Neuropeptides 53 (2015) 71–77

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/ynpep

Behavioural characteristics of the Prader–Willi syndrome related biallelic *Snord116* mouse model

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ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 4 May 2015
Accepted 30 June 2015
Available online 12 July 2015

Keywords:
SNORD116
MBII-85
HBII-85
Prader–Willi syndrome
Mouse model
Behaviour
Anxiety
Cognition
Social preference

ABSTRACT

Prader–Willi syndrome (PWS) is the predominant genetic cause of obesity in humans and is associated with several behavioural phenotypes such as altered motoric function, reduced activity, and learning disabilities. It can include mood instability and, in some cases, psychotic episodes. Recently, the *Snord116* gene has been associated with the development of PWS, however, it's contribution to the behavioural aspects of the disease are unknown. Here we show that male and female mice lacking *Snord116* on both alleles exhibit normal motor behaviours and exploration but do display task-dependent alterations to locomotion and anxiety-related behaviours. Sociability is well developed in *Snord116* deficient mice as are social recognition memory, spatial working memory, and fear-associated behaviours. No sex-specific effects were found. In conclusion, the biallelic *Snord116* deficiency mouse model exhibits particular endophenotypes with some relevance to PWS, suggesting partial face validity for the syndrome.

1. Introduction

SNORD116, which is also known as *HBII-85*, is a non-coding ribonucleic acid (RNA) molecule. It plays a role in the modification of other small nuclear RNAs and is often referred to as a guide RNA or a small nucleolar RNA (snoRNA), as it is located in the nucleus of eukaryotic cells. Cavaille and co-workers discovered that *SNORD116* in wild type-like mice is exclusively expressed in the brain and that it maps to chromosome 15q11–q13 in humans (Cavaille et al., 2000). This region and micro-deletions to the *SNORD116* snoRNA cluster have been associated with the Prader–Willi syndrome (PWS) including the typical hyperphagia and obesity ((Sahoo et al., 2008; de Smith et al., 2009) but see also (Runte et al., 2005)). In line with this, *SNORD116* is absent from the brain of patients with PWS and work utilising *Snord116* knockout mice has suggested that the snoRNA *Snord116* gene cluster is a critical element in PWS formation (Ding et al., 2008; Sahoo et al., 2008; de Smith et al., 2009).

PWS is one of the most common genetic obesity disorders and is associated with a variety of symptoms including behavioural alterations such as delayed motor and language development, excessive eating and gradual development of morbid obesity (from early childhood onwards). Furthermore, affected patients can develop cognitive disabilities as well as temper tantrums and compulsive behaviour later in life (Cassidy et al., 2012). Some of the more the specific characteristics of human PWS are of short stature, low muscle tone, poor suckling reflex, incomplete sexual development, cognitive impairments and extreme and insatiable appetite, which can lead to excessive food consumption and consequently morbid obesity (Cassidy et al., 2012). Furthermore, PWS patients can suffer from compulsive behaviours (e.g. skin-picking), psychiatric symptoms, motor function deficiencies, and enhanced levels of anxiety (Feurer et al., 1998; Reddy and Pfeiffer, 2007).

The PWS locus is subject to parent-of-origin imprinting. The maternal allele of the gene(s) of interest is imprinted and thus silenced via epigenetic mechanisms whereas the paternal allele is mutant and therefore non-functional (human: (Cassidy et al., 2012) mouse: (Ding et al., 2008)). If the mutant allele is maternally derived, individuals do not develop PWS but the related Angelman syndrome (Saitoh et al., 1997).

The mouse PWS locus is highly homologous to the one in humans. Mouse models for *Snord116* deficiency show similar symptoms to humans suffering from PWS. Skyrabin and co-workers describe that a

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http://dx.doi.org/10.1016/j.npep.2015.06.009
0143-4179/

deletion in *Snord116* snoRNA (or more precisely, the so-called *MBII-85* snoRNA cluster on one allele) results in postnatal growth retardation (Skryabin et al., 2007). Mice with no parental copy of the *Snord116* snoRNA cluster were significantly smaller on postnatal day 10 than wild type-like siblings. The postnatal growth retardation was evident across six generations and independent of the genetic background. The differences in growth dynamics continued into adulthood, but contrary to humans with PWS these *Snord116* deficient mice do not develop an obese phenotype. Interestingly, there appears to be a moderate effect of sex with female knockout mice developing a less pronounced phenotype than males (Skryabin et al., 2007). No weight differences were detected during embryonic development or late gestation, which suggest that poor sucking behaviour of knockout offspring might be responsible for early growth retardation. Postnatal lethality of knockout mice was dependent on the genetic background and relatively low (i.e. around 15% in mice on 129SvJxC57BL/6J background). Fertility and the expression of other snoRNA genes (*MBII-436*, *MBII-13*, and *MBII-52*) as well as other genes with relevance to PWS (i.e. *Necdin*, *Magel2*, *Mkrn3*, *Frat3*, and *Snurf-Snrpn*) were not significantly altered suggesting that deletion of the *MBII-85* snoRNA cluster does not affect imprinting of neighbouring genes (Skryabin et al., 2007).

