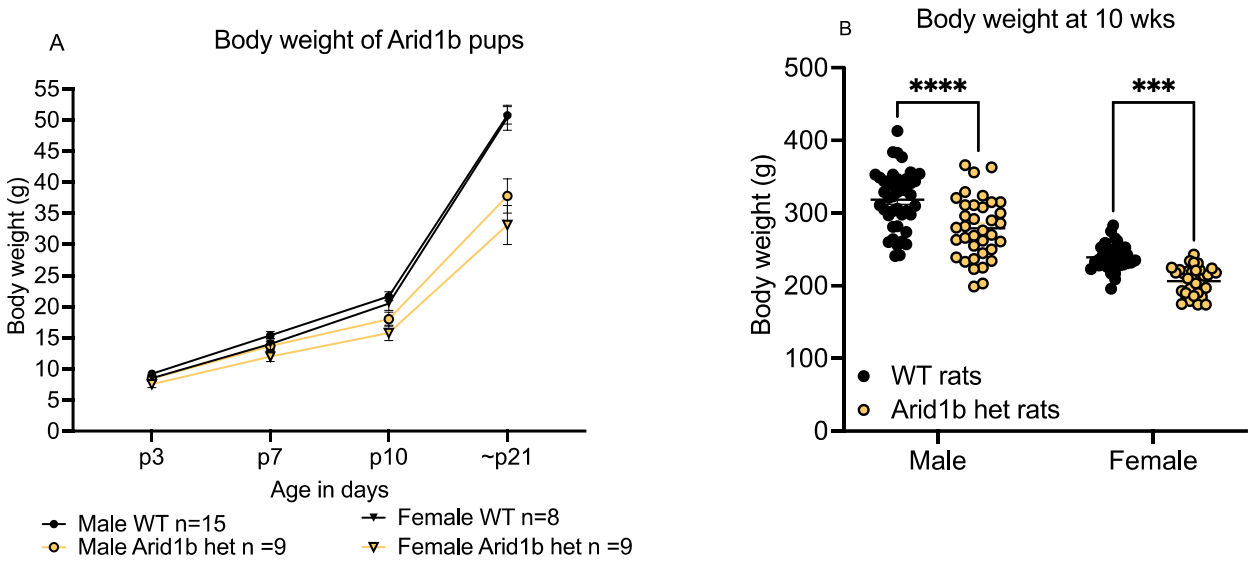


Figure 2. Body weight is reduced in Arid1b het rats. Body weights (mean ± SEM) *A) from postnatal day (P) 3 until approx. P21 and B) at 10 wks of age in male and female rats on a weight control diet to maintain at 85-95% free feeding weight. *** P < 0.001, **** P< 0.0001 post hoc Fisher's LSD, Edinburgh pipeline, same effect found in Bangalore pipeline.*

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 the sample and test trials are shown in Fig. 3 B and C respectively. Group mean discrimination indices in the test trial did not significantly differ between genotypes (2-way ANOVA: genotype effect ns). And the DI for all groups was significantly above chance (one sample t-test against the null hypothesis that DI = 0; all groups: $t_9 > 5.7$, $P < 0.0003$; 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 *Arid1b* 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 and G respectively. Group mean discrimination indices in the test trial did not significantly differ between genotypes (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: $t_9 > 3.3$, $P < 0.005$; Fig. 3I) showing that all groups are preferentially exploring the novel object, demonstrating good object recognition memory in the long-term test in both WT and *Arid1b* het rats (24 hr ITI; Fig. 3 H-I)

