Behavioural consequences of *Setd1a* haploinsufficiency in mice: evidence for heightened emotional reactivity and impaired sensorimotor gating

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ABSTRACT

A number of studies implicate the loss of function (LoF) mutations affecting the histone methyl transferase *SETD1A* in the aetiology of a range of neurodevelopmental disorders including schizophrenia. Here, we examined the behavioural consequences of haploinsufficiency of *Setd1a* in a mouse model. We find evidence for changes in a number of phenotypes of relevance to schizophrenia, including increased anxiety-related behaviour, enhanced acoustic startle response, and decreased pre-pulse inhibition of acoustic startle. The sensorimotor gating deficits in *Setd1a*$^{+/-}$ mice could not be rescued by haloperidol or risperidone, suggesting that these antipsychotics are ineffective for ameliorating schizophrenia-relevant phenotypes in *Setd1a*$^{+/-}$ mice and point to deficits in neural systems other than the monoamine system. These phenotypes are emerging as key features of a number of other mouse models of rare neurodevelopmental disorders caused by LoF mutations in genes encoding epigenome modifiers suggesting they may act in a network to modulate brain development. Taken together these data strengthen the support for the use of *Setd1a* haploinsufficient mice as a model for the biological basis of schizophrenia, and point towards possible underpinning neural mechanisms.

Keywords: schizophrenia; developmental delay; startle response; pre-pulse inhibition; haloperidol; risperidone

INTRODUCTION

Exome sequencing studies have implicated loss of function (LoF) mutations in the *SETD1A* (SET Domain Containing 1A) gene that increase susceptibility for schizophrenia (1-5). *SETD1A* LoF mutations have also been reported in children with diverse neurodevelopmental disorders (4, 6), childhood apraxia of speech (7), and early-onset epilepsy (8). While these genetic variants are rare (occurring in 0.13 % of schizophrenia cases), they are highly penetrant and their effects on gene function (i.e. haploinsufficiency) can be recapitulated in model systems. Consequently, *SETD1A* provides a biologically tractable target for disease modelling.

SETD1A encodes a subunit of a histone methyltransferase complex that catalyses methylation of lysine residue 4 on histone 3 (H3K4) (9). Previous studies have implicated a role for *SETD1A* in a range of biological functions, including cell cycle regulation (10-12), maintenance of pluripotency in embryonic stem cells (13-15) and neuronal progenitors (16, 17), and DNA repair (11, 18, 19). In terms of the role of *SETD1A* in brain and behaviour, previous work in mice has shown that *Setd1a* haploinsufficiency causes working memory impairments, axonal branching deficits, impaired synaptic plasticity, and abnormal cortical ensemble activity (20, 21). Further evidence for impaired working memory in mice haploinsufficient for *Setd1a* has been reported by Nagahama *et al*. (22), who also observed a broader range of behavioural phenotypes, including hyperactivity, altered social behaviour, and some evidence for impaired sensorimotor gating. Other work in *Drosophila* has shown that short- and long-term courtship memory is impaired by conditional knockdown of Set1 in neurons of the mushroom body (6).

Here, we further explore behavioural consequences of *Setd1a* haploinsufficiency in a mouse model. We find evidence for increased anxiety-related behaviour, enhanced acoustic startle response, and decreased pre-pulse inhibition of acoustic startle. These behavioural changes, and the sensorimotor gating impairments in particular, are phenotypes of particular relevance to schizophrenia. However, the sensorimotor gating deficits could not be rescued by haloperidol or risperidone, suggesting that these antipsychotics are ineffective for ameliorating schizophrenia-relevant phenotypes in *Setd1a*$^{+/-}$ mice. Taken together these data strengthen the support for the use of *Setd1a* haploinsufficient mice as a model for the biological basis of schizophrenia, and point towards possible underpinning neural mechanisms.

MATERIALS AND METHODS

Animals

All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. *Setd1a*$^{+/-}$ mice were produced using a strain created by the Knockout Mouse Phenotyping Consortium (23). To generate a model with a constitutive germline transmissible knockout allele, male C57BL/6NTac-Setd1a$^{tm1c(EUCOMM)Wtsi}$/WtsiCnrm mice (obtained from MRC Harwell) were paired with female B6.C-Tg(CMV-cre)1Cgn/J mice (obtained from The Jackson Laboratory) (24). F1 male progeny were genotyped to identify animals heterozygous for the Setd1a$^{CMV\text{-}cre/+}$ allele. These were crossed with C57BL/6J females (obtained from Charles River) to enable removal of the X-linked CMV-cre transgene from males in the F2 generation. Experimental cohorts were generated by pairing male F2 *Setd1a*$^{+/-}$ mice with C57BL/6J females.

Animals were weaned on postnatal day 28 and housed in mixed-genotype cages with littermates of the same sex (2-5 per cage). Standard laboratory chow and water was available *ad libitum* throughout all experiments. Holding rooms were maintained on a 12-hour light-dark cycle (lights on from 08:00-20:00) at a temperature of 21 (± 2) °C and 50 (± 10) % humidity.

Model validation

To confirm *Setd1a* haploinsufficiency in our model, levels of *Setd1a* mRNA and protein were quantified in whole brains dissected at E13.5. RNA extraction was performed using a Direct-zol™ RNA Miniprep Kit (Zymo, UK). 1 µg total RNA was used for cDNA synthesis using RNA to cDNA EcoDry™ Premix (double-primed) kits (Clontech, UK). qRT-PCR reactions were performed in triplicate using a Corbett Rotorgene 6000 Real-Time PCR machine with Sensimix SYBR No-Rox (Bioline, UK) and intron-spanning primers (Table S1). The geometric mean of Ct values across three housekeeping genes (*Hprt*, *Dynein*, and *B2m*) were used as endogenous controls to normalise *Setd1a* expression levels using the ΔΔCT method (25).

Protein was extracted from brain homogenates in RIPA buffer (Sigma, UK) containing cOmplete™ Mini Protease Inhibitor Cocktail (Roche, Switzerland). A Pierce™ BCA Protein Assay kit (Thermo Scientific, UK) was used to quantify protein concentration. Samples were diluted in protein loading buffer (LI-COR, UK) containing 0.05 % (v/v) 2-Mercaptoethanol (Sigma, UK) and denatured by heating at

95 °C for five minutes. 20 µg total protein per sample was separated by SDS-PAGE using a NuPAGE™ 4-12 % Tris-Acetate gel (Invitrogen, UK) and NuPAGE™ Tris-Acetate SDS Running Buffer (Invitrogen, UK). Proteins were transferred to a 0.45 µm pore size nitrocellulose membrane (Invitrogen, UK) in NuPAGE™ Transfer Buffer (Invitrogen, UK) containing 10 % (v/v) methanol (Fisher Scientific, UK). To enable normalisation of *SETD1A* protein abundance, membranes were stained for total protein using REVERT™ Total Protein Stain (LI-COR, UK). Odyssey TBS Blocking Buffer (LI-COR, UK) was used to block membranes for one hour at room temperature. Membranes were incubated overnight at 4 °C with 1:1,000 polyclonal *Setd1a* antibody (Bethyl Laboratories, USA). TBS-T (1M NaCl, 1M Tris-HCl, 0.2 % (v/v) Tween 20) was used to wash the membrane four times (5 minutes per wash) before incubation in 1:10,000 IRDye 800CW goat anti-rabbit secondary antibody (LI-COR, UK) for one hour at room temperature. Wash steps were repeated prior to imaging using an Odyssey CLx and protein quantification using Image Studio software (LI-COR, UK).