Another study investigated the effects of a paternally derived deletion of *Snord116* in male and female mice (Ding et al., 2008). The knockout mice also exhibited growth delay in the first three postnatal weeks (no lethality) but exhibited normal fertility and lifespan. Furthermore, at 3 months of age, knockout mice developed hyperphagia but stayed lean on normal and high fat diets. These mice also showed normal energy homeostasis maintenance. Behavioural testing of 2–6 months old male mice revealed a defect in motor learning but not in baseline motor coordination or balance (i.e. tested in the accelerod test). Muscle tone and strength were unaltered in *Snord116* knockout mice as were locomotion and exploration in the open field test. Furthermore, knockout mice had no deficits in working memory and spatial memory in two versions of the Y-maze test and showed normal pain sensitivity in the hot plate test. In contrast, *Snord116* deficient mice displayed increased anxiety and locomotion in the elevated plus maze and also developed hyperphagia, elevated levels of plasma ghrelin and altered metabolism in adulthood, although energy homeostasis regulation was normal (Ding et al., 2008).

As *Snord116* is a paternally imprinted gene, most studies assume a simple pattern of imprinting (i.e. expression of paternally inherited copy but silencing of maternal copy). However, more complex patterns of imprinted genes exist, which depend on genetic information derived from both parents (Wolf et al., 2008). Thus, we evaluate here for the first time a novel homozygous mouse model for *Snord116* for its face validity (Takao et al., 2007). For this, we carried out a comprehensive battery of behavioural paradigms with relevance to PSW symptoms in biallelic *Snord116* deficient mice. Mice were tested for motor coordination and muscle strength, balance, locomotion and exploration, and anxiety behaviour, as well as cognitive domains (i.e. spatial and recognition memory as well as fear-associated memory).

2. Materials and methods

2.1. Animals

In order to determine the behavioural consequences of a complete germline deletion of the *Snord116* cluster in mice, we crossed floxed *Snord116* mice (*Snord116*$^{lox/lox}$) (Ding et al., 2008) with a germline oocyte-specific Cre-line (Schwenk et al., 1995). The resultant heterozygous *Snord116* knockout mice were crossed to generate homozygous mice (*Snord116*$^{-/-}$ or SNORD KO). All mice were on a pure C57BL/6J background. The successful deletion of the *Snord116* gene was then confirmed by PCR and in situ hybridisation of brain sections from *Snord116* KO mice and wild type-like (WT) controls. In short, fresh frozen brains were sectioned at 30 μm thickness and thaw-mounted on Superfrost Plus® glass microscope slides (Lomb Scientific Pty Ltd., NSW 2229, Australia). In situ hybridisation was performed, as previously described (Parker and Herzog, 1999). Briefly, matching hypothalamic sections of deletion and control mice were hybridised with candidate mRNAs, which were labelled with [^{35}S] thio-dATP (Amersham Pharmacia Biotech, Buckinghamshire, UK) using terminal deoxynucleotidyltransferase (Roche, Mannheim, Germany). Silver grain densities of labelled mRNAs were analysed and compared using ImageJ software (US National Institutes of Health). DNA oligonucleotides used included those complementary to the mRNAs of mouse *Snord116* 5′-GTTCAGCTTTTCCAAGGA ATGTTTGACTGGGAATCATCATAGATCC-3′.

WT as well as biallelic *Snord116* deficient mice of both sexes (data were pooled across sex as no main effects of 'sex' were found: N = 12–17 per genotype) were transported to the Garvan Institute of Medical Research (Garvan) at 17–20 weeks of age, where they were group-housed in Polysulfone cages (1144B: Techniplast, Rydalmere, Australia) equipped with some tissues for nesting. Mice were kept under a 12:12 h light:dark schedule [light phase:white light (illumination: 124 lx) — dark phase: red light (illumination: < 2 lx)] for at least 2 weeks of habituation before behavioural testing started. Food and water were provided ad libitum, except where specified. Adult A/J mice from Animal Resources Centre (Canning Vale, Australia) were used as standard opponents for the social preference test.

Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Behavioural phenotyping

All experiments were carried out at least 1 h after onset of the light phase and completed within the first 6 h of the light phase. At the conclusion of each test trial, the test device was cleaned with 70% ethanol solution. Test order was as follows: open field, elevated plus maze, motor function tests, social preference test, Y-maze, and fear conditioning (inter-test interval of at least 48 h) (see also Table 1).

2.2.1. Open field test (OF)

In this test, the conflict between the drive to explore a new environment and a natural aversion to illuminated open areas is used to examine both anxiety and motor activity (Crawley, 1985). Mice were tested in an automated, photobeam-controlled OF, 43.2 × 43.2 cm (MedAssociates Inc., Vermont, USA). The arena was divided into a central and a peripheral zone (central zone photobeam coordinates 3/3, 3/13, 13/3, 13/13 (Long et al., 2012)). Mice were placed in a corner of the arena (illumination level: 20 lx) and were allowed to explore the arena for the following 30 min, while their activity was measured automatically (software settings: box size: 4; ambulatory trigger: 2; resting delay: 1500 ms). Measures of anxiety include the time spent in the central area of the open field and distance travelled in the centre as a percentage of overall distance travelled. Distance travelled, time spent '*resting*' (no photobeam-detectable movement), and small motor movements (photobeam breaks without ambulation, i.e. only 1 beam break within 1.5 s) were recorded as measures of motor activity and overall activity. Vertical activity (*rearing*) was used as a measure of exploration.