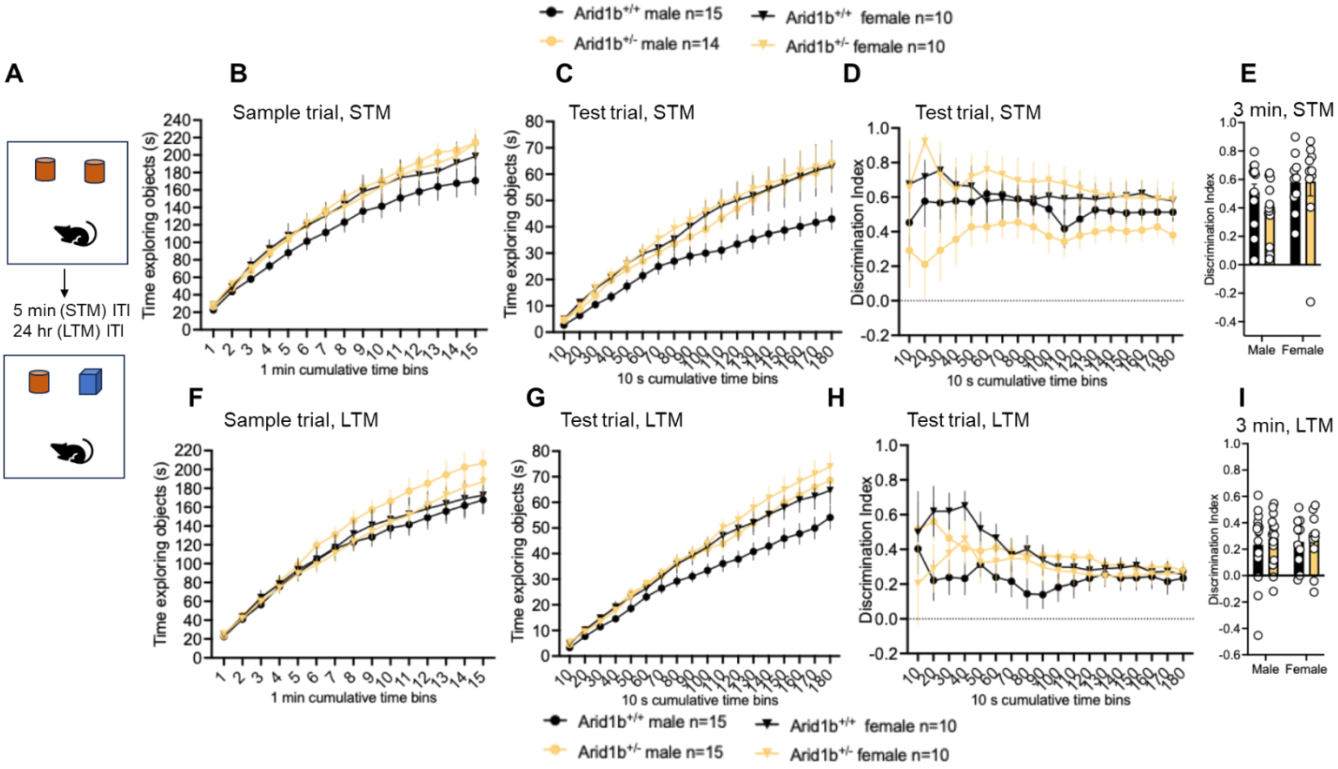


Figure 3. No impairment in long- or short-term object recognition memory in het rats. Object recognition; performance measures (mean ± SEM) in the object recognition task*. A) Task Schematic of the short-term (5 min interval) and long-term (24 hr interval) memory tasks. Rats received the sample trial (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.*

4 OBJECT LOCATION MEMORY

Male data, 1 hr intertrial interval, Bangalore pipeline

Memory was assessed in the long-term object location task (1 hr intertrial interval in Bangalore pipeline). Data collected in Bangalore showed that *Arid1b* het rats had impaired memory in this task relative to WT littermates with 1 hr between trials (Fig. 4.1).