Behavioural testing

Male and female *Setd1a*$^{+/-}$ mice and their wild type (WT) littermates were tested in adulthood (aged 2-3 months at start of testing). In Cohort 1, 39 males (21 WT and 18 *Setd1a*$^{+/-}$) and 38 females (20 WT and 18 *Setd1a*$^{+/-}$) were tested in the following order: elevated-plus maze (EPM), open field test (OFT), locomotor activity, sensorimotor gating, rotarod test, and novel object recognition. All apparatus was cleaned with 70 % (v/v) ethanol between animals.

Cohort 2 was used to test the effects of antipsychotics (haloperidol and risperidone) on sensorimotor gating impairments observed in Cohort 1. A power calculation performed on the pre-pulse inhibition data from Cohort 1 showed that a minimum sample size of 22 was required ($1 - \beta = 0.8$, $\alpha = .05$) for Cohort 2, which comprised 26 males (16 WT and 10 *Setd1a*$^{+/-}$) and 30 females (17 WT and 13 *Setd1a*$^{+/-}$). First, all mice completed a pre-test session to test whether the basic effects of *Setd1a* haploinsufficiency were replicated. Then, mice were randomly divided into two approximately equal groups (balanced for sex and genotype) and allocated to either the haloperidol or risperidone condition (Table 1).

Table 1. Number of animals allocated to drug condition in Cohort 2.

	Haloperidol		Risperidone	
	WT	*Setd1a*$^{+/-}$	WT	*Setd1a*$^{+/-}$
Male	8	5	8	5
Female	9	7	8	6
Total	17	12	16	11

Anxiety-related behaviour

The EPM and OFT were used to assess anxiety-related behaviour under identical lighting conditions (15 lux). Each animals' position in the apparatus during the trial was determined using EthoVision XT software (Noldus Information Technology, Netherlands) at a tracking rate of 12 frames per second via a camera mounted above the apparatus.

The EPM was constructed of white Perspex and consisted of four arms of equal size (175 x 78 mm) extending from a central square region (78 x 78 mm) and positioned 450 mm above the floor. Two of the arms were 'open' (no walls) and two were enclosed by 150 mm high opaque walls. Arms of the same type were diametrically opposed. Each animal was placed in the same enclosed arm at the start of the trial and allowed to explore the apparatus for five minutes. Measures of anxiety were: i) total distance moved (cm), ii) proportion of time spent on either of the open arms, iii) latency of first entry into an open arm, and iv) number of entries into the open arms (summed across both arms).

The OFT comprised a 750 x 750 mm arena with 45 cm white Perspex walls. Each animal was placed in the same corner of the arena and allowed to explore freely for 10 minutes. The arena was divided into two, concentric virtual zones: the 'inner zone' (central 600 x 600 mm) and the 'outer zone' (surrounding 150 mm). Measures of anxiety were: i) total distance moved (cm), ii) proportion of time spent in the inner zone, iii) latency of first entry into the inner zone, and iv) number of entries into the inner zone.

Locomotor activity

Locomotor activity levels were assessed using clear Perspex chambers (210 x 360 x 200 mm) with two transverse infrared beams positioned 30 mm from either end of

the chamber and 10 mm above the floor of the chamber. Each animal completed three 120 minute sessions over consecutive days at the same time of day. The apparatus was linked to a computer using ARACHNID software (Cambridge Cognition Ltd., UK). Activity levels were recorded as the number of beam breaks during each session using a custom programme (BBC BASIC Version 6).

Sensorimotor gating

The acoustic startle response (ASR) and pre-pulse inhibition (PPI) were measured using apparatus from SR-Lab (San Diego Instruments, USA). Animals were placed in a clear Perspex tube (35 mm internal diameter) mounted on a Perspex plinth in a sound-attenuating chamber. A 70 dB (A scale) white noise stimulus was continuously played throughout the session via a loudspeaker positioned 120 mm above the tube. Each session started with a five minute habituation period. Acoustic stimuli were presented with a mean intertrial interval of 16 seconds (pseudorandomly varied between 6 and 24 seconds). Each pulse-alone trial consisted of a 40 ms acoustic startle stimulus. Pre-pulse trials consisted of a 20 ms acoustic pre-pulse stimulus followed by a 40 ms acoustic startle stimulus 80 ms after pre-pulse offset.

In Cohort 1 and 3, the session was split into three blocks. Pulse amplitude was set to 120 dB and 105 dB (above background) in block 1 and block 2, respectively. In each block, 6 consecutive 120 dB pulse-alone trials were presented followed by 7 additional pulse-alone trials interspersed with 18 pre-pulse trials (either 4, 8 or 16 dB (above background) with six trials of each pre-pulse amplitude). The third block comprised a range of pulse-alone trials (80 – 120 dB (above background) in 10 dB increments), with three of each trial type. A shortened version of task was used for Cohort 2. In the basal session, all 4 dB pre-pulse trials and all 105 dB trials were removed. In the drug experiment, block 3 was also removed from the session. For all experiments, only the data from 120 dB pulse alone and 8 and 16 dB pre-pulse trials are reported here for brevity but all raw data are available in the online repository.

The whole-body startle response was detected on each trial by a piezoelectric sensor attached to the plinth, which transduced flexion in the plinth into a digitised signal. The average startle response (Vavg) was recorded in arbitrary startle units using SR-Lab software over the 65 ms period following stimulus onset. Startle data were weight-adjusted by dividing Vavg by body weight recorded immediately after

the test session. PPI was calculated as the percentage reduction in startle amplitude between pre-pulse and pulse-alone trials (excluding the first three pulse-alone trials).

Rotarod test

Motor learning and coordination were assessed using Rotarod 47600 apparatus (Ugo Basile, Italy) designed for use in mice. The rod (30 mm diameter) was coated with rubber grooves to provide grip and divided into 570 mm chambers. Each animal completed five trials across two days (three trials on day one and two trials the next day). During each 300 second trial, the speed of rotation increased from 5 – 50 rpm at a constant rate of 0.15 rpm per second. The latency to fall was recorded on each trial by levers (160 mm below the rod) that caused a timer to stop when triggered by the animal falling off the rod. On trials where the animal stayed on the rod for the duration of the trial, the latency to fall was recorded as the maximum trial length.

Novel object recognition

Recognition memory was assessed using the novel object recognition (NOR) paradigm. Testing was conducted under dimmed lighting (15 lux) in a white Perspex arena (300 x 300 mm) with 300 mm high walls. Prior to testing, mice were habituated to the empty arena for 10 minutes per day over three consecutive days.

Each test session comprised three phases. In the ‘habituation phase’, mice explored the empty arena for 10 minutes. Mice were returned to a holding cage while the experimenter placed two identical objects in diagonally opposite quadrants of the arena (105 mm from the corner). In the ‘acquisition phase’, mice were placed back into the arena and allowed to explore the objects for up to 15 minutes. To control for potential differences in object neophobia that could contribute to subsequent memory performance, the total amount of object exploration (defined as when the animal’s head was within 20 mm and oriented towards the object) was timed during the trial by the experimenter. The acquisition phase was ended once 40 seconds of object exploration was achieved or the maximum trial length had elapsed. Animals were then returned to either a holding cage or their home cage for a retention interval of 30 minutes or 24 hours. In the ‘test phase’, mice were placed back in the arena with one of the objects that they had been exposed to during the acquisition phase (‘familiar’) and another object that had not been encountered previously (‘novel’). The objects were presented in the same locations as the acquisition phase.

Animals were allowed to explore freely for five minutes and the amount of familiar and novel object exploration was recorded manually via the keyboard by the experimenter using EthoVision XT software (Noldus Information Technology, Netherlands). A discrimination ratio was calculated by dividing the amount of time the animal spent exploring the novel object by the total amount of object exploration (novel and familiar summed).

All animals completed the experiment at both the 30 minute and 24 hour retention interval, with order counterbalanced across animals and at least 24 hours between each condition. For the second test session, new objects that had not been encountered previously were placed in the quadrants of the arena that were unoccupied in the previous session. Location of the objects and allocation of objects to retention interval were counterbalanced across animals.

Drug preparation and administration

Haloperidol (Sigma, UK) and risperidone (Sigma, UK) were initially dissolved in 1M glacial acetic acid and then diluted in 0.9 % saline such that an equal volume (100 µL per gram of body weight) was administered for each injection. Drugs were administered by intraperitoneal injection with a 30 minute pre-treatment delay before the sensorimotor gating test. All animals completed three sessions (vehicle, 0.5 mg/kg and 1.0 mg/kg) with seven days washout between sessions. These dosages were selected based on pilot dose-response data showing that a 0.5 mg/kg dose of either haloperidol or risperidone did not have significant effects on sensorimotor gating in WT C57BL/6J mice (Figure S1). At 1.0 mg/kg, haloperidol caused a significant increase in PPI and risperidone caused a significant reduction in the ASR. Therefore, 0.5 mg/kg was selected as a sub-threshold dose to explore drug effects in *Setd1a*$^{+/-}$ mice in the absence of non-specific effects of the drugs on sensorimotor gating that occur in WT mice. Dose order was counterbalanced across animals for vehicle and 0.5 mg/kg. All mice received the 1.0 mg/kg dose in the final session to explore drug effects at a dose that was known to affect sensorimotor gating in WT animals to rule out the possibility that the lack of effect in *Setd1a*$^{+/-}$ mice was due to an insufficient dose of drug.

Data analysis

All data are available at: https://osf.io/gt7ew/. Data were analysed using IBM SPSS software (version 25). *Setd1a* expression levels (mRNA and protein) in E13.5 brain were analysed using independent samples t-tests (*Setd1a*$^{+/-}$). The between-subjects factors of genotype and sex were included in analyses of all behavioural data. Data from the EPM and OFT were analysed using MANOVA with four dependent variables; distance moved, proportion of time spent in anxiogenic region, latency of first entry into the anxiogenic region (log transformed to remove positive skew), and number of entries into the anxiogenic region. Locomotor activity data were analysed using a mixed ANOVA with day as a within-subjects factor. ASR data were analysed using ANOVA with sex and genotype as between-subjects factors. PPI data were analysed using a mixed ANOVA with pre-pulse amplitude (8 dB and 16 dB) as a within-subjects factor. The additional within-subjects factor of drug condition (vehicle, 0.5 mg/kg and 1.0 mg/kg) was included in the model for analysis of the pharmacological challenge experiment. Rotarod data were analysed using a mixed ANOVA with the within-subjects factor of trial number (5 levels). NOR data were analysed using a mixed ANOVA with the within-subjects factor of retention interval (30 minutes and 24 hours). Data from one male WT subject (Cohort 1) were missing for NOR due to a technical issue during testing. Data from one female WT animal (Cohort 3) were excluded from analysis of the OFT because it was identified as a multivariate outlier. Greenhouse Geisser corrected results are reported for locomotor activity and the Rotarod test because a significant Mauchly's test result indicated that the assumption of sphericity had been violated.

RESULTS

Confirmation of *Setd1a* haploinsufficiency in the *Setd1a*$^{+/-}$ model

Setd1a mRNA expression was reduced by 48.8 % in *Setd1a*$^{+/-}$ brain at E13.5 compared to WTs ($t(14) = 9.18$, $p < .001$; Figure 1A). Consistent with this, levels of SETD1A protein were also reduced by 46.3 % ($t(8) = 2.71$, $p = .03$; Figure 1B). This magnitude of reduction is consistent with haploinsufficiency and confirms that *Setd1a* knockdown to half of WT levels was successfully achieved.

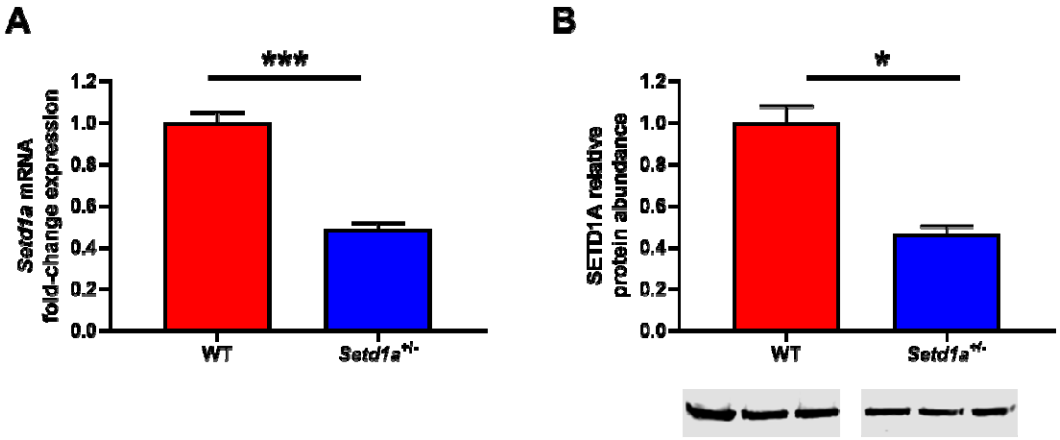

Figure 1. Reduced *Setd1a* dosage in E13.5 *Setd1a*$^{+/-}$ brain. Mean (± SEM) fold-exchange expression (relative to WT) **A)** *Setd1a* mRNA (N = 8 per genotype) and **B)** SETD1A protein abundance (N = 5 per genotype).

Setd1a$^{+/-}$ mice show increased anxiety-related behaviour in the OFT but not the EPM

Analysis of the EPM data revealed a significant multivariate effect of genotype ($F(4, 70) = 2.52$, $p = .049$, Wilk's $\lambda = 0.87$). However, univariate follow-up analyses revealed no significant effects of genotype for total distance moved ($p = .99$; Figure 2A), proportion of time on the open arms ($p = .75$; Figure 2B), log latency of first entry into one of the open arms ($p = .06$; Figure 2C) or number of entries into the open arms ($p = .20$; Figure 2D). There was no significant multivariate effect of sex ($F(4, 70) = 1.39$, $p = .25$, Wilk's $\lambda = 0.93$) and no interaction between genotype and sex ($F(4, 70) = 1.33$, $p = .27$, Wilk's $\lambda = 0.93$).