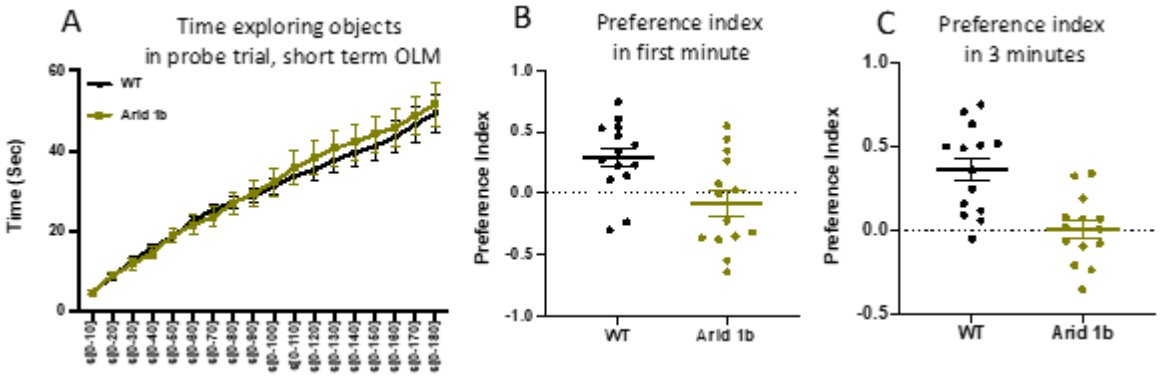


Figure 4.1. Impaired object location memory after 1 hr inter trial interval in Arid1b het rats. Object location; performance measures *(mean ± SEM) in the short- term task (1 hr inter trial interval). A) Accumulated time exploring the objects in the probe trial, B) preference index for the moved object in the first minute of the probe trial (one-sample t- test: WT, P = 0.002; Het, P = 0.442) and in the C) full 3 mins of the probe trial (one-sample t- test: WT, P = 0.002; Het, P = 0.672). N = 14 per group. Data from Bangalore pipeline.*

5 MARBLE INTERACTION

To investigate the impact of a reduction in ARID1B levels on interest in novel objects, we tested the rats in the marble interaction paradigm (Fig 5A). Both male and female *Arid1b* het rats spent less time manipulating marbles with their forepaws than WT littermates during the trial (Fig. 5B; 2-way ANOVA, genotype: $F_{1,62}$= 6.3, P = 0.0149).

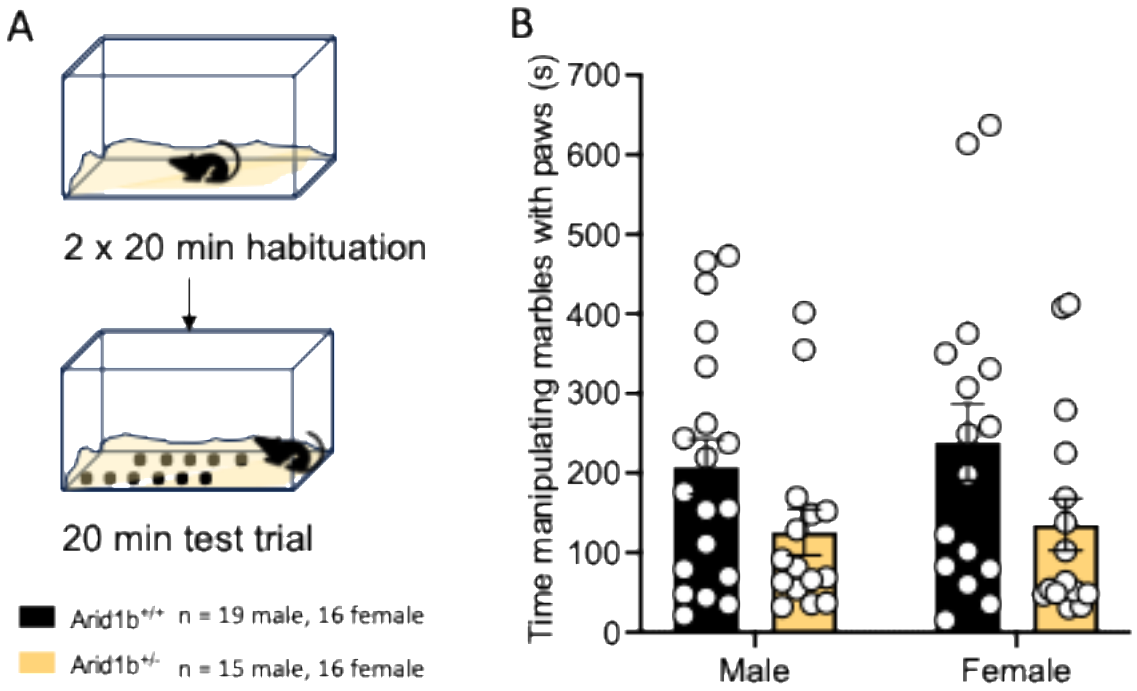


Figure 5. Arid1b het rats manipulate marbles less that WT rats. Performance measures (mean ± SEM) in the marble interaction task*. A) Task Schematic of the two habituation sessions and the test trial of the marble interaction task on three separate days. B) Time (s) mean ± SEM rats spent manipulating marbles with their forepaws out of the 20-min test trial. Data from Edinburgh pipeline.*

6 WATER MAZE

Prior to assessing spatial memory, we first ensured that the rats have the basic motor and visual capabilities to perform in the water maze using a visible platform protocol (no spatial cues available, beacon on top of the platform). There were no differences in time (or distance) to reach the platform during visible training (data not shown) between male or female WT and *Arid1b* het rats.

To assess spatial long-term memory (reference trials), the platform remained in the same location over trials and rats received 4 daily trials for 4 days, each trial starting from a different location and spatial cues were available. In the first trial of each day the (Atlantis) platform was not available to the rats for the first 60 s of the trial, which allowed for assessment of dwell time in the target zone.

Time to find the platform in the trials 2-4 for each day significantly reduced across trials in all groups (Fig. 6A, 2-way RM ANOVA: main effect of trial: $F_{5.5,249.4}= 5.8$, $P < 0.0001$), suggesting intact spatial learning in *Arid1b* het and WT rats.

Increased time spent in the quadrant of the pool that contained the platform during first daily trial of each day revealed that rats learned the location of the platform over days, and that *Arid1b* het rats learned this as well as WT rats (Fig. 6B, 2-way RM ANOVA: main effect of day: $F_{2.8,128.3}= 12.7$, $P < 0.0001$; genotype: ns). % time in the quadrant opposite the platform decreased over days in WT and Arid1b het rats (Fig. 6B, dotted lines).

To assess extinction of the old location and learning of a new spatial location (reversal trials), the rats then received 4 more days of testing with 4 trials per day with the platform now located in the opposite quadrant to during previous training. There was no significant difference amongst the groups in the learning of this new location, as shown by decreased time to find the platform over trials in all groups (Fig. 6C, 2-way RM ANOVA: main effect of trial: $F_{4.7,214.6}= 6.1$, $P < 0.0001$).

During the first daily trial (in which the platform is not available for the first 60 s), all groups increased the % of time spent in the quadrant of the pool that contained the platform (Fig. 6D, 2-way RM ANOVA: main effect of day: $F_{2.3,107.4}= 22.8$, $P < 0.0001$).

Distance to find the platform, swim speed and levels of thigmotaxis (time in outer 12cm perimeter of the pool, an anxiety corelate) did not differ amongst the groups for any trials (data not shown).