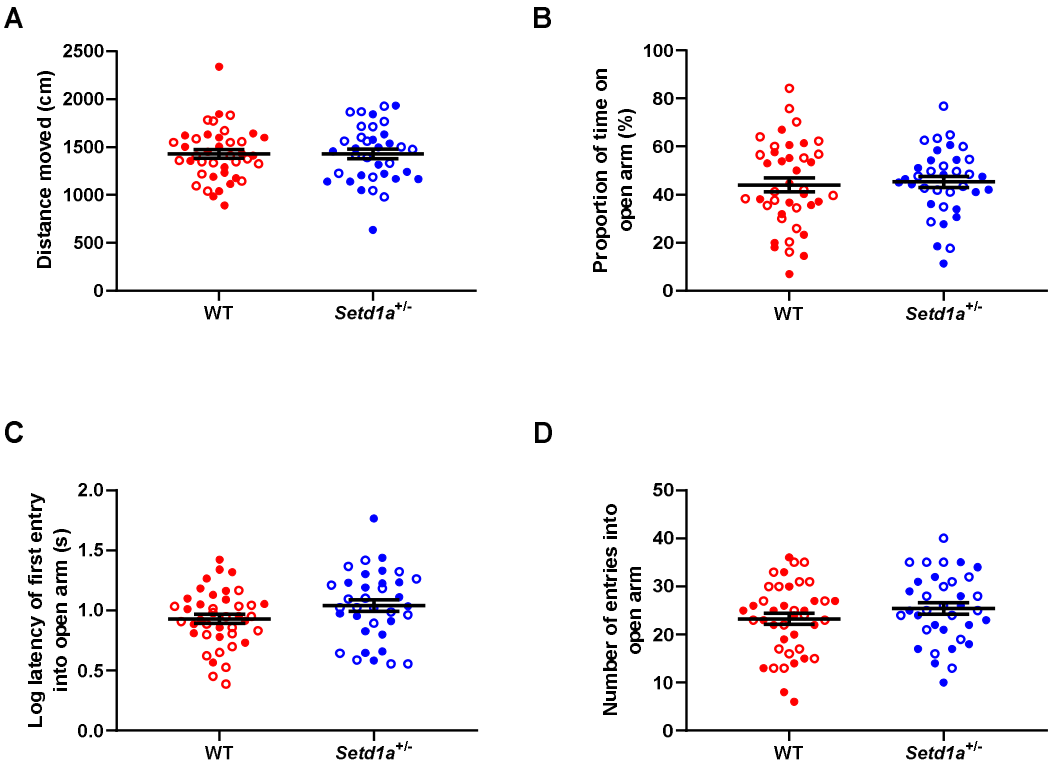

Figure 2. *Setd1a*$^{+/-}$ mice do not show increased anxiety-related behaviour on the EPM. Mean (± SEM) **A)** distance moved (cm), **B)** proportion of time spent on the open arm (%), **C)** log latency of first entry into an open arm (s), and **D)** number of entries into an open arm. Males = filled symbols and females = open symbols.

Analysis of the data from the OFT revealed a significant multivariate effect of genotype ($F(4, 70) = 3.43$, $p = .01$, Wilk's $\lambda = 0.84$). Follow-up univariate analyses revealed that, compared to their WT littermates, *Setd1a*$^{+/-}$ mice made significantly fewer entries into the inner zone ($p = .04$; Figure 3D) and spent significantly less time in this region ($p = .02$; Figure 3B). There were no differences between *Setd1a*$^{+/-}$ and WT mice in the total distance moved during the test ($p = .11$; Figure 3A) or log latency of first entry into the inner zone ($p = 18$; Figure 3C). There was no significant multivariate effect of sex ($F(4, 70) = 1.31$, $p = .27$, Wilk's $\lambda = 0.93$) or interaction between sex and genotype ($F(4, 70) = 0.13$, $p = .97$, Wilk's $\lambda = 0.99$), indicating that both male and female *Setd1a*$^{+/-}$ mice showed increased anxiety-related behaviour in the OFT.

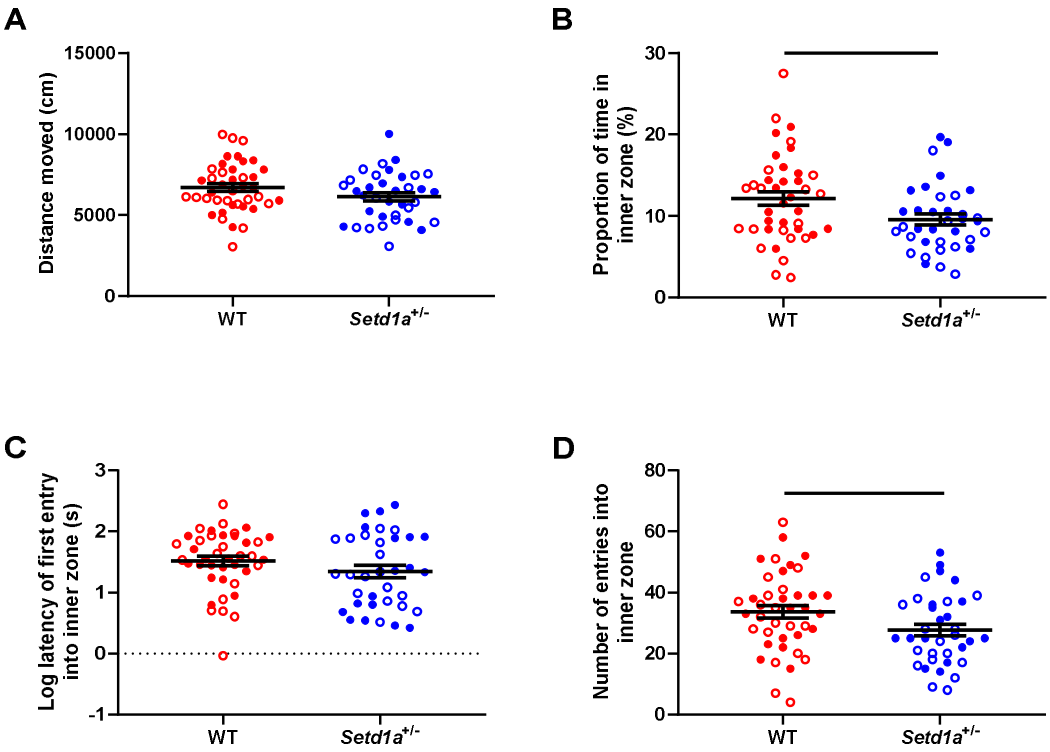

Figure 3. *Setd1a*$^{+/-}$ mice show increased anxiety-related behaviour in the OFT. Mean (± SEM) **A)** distance moved (cm), **B)** proportion of time spent in the inner zone (%), **C)** log latency of first entry into the inner zone (s), and **D)** number of entries into the inner zone. Males = filled symbols and females = open symbols. * $p < .05$.

Locomotor activity levels, motoric function, and novel object recognition are not altered in *Setd1a*$^{+/-}$ mice

Locomotor activity levels were assessed as the number of beam breaks made over three consecutive days (Figure 4A). The number of beam breaks made by *Setd1a*$^{+/-}$ mice was not significantly different from WTs ($F(1, 73) = 0.46$, $p = .50$), indicating no effect of *Setd1a* haploinsufficiency on locomotor activity levels. Moreover, there was a significant main effect of day ($F(1.69, 123.08) = 28.63$, $p < .001$) and no significant interaction between day and genotype ($F(1.69, 123.09) = 0.18$, $p = .80$). This shows that the degree of habituation of locomotor activity levels across days was equivalent in *Setd1a*$^{+/-}$ and WT mice. There was also no significant main effect of sex ($F(1, 73) = 0.40$, $p = .84$). No significant interactions were observed between sex and genotype ($F(1, 73) = 2.45$, $p = .12$) or between genotype, day, and sex ($F(1.69, 123.09) = 0.62$, $p = .92$). These results show that both male and female *Setd1a*$^{+/-}$