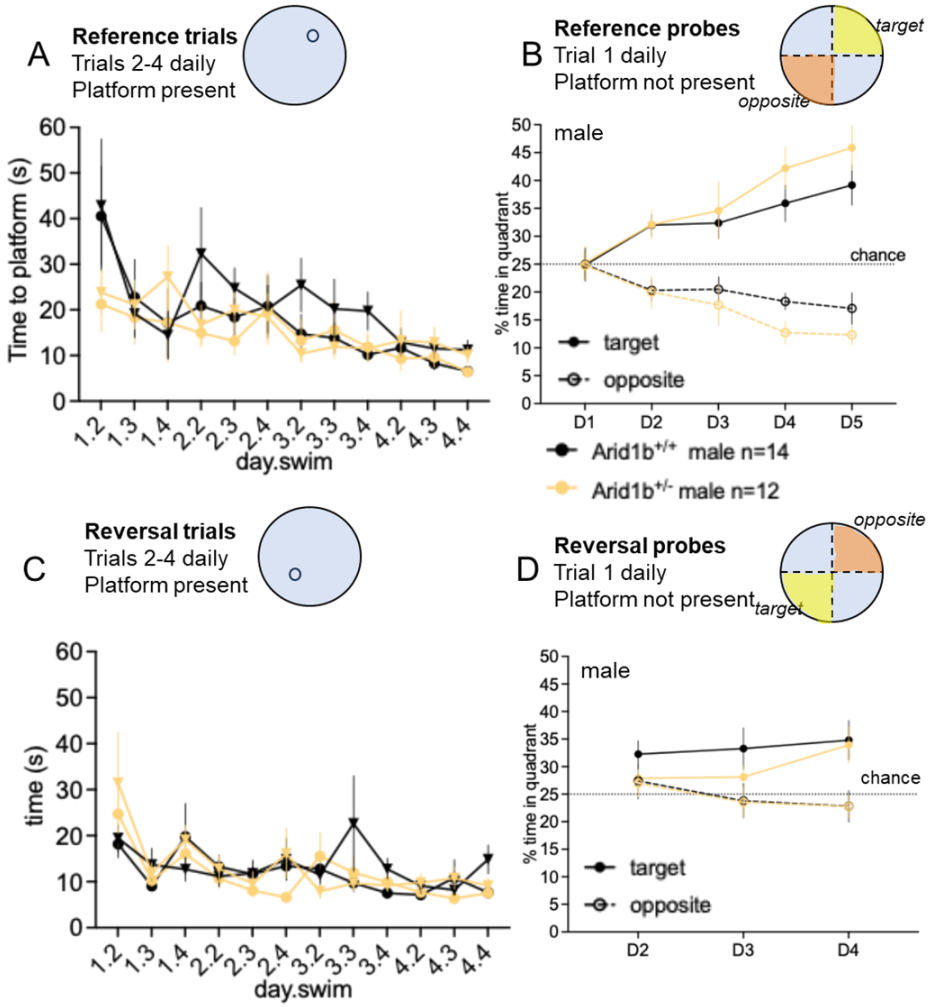


Figure 6. Spatial learning and reversal learning unaltered in Arid1b het rats. Water maze performance parameters (mean ± SEM) for Arid1b het and WT male and female rats. *A) Time to find the platform in trials 2-4 for days 1-4. B) The % of the 60s trial that the rats spent in the first trial of each day in the quadrant that contained the platform (solid line) and time in the quadrant opposite to the platform (dotted line); the platform was not present for the first 60 s of the trial. C)Time to find the platform in trials 2-4 for days 2-8 (reversal trials). D) The % of the 60s trial that the rats spent in the first trial of each day in the quadrant that contained the platform (solid line) and the opposite quadrant (dotted line).*

7 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 *Arid1b* het and WT male and female rats.

Both male and female WT and *Arid1b* het rats displayed increased freezing levels to the tone during conditioning trials (Fig. 9B; 2-way RM ANOVA; tone: $F_{1.9,136.4}$=190.2, P < 0.0001).

Both and male and female *Arid1b* het rats displayed higher freezing during recall 24 hrs later (tones 1 – 4, Fig. 7C; 2-way RM ANOVA; tone*genotype: $F_{13,897}$= 5.0, P < 0.0001). Both WT and *Arid1b* het rats displayed reduced freezing in tones 5-13, suggesting intact extinction learning (tones 5 –13; Fig. 7C).

All groups showed high extinction indices (EI) (all significantly greater than null hypothesis of EI = 0), indicating successful extinction learning, there was a trend for the Arid1b het rats to have higher extinction index that WT rats, reflecting the higher freezing levels in the early recall trials (Fig 7D, 2-way ANOVA; genotype: $F_{1,69}$=4.0, P = 0.049).

Freezing levels during the recall trials on Day 2 remained low in all groups and did not significantly differ between the groups (Fig. 7E; 2-way RM ANOVA: effect of genotype and sex ns) suggesting intact extinction memory in all groups.

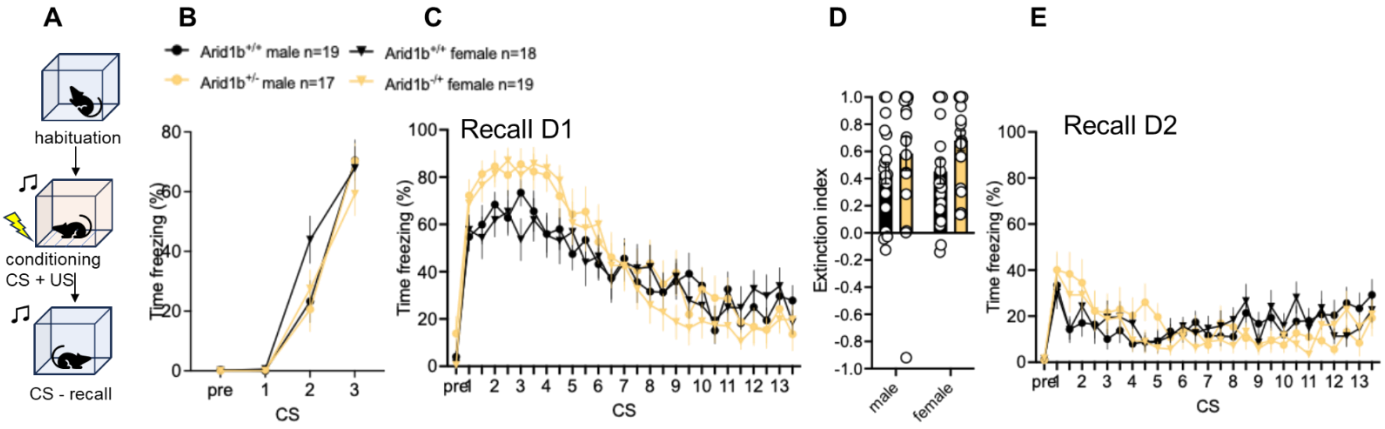


Figure 7. Arid1b het rats show increased freezing during fear recall but typical extinction leaning in auditory fear conditioning task. Auditory fear conditioning parameters *A) Task schematic of the 3 stages of the task: habituation, conditioning in which the conditional stimuli (auditory tone) and 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. Data from Edinburgh pipeline. Analyses were performed on freezing to tone (not freezing during inter tone interval) but data plotted in full.*

8 PREY CAPTURE

We investigated the impact of a reduction of ARID1B 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. 8A). 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).

We tracked the distance each rat moved during the second habituation trial (10 min alone in the arena) using ANYmaze. Females travelled greater distances than males, regardless of genotype (Fig. 8B, 2-way RM ANOVA, sex*time bin: $F_{9,414}$=11.12, P < 0.0001).

During hunting, *Arid1b* het rats were at least 5 times less likely to catch crickets in all trials than WT rats (Fig 8C; Binomial GLM for proportions, genotype: p < 0.002). All 25, except one WT rat, caught in all trials, and six out of 25 *Arid1b* het rats did not catch in all trials.

The time rats took to catch crickets decreased over days, in a genotype dependent manner (Fig. 8D). Capture times decreased over days for WT rats, but not in *Arid1b* het rats, regardless of sex (LMM; day*genotype: P < 0.009). There was a significant difference in time to capture crickets between WT and *Arid1b* het rats on D2 –D5 (LMM, post hoc analyses P< 0.0025).

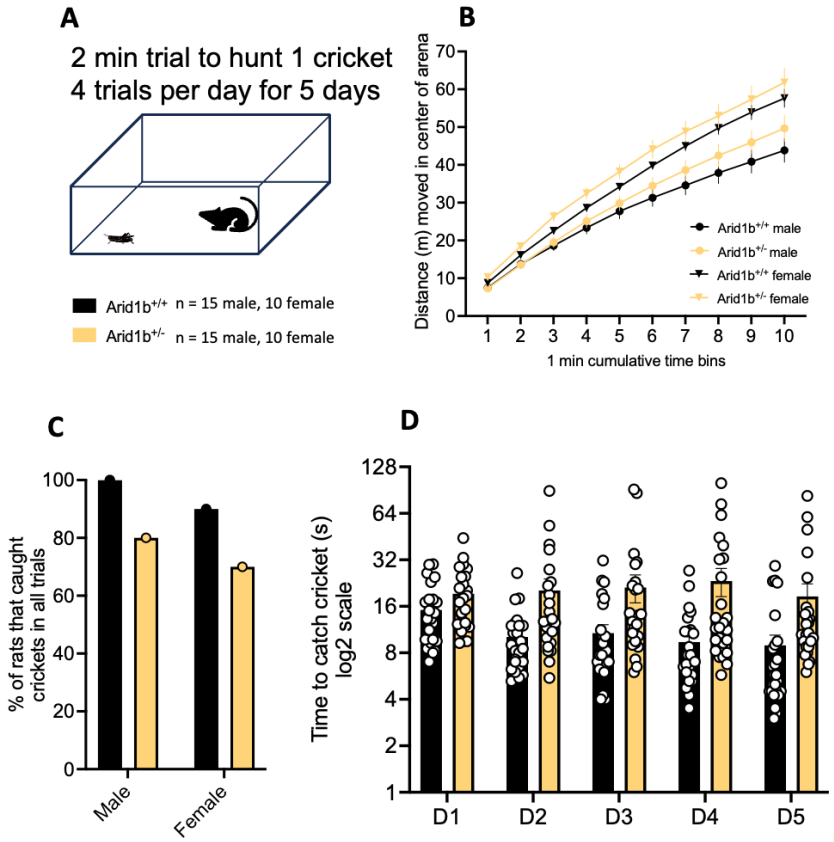


Figure 8. Arid1b het rats are less likely to catch crickets and take longer to catch them than WT rats. Prey capture performance (mean ± SEM). *A) Task schematic of prey capture paradigm and sample sizes. B) Distance (m) moved in the arena, during the 10 min habituation on D2, during which no hunting takes place, in 1-min cumulative time bins, tracked using ANYmaze software. C) % of rats that caught crickets in all trials. D) Time rats take to catch cricket (s) on a log2 scale, male and female pooled. If rats did not catch, the trial was not included. Data from Edinburgh Pipeline.*