353 mice show normal locomotor activity levels and habituation of activity levels across
354 test sessions.
355 Assessment of motor coordination using the Rotarod test (Figure 4B) revealed that
356 the latency to fall over five accelerating trials was not significantly different between
357 *Setd1a*$^{+/-}$ and WT mice ($F(1, 73) = 2.70$, $p = .11$). A significant main effect of trial was
358 observed ($F(2.97, 217.0) = 16.99$, $p < .001$), indicating that the latency to fall
359 increased with training. There was no significant interaction between genotype and
360 trial ($F(2.97, 217.0) = 0.50$, $p = .68$), which shows that motor learning was not
361 impaired in *Setd1a*$^{+/-}$ mice. There was also no significant main effect of sex ($F(1, 73)$
362 $= 0.33$, $p = .57$) and no significant interaction between sex and genotype ($F(1, 73) =$
363 0.001, $p = .98$). The three way interaction between genotype, sex, and trial was also
364 not significant ($F(2.97, 217.0) = 2.41$, $p = .07$). These data indicate normal motor
365 coordination and motor learning in male and female *Setd1a*$^{+/-}$ mice.
366 Novel object recognition memory performance was assessed after a 30 minute and
367 24 hour retention interval (Figure 4C). As expected, discrimation ratios were higher
368 after 30 minutes compared to 24 hours ($F(1, 72) = 19.61$, $p < .001$). There was no
369 significant main effect of genotype ($F(1, 72) = 0.05$, $p = .82$) and no interacton
370 between genotype and retention interval ($F(1, 72) = 1.68$, $p = .20$). There was also
371 no significant main effect of sex ($F(1, 72) = 0.32$, $p = .57$), no interaction between sex
372 and genotype ($F(1, 72) = 0.45$, $p = .51$) or between sex, genotype and delay ($F(1,$
373 $72) = 0.59$, $p = .45$). These findings indicate intact short- and long-term object
374 recognition memory in both male and female *Setd1a*$^{+/-}$ mice.
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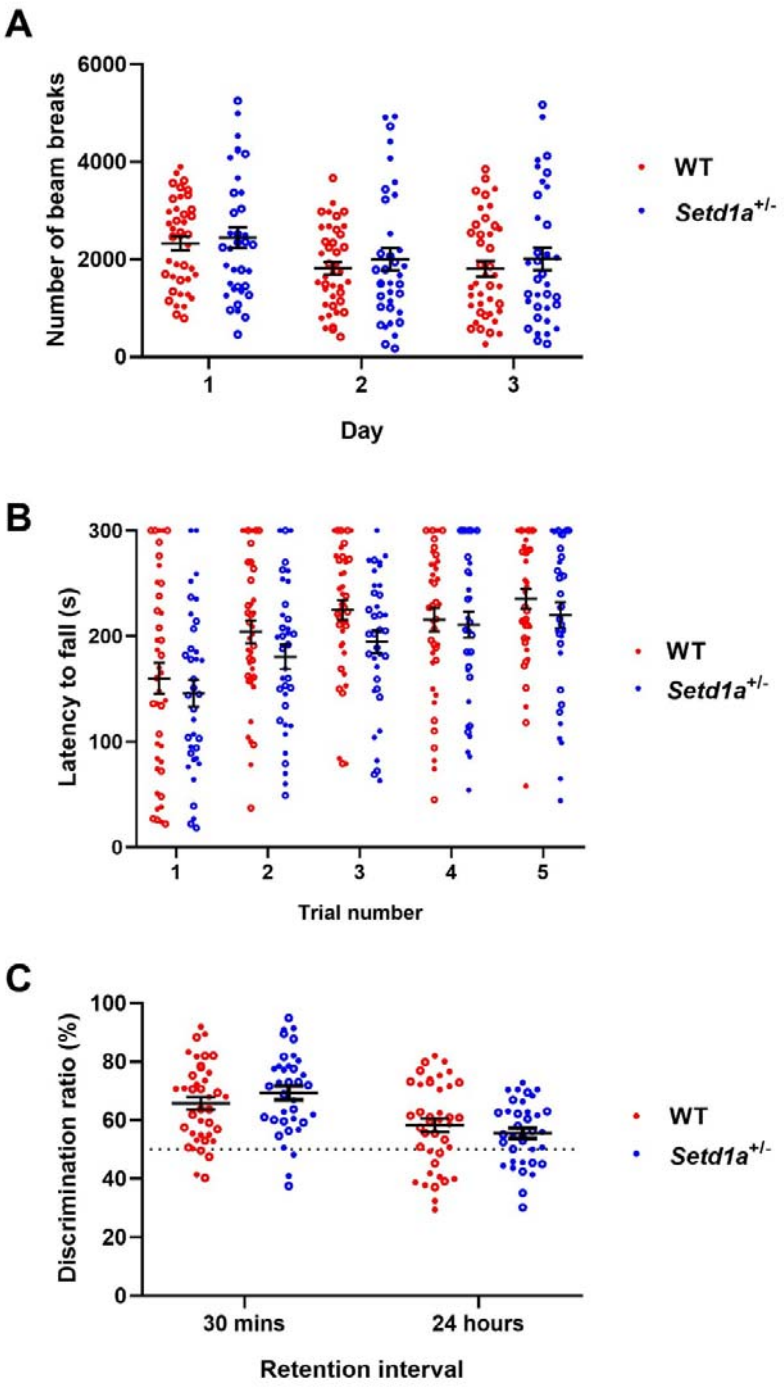

Figure 4. *Setd1a*$^{+/-}$ mice show normal locomotor activity levels, motoric function, and novel object recognition memory. Mean (± SEM) **A)** number of beam breaks recorded in locomotor activity chambers during two hour sessions over three consecutive days, **B)** latency to fall (seconds) over five accelerating Rotarod trials, and **C)** novel object discrimination ratios (%) at 30 minute and 24 hour retention intervals (dotted line shows chance performance). Males = filled symbols and females = open symbols.

385 **_Setd1a_$^{+/-}$ mice show an elevated acoustic startle response and diminished pre-**
386 **pulse inhibition**

387 The ASR was measured in response to a 120 dB (above background) acoustic
388 stimulus (Figure 5A). A significant main effect of genotype was observed (F(1, 73) =
389 5.35, p = .02), indicating that the magnitude of ASR was significantly greater in
390 _Setd1a_$^{+/-}$ mice compared to WTs. The main effect of sex was not significant (F(1, 73)
391 = 3.25, p = .08) and there was no significant interaction between sex and genotype
392 (F(1, 73) = 1.98, p = .16). This shows that the hyperstartling effect of _Setd1a_
393 haplosufficiency was consistent in males and females.

394 PPI of the 120 dB ASR was assessed in response to an 8 dB and 16 dB (above
395 background) pre-pulse stimulus (Figure 5B). A significant main effect of pre-pulse
396 amplitude was observed (F(1, 73) = 253.07, p < .001) indicating that the magnitude
397 of PPI was greater in response to the louder pre-pulse stimuli. There was a
398 significant main effect of genotype (F(1, 73) = 26.64, p < .001) and no significant
399 interaction between genotype and pre-pulse amplitude (F(1, 73) = 2.00, p = .16),
400 indicating reduced PPI in _Setd1a_$^{+/-}$ mice relative to WTs at both pre-pulse
401 amplitudes. There was no signficant main effect of sex (F(1, 73) = 2.62, p = .11), or
402 interaction between sex and genotype (F(1, 73) = 1.98, p = .16) or sex, genotype,
403 and pre-pulse amplitude (F(1, 73) = 0.001, p = .97). These results demonstrate that
404 PPI of the ASR was reduced at both pre-pulse amplitudes in male and female
405 _Setd1a_$^{+/-}$ mice.

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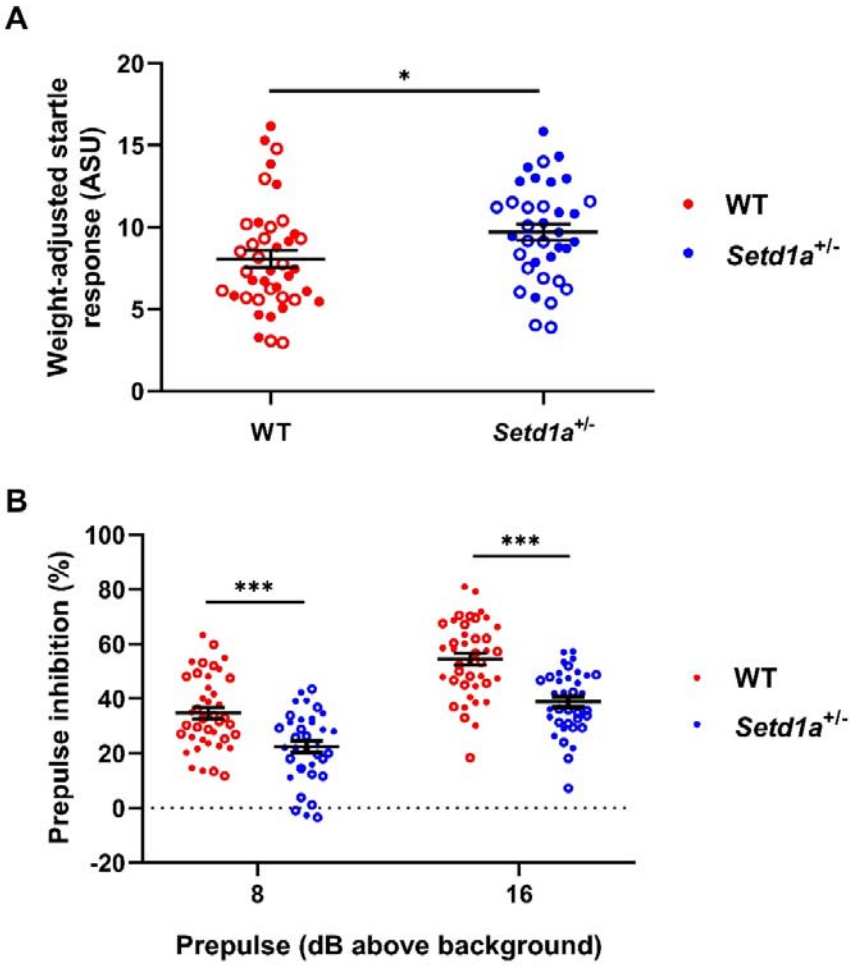

Figure 5. *Setd1a*$^{+/-}$ mice show an elevated ASR and reduced PPI of the ASR. Mean (± SEM) **A)** weight-adjusted ASR (arbitrary startle units) evoked by a 120 dB (above background) acoustic stimulus and **B)** percentage reduction of the ASR by an 8 dB and 16 dB (above background) pre-pulse stimulus. Males = filled symbols and females = open symbols. * $p < .05$, *** $p < .001$.

Aberrant sensorimotor gating in *Setd1a*$^{+/-}$ mice cannot be rescued by haloperidol or risperidone

Prior to the drug challenge experiment, the effects of *Setd1a* haploinsufficiency on sensorimotor gating were re-examined in an independent batch of animals (Cohort 2). This pre-test session revealed a significant main effect of genotype on ASR ($F(1, 52) = 15.58$, $p < .001$; Figure 6A) and PPI ($F(1, 52) = 15.58$, $p < .001$; Figure 6B). This indicates that the observation of increased ASR and reduced PPI in *Setd1a*$^{+/-}$ mice was robust and could be replicated in a separate cohort.

In the animals that received haloperidol, there was a significant main effect of genotype on ASR ($F(1, 25) = 14.01$, $p = .001$; Figure 6C). There was no significant

interaction between genotype and dose ($F(2, 50) = 1.72$, $p = .19$), indicating that the elevated startle response in *Setd1a*$^{+/-}$ mice was not normalised by haloperidol. There was a significant main effect of genotype on PPI ($F(1, 25) = 21.92$, $p < .001$; Figure 6D), which was qualified by a significant interaction between sex and genotype ($F(1, 25) = 9.16$, $p = .01$). Simple effects post-hoc tests revealed that the main effect of genotype was significant in females ($F(1, 25) = 33.86$, $p < .01$) but not males ($F(1, 25) = 1.22$, $p = .28$). In addition, WT females showed significantly greater PPI than WT males ($F(1, 25) = 6.26$, $p = .02$) but there was no significant sex difference in *Setd1a*$^{+/-}$ mice ($F(1, 25) = 3.45$, $p = .08$). The main effect of dose was not significant ($F(2, 50) = 3.08$, $p = .06$) but indicated a dose-related increased in PPI. However, the interaction between dose and genotype was not significant ($F(2, 50) = 0.02$, $p = .98$). This shows that the non-significant enhancement of PPI by haloperidol occurred in both WT and *Setd1a*$^{+/-}$ mice, rather than restoring PPI of *Setd1a*$^{+/-}$ mice to WT levels.

In the risperidone group, there was a significant main effect of genotype on ASR ($F(1, 23) = 63.38$, $p < .001$; Figure 6E). A significant main effect of dose was observed ($F(2, 46) = 25.25$, $p < .001$) and this was qualified by a significant three way interaction between dose, genotype, and sex ($F(2, 46) = 5.51$, $p = .01$). In males, the main effect of dose ($F(2, 22) = 7.32$, $p = .004$) was qualified by a significant interaction between dose and genotype ($F(2, 22) = 6.26$, $p = .01$). Bonferroni-corrected pairwise comparisons revealed a dose-dependent reduction of ASR in WT (relative to vehicle $p = .05$ at 0.5 mg/kg and $p = .01$ at 1.0 mg/kg) but not *Setd1a*$^{+/-}$ males (relative to vehicle $p = .62$ at 0.5 mg/kg and $p = .42$ at 1.0 mg/kg). Conversely, in females the main effect of dose was significant ($F(2, 24) = 23.09$, $p < .001$) but there was no interaction between genotype and dose ($F(2, 24) = 1.32$, $p = .29$). These findings suggest that the startle-inhibiting effect of risperidone was reduced in male (but not female) *Setd1a*$^{+/-}$ mice (Figure S2). There was also a significant main effect of genotype on PPI ($F(1, 23) = 6.36$, $p = .02$; Figure 6F) and no significant interaction between genotype and sex ($F(1, 23) = 0.24$, $p = .63$). The main effect of dose was not significant ($F(2, 46) = 2.71$, $p = .08$) and there was no interaction between genotype and dose ($F(2, 46) = 1.38$, $p = .26$). These findings show that deficient PPI in *Setd1a*$^{+/-}$ mice was not be rescued by risperidone.