9	ONE TRIAL ADULT SOCIAL INTERACTION

To investigate the impact of reduced levels of ARID1B on social behaviour, we exposed WT and *Arid1b* 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 etc. Fig. 9A).

Male rats spent more time than females investigating an unfamiliar conspecific, but there was no difference between *Arid1b* het and WT rats in the time spent socially exploring in either sex (Fig. 9B, 2-way ANOVA: main effect of sex: $F_{1,42} = 4.7$, P = 0.035, all other effects: ns).

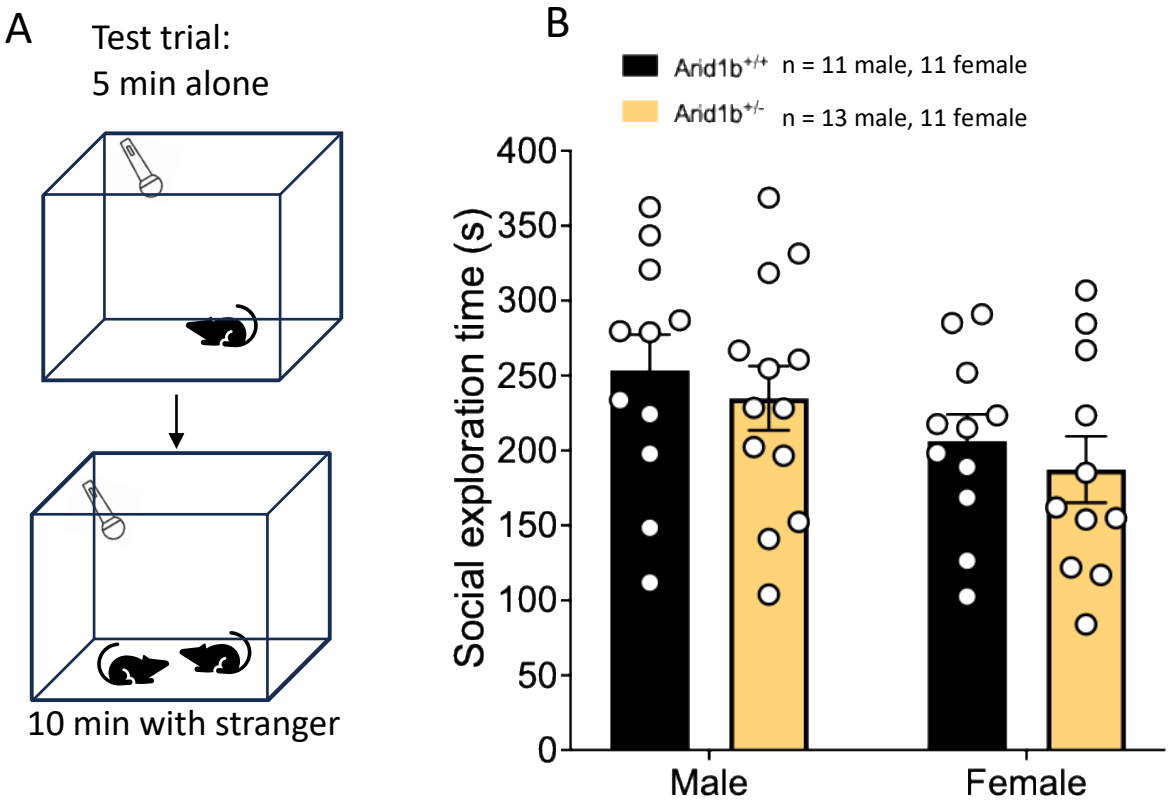


Figure 9. No difference in time spent engaged in social exploration of a novel rat between Arid1b het and WT rats. Time (mean ± SEM) spent socially investigating an unfamiliar rat in the 1 trial social interaction paradigm*. N = 11 male and 11 female pairs per group. Data from Edinburgh Pipeline.*