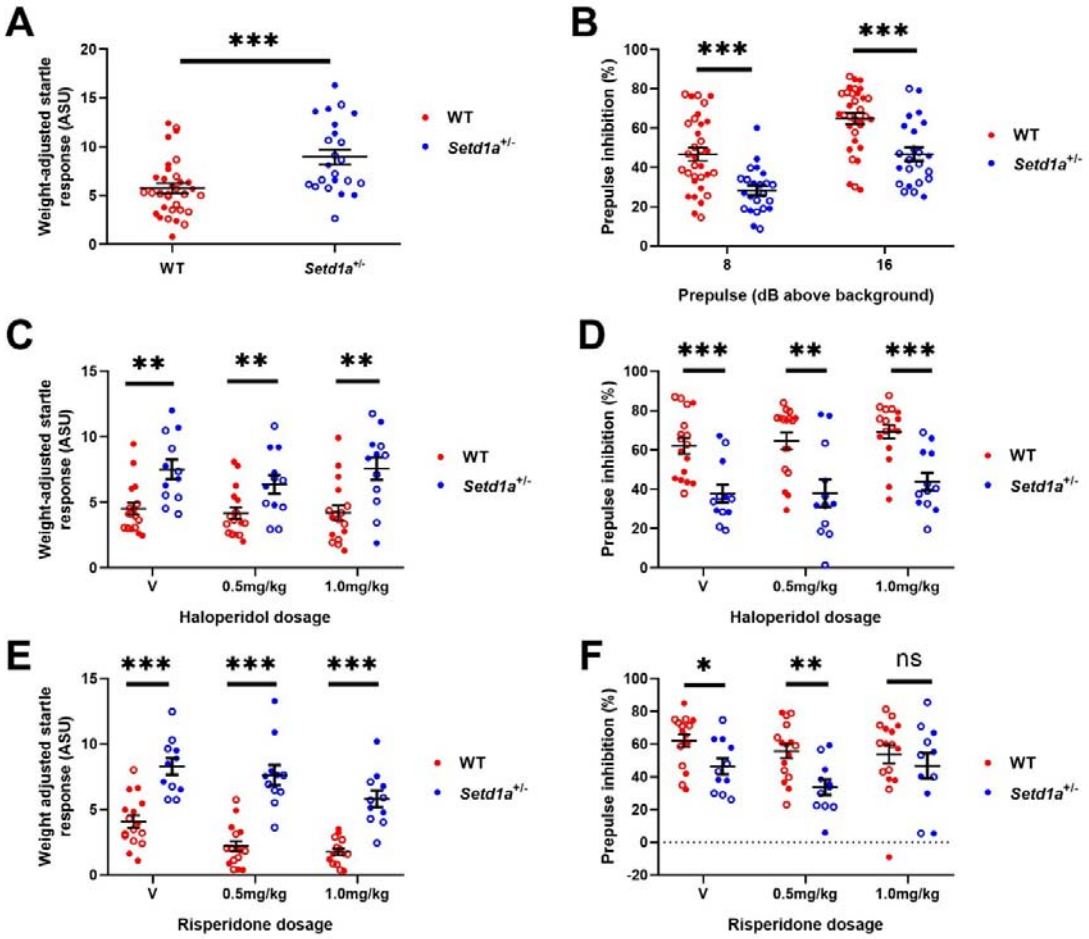

Figure 6. Abnormal sensorimotor gating in *Setd1a*$^{+/-}$ mice is not rescued by haloperidol or risperidone. A) weight-adjusted ASR at 120 dB (above background) and **B)** pre-pulse inhibition (%) by an 8 dB and 16 dB (above background) pre-pulse stimulus under basal conditions in the pre-test session. **C)** ASR and **D)** PPI (averaged across 8 dB and 16 dB pre-pulse) following intraperitoneal injection with vehicle, 0.5 mg/kg, and 1.0mg/kg haloperidol. **E)** ASR and **F)** PPI (averaged across 8 dB and 16 dB pre-pulse) following intraperitoneal injection with vehicle, 0.5 mg/kg, and 1.0mg/kg risperidone. All data are mean (± SEM). Males = filled symbols and females = open symbols. * $p < .05$, ** $p < .01$, *** $p < .001$.

DISCUSSION

Here, we examined the behavioural consequences of haploinsufficiency of the schizophrenia candidate gene *Setd1a* in a mouse model. We find evidence for changes in a number of phenotypes of relevance to schizophrenia, including increased anxiety-related behaviour, enhanced acoustic startle response, and decreased pre-pulse inhibition of acoustic startle. These sensorimotor gating deficits could not be rescued by haloperidol or risperidone, suggesting that these antipsychotics are ineffective for ameliorating schizophrenia-relevant phenotypes in *Setd1a*$^{+/-}$ mice and point to deficits in neural systems other than the monoamine system. Taken together these data strengthen the support for the use of *Setd1a* haploinsufficient mice as a model for the biological basis of schizophrenia, and point towards possible underpinning neural mechanisms.

We have developed and characterised a new *Setd1a*$^{+/-}$ mouse model. After demonstrating that *Setd1a* haploinsufficiency led to a robust ~50% reduction in Setd1a protein levels, we characterised a number of behavioural phenotypes of relevance to neurodevelopmental disorders and schizophrenia in a large experimental cohort of both sexes. We measured anxiety using two separate tests, but only found changes, specifically increased anxiety, in the open field test, with behaviour on the elevated plus maze being equivalent to wild-type littermates. The reduced time spent in the centre of the open field is not due to generalised effects on activity, as *Setd1a*$^{+/-}$ mice showed equivalent behaviour on a simple locomotor activity test and on the Rotarod. These data therefore suggest a subtle, but specific deficit in anxiety. This contrasts with a previous study of a similar *Setd1a*$^{+/-}$ mouse model that showed no difference in the open field test (21). However, we note that these authors used a much longer test time (60 minutes) which may mask a subtle anxiety phenotype as demonstrated here.

Perhaps surprisingly our *Setd1a*$^{+/-}$ mouse model did not show any deficits on the novel object test of learning and memory. In contrast, memory deficits have been demonstrated in a both mouse (22) and Drosophila (6) models of Setd1a LoF mutations. Nevertheless, Mukai et al (21) also did not see any deficits on the novel object test using a *Setd1a* haploinsufficient mouse model very similar to ours. Moreover, the deficits seen in the Nagahama et al (22) study affected only one test point in the novel object recognition test (1 hour, but not 24 hours), possibly

499 indicating a subtle phenotype that is subsumed by the difference between their
500 model, and the ones used here and by Mukai et al. (21).
501 One the most robust behavioural abnormalities found in the *Setd1a*$^{+/-}$ mice were
502 seen in measures of sensorimotor gating. Two separate cohorts demonstrated
503 enhanced acoustic startle response (ASR) and reduced pre-pulse inhibition of the
504 ASR. It is unsurprising therefore that our observation of sensorimotor deficits is
505 supported by other studies of models of *Setd1a* LoF mutations showing similar
506 changes in pre-pulse inhibition (22) and abnormalities in the neural circuitry
507 underlying information processing (26). Deficits in attention and gating, or filtering out
508 intrusive stimuli are prominent features shared by both psychiatric and
509 developmental disorders (26, 27), with which haploinsufficiency of *SETD1A* is
510 associated (4, 6, 7). These are also emerging as key features of a number of other
511 rare neurodevelopmental disorders caused by LoF mutations in genes encoding
512 epigenome writers, readers and erasers. For instance, mouse models
513 haploinsufficient for the histone methyltransferase *Ehmt1* (Glp), which is linked to
514 Kleefstra syndrome, also show deficits in sensorimotor gating and information
515 processing across a number of different studies (28-31). This could suggest these
516 genes act in a network to modulate brain development, with common transcriptional
517 targets and/or by regulating each other. This idea is supported by recent RNA-seq
518 evidence from another mouse model demonstrating that *Setd1a*$^{+/-}$ haploinsufficiency
519 leads to differentially expressed genes in the mPFC enriched for genes associated
520 with neurodevelopmental disease and schizophrenia, including, for instance, *Ehmt1*
521 (22).
522 In light of the changes in acoustic startle response and pre-pulse inhibition of startle
523 seen in our *Setd1a* haploinsufficient model we decided to test whether these
524 sensorimotor gating deficits could be rescued by administering commonly used
525 antipsychotic drugs, namely haloperidol and risperidone. Although both anti-
526 psychotics have previously been shown to reverse PPI deficits in several rodent
527 models of schizophrenia (32), generally neither rescued the sensorimotor gating
528 deficits seen in the *Setd1a*$^{+/-}$ mice. However, there was an interesting difference in
529 the effectiveness of risperidone between male and female *Setd1a*$^{+/-}$ mice, with no
530 attenuation of startle response in males. Whether this is a real difference needs
531 further testing, particularly as numbers for these experiments were low. Despite this
532 possible sex difference, generally these data suggest that the neurobiological

changes that cause deficient PPI in *Setd1a*$^{+/-}$ mice are not appropriately targeted by either haloperidol (mainly dopaminergic) or risperidone (mainly serotonergic). This could indicate that the monoamine circuitry remains largely intact in the *Setd1a*$^{+/-}$ mice, and that baseline behavioural changes are caused by changes in other neural systems. Of particular relevance here is that the Nagahama et al (22) report that glutamate release probability is reduced when *Setd1a* is knocked down in postsynaptic pyramidal neurons. Future studies should investigate the ability of glutamatergic drugs in ameliorating the behavioural deficits seen in our *Setd1a* haploinsufficiency model.

Overall, these data add to our growing understanding of the role of *Setd1a* in brain and behaviour. Our findings, coupled with those from others investigating different *Setd1a* models, demonstrate replicable common phenotypes of relevance that could be used for the development and testing of therapies in rescue studies. Moreover, the shared deficits seen in other mouse models of LoF mutations may be indicative of shared mechanisms between different genes encoding epigenome modifiers.

Data Availability

All data are publicly available at the following 'Open Science Framework' link: https://osf.io/gt7ew/

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Author contribution

MLB, ARI, LSW and TH conceived the project. MLB and TH planned the behavioural experiments, all of which were performed by MLB; Tissue collection, processing and analysis was performed by MLB, with input from ARI. MLB wrote the paper, with editing input from ARI, LSW and TH.

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The C57BL/6NTac-Setd1a$^{tm1a(EUCOMM)Wtsi}$/WtsiCnrm mouse strain was originally created by KOMP using ES cell clone generated by the Wellcome Trust Sanger Institute and distributed by EMMA. Subsequent FLPo deletion to create the C57BL/6NTac-Setd1a$^{tm1c(EUCOMM)Wtsi}$/WtsiCnrm allele was conducted at the MRC Harwell Institute.

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686

SUPPLEMENTARY INFORMATION

Table S1. qRT-PCR primer sequences and product sizes for *Setd1a* and three housekeeping genes (*Hprt*, *Dynein*, and *B2m*).

Target	Forward primer	Reverse primer	Product size (bp)
Setd1a	CCCTCCCGGTTCCTAAGTTT	CATTGTCATTGAGCCTCGCA	90
Hprt	GCGATGATGAACCAGGTTATGA	GCCTCCCATCTCCTTCATGA	146
Dynein	GACCTCAGGCTCAGACGAAGAC	AAGACGCTCATGGCATCACA	116
B2m	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC	104

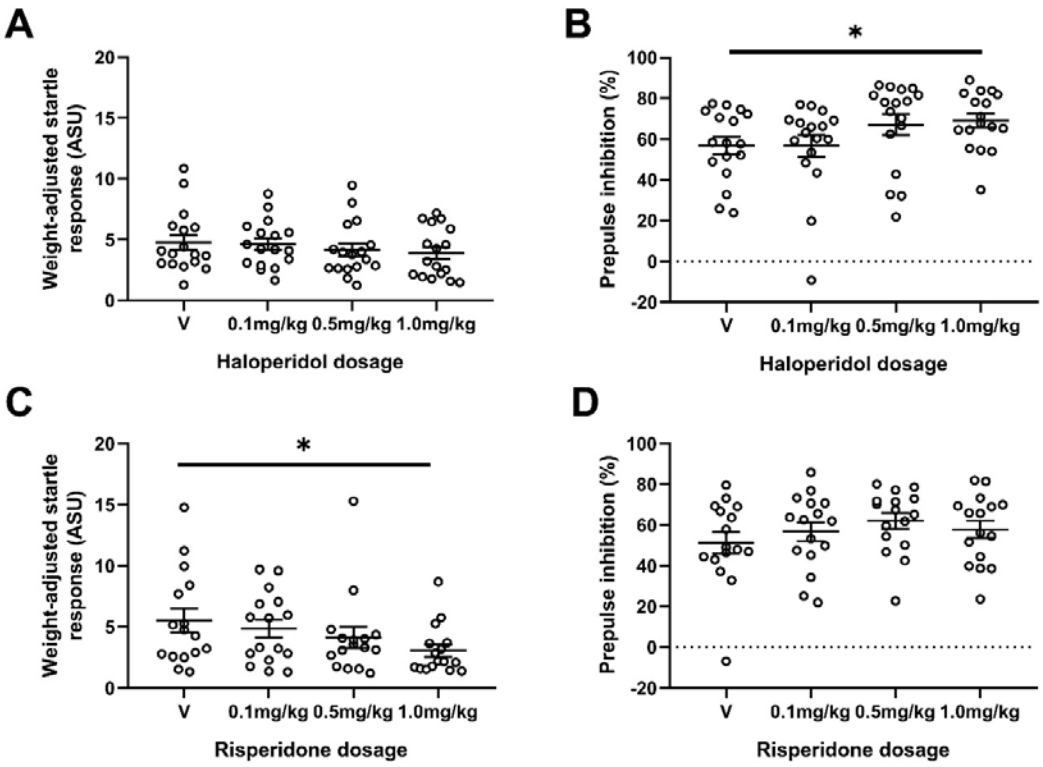
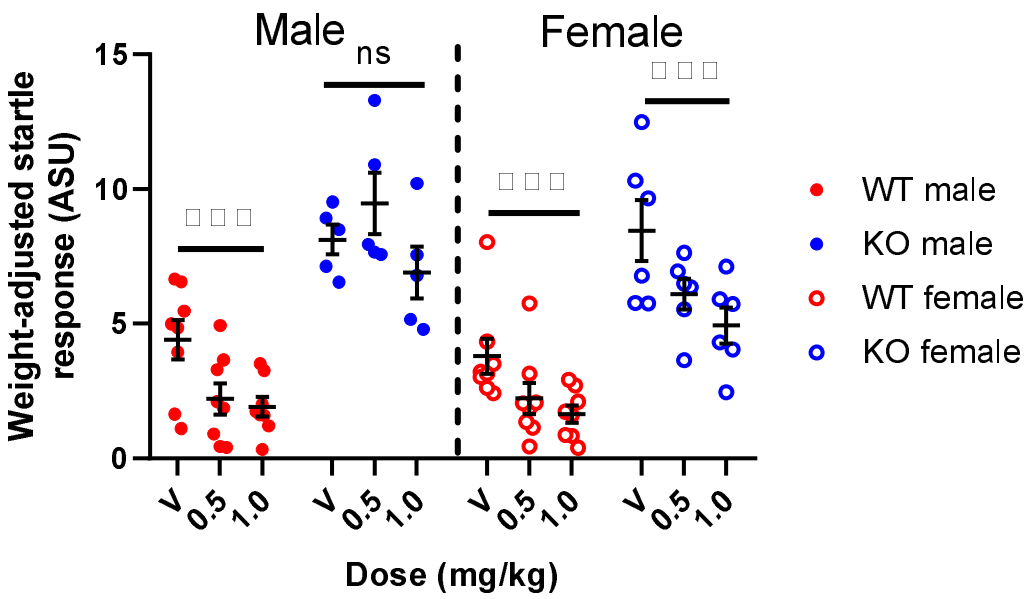


Figure S1. Effects of haloperidol (N = 17) and risperidone (N = 16) on sensorimotor gating in male C57BL/6J mice. A) No significant main effect of haloperidol dose on ASR ($F(3, 48) = 1.43$, $p = .25$). **B)** Significant main effect of haloperidol dose on PPI ($F(3, 48) = 4.11$, $p = .01$). Bonferroni-corrected post-hoc tests revealed significantly increased PPI at 1.0 mg/kg compared to vehicle ($p = .04$) but not at 0.1 mg/kg ($p = .99$) or 0.5 mg/kg ($p = .18$). **C)** Significant main effect of risperidone dose on ASR ($F(3, 45) = 6.42$, $p = .001$). Bonferroni corrected post-hoc tests revealed significantly decreased ASR at 1.0 mg/kg relative to vehicle ($p = .03$) but not at 0.1 mg/kg ($p = .46$) or 0.5 mg/kg ($p = .10$). **D)** No significant main effect of risperidone dose on PPI ($F(3, 45) = 2.12$, $p = .11$).



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704 **Figure S2. Effect of risperidone on ASR in *Setd1a*$^{+/-}$ and WT mice separated by**

705 **sex.** Mean (+/- SEM) ASR at 120 dB (above background) following IP administration

706 of vehicle, 0.5 or 1.0 mg/kg risperidone, presented separately for males and females.

707