Table 1
Test biography: test age [d] ± 3 days and test order of control (WT) and *Snord116* knockout mice (SNORD KO) are shown (N = 12–17 per genotype).

Test age [d]	Behavioural paradigm
130	Open field (OF)
131	Elevated plus maze (EPM)
135	Motor function (pole test and wire hang test)
137	Social preference test (SPT)
140	Y-maze test (YM)
144	Contextual and cued fear conditioning (FC)

2.2.2. Elevated plus maze (EPM)

The EPM assesses the natural conflict between the tendency of mice to explore a novel environment and avoidance of a brightly lit, elevated and open area (Montgomery, 1955; Montgomery and Monkman, 1955). The grey plus maze was "+" shaped (for details of apparatus see (Boucher et al., 2007)). Mice were placed at the centre of the + (faced towards an enclosed arm) and were allowed to explore the maze for 5 min. The time spent and distance travelled in the open and enclosed arms as well as *grooming*, *head dipping*, *rearing*, and *stretch-attend postures* was recorded using AnyMaze™ (Stoelting, Wood Dale, USA) tracking software.

2.2.3. Motor function tests (i.e. pole test and wire hang test)

Motor function/coordination can impact on animals' behavioural performance and present test confounders (Crawley and Paylor, 1997; Karl et al., 2003). Thus, we evaluated mice's motor functions in the pole test and the wire hang test (Table 2).

Pole test: a wooden stick (diameter: 1 cm; length: 50 cm - wrapped in fine sand paper) with a cork ball on its top (diameter: 1.5 cm) is installed vertical on a heavy platform. The mouse is placed on top of the pole, placed directly under the ball at the top — the head held upwards. The latency to turn round and to reach the platform at the bottom is measured (cut-off time: 120 s). If the animal slides down the wooden stick without active climbing or turning round, both parameters are recorded as 120 s. The apparatus is cleaned after each trial (three trials in total) with 70% ethanol.

Wire hang test: the mouse is placed on a wire in a way that it grips the wire with its front paws. The wire is raised approximately 50 cm from the surface. The latency to fall down is recorded (cut-off time: 60 s). The apparatus is cleaned after each trial (two trials in total) with 70% ethanol.

2.2.4. Social preference test (SPT)

The SPT was used to assess sociability and social novelty preference (i.e. social recognition memory) in test mice (Cheng et al., 2013; Cheng et al., 2014). The apparatus consisted of 3 chambers, a central chamber (length: 9 cm, width: 18 cm, depth: 20 cm) and two outer chambers (6 cm × 18 cm × 20 cm). The dividing walls were made of clear Plexiglas, with square passages, 4 cm high and 4 cm wide. One circular cage (i.e. mouse enclosure) was placed into each outer chamber. The mouse enclosures were 15 cm in height with a diameter of 7 cm and bars spaced 0.5 cm apart to allow nose contact between mice (i.e. test mouse and A/J mouse) but prevent fighting. The chambers and enclosures were cleaned with 70% ethanol in-between trials and fresh bedding was added prior to each test trial.

Table 2
Motor functions, locomotion and exploration, and cognitive behaviours: Behavioural performance of control (WT) and *Snord116* deficient (SNORD KO) mice in the pole test, the wire hang test, the open field (OF), the elevated plus maze (EPM), and the Y-maze (YM). Data are shown as mean ± SEM (N = 12–17 per genotype). Significant effects of 'genotype' versus WT mice are indicated with '*' (*$p < .05$).

	WT	SNORD KO
Wire hang		
Latency to fall [s]	36.3 ± 5.6	28.7 ± 5.2
Pole test		
Latency to climb down [s]	44.6 ± 8.0	29.1 ± 7.9
OF		
Small motor movements [n]	2376.8 ± 30.6	2236.0 ± 41.9*
EPM		
Rearing [n]	15.3 ± 2.7	14.7 ± 1.5
Head dipping [n]	29.5 ± 3.4	22.9 ± 2.6
Distance travelled in enclosed arm [m]	9.2 ± 0.5	11.0 ± 0.6*
Entries into enclosed arm [n]	19.8 ± 1.0	22.7 ± 1.6
YM		
Novel arm distance [%]	41.2 ± 2.1	40.8 ± 1.5
Novel arm entries [%]	39.9 ± 1.5	39.3 ± 1.3
Novel arm time [%]	38.5 ± 2.5	39.4 ± 1.5

Test animals were isolated for an hour prior to the start of testing. During the habituation trial, WT and SNORD KO mice were placed individually in the central chamber and allowed to freely explore the apparatus and the two empty enclosures for 5 min. For the sociability test an unfamiliar adult same-sex A/J mouse was placed in one of the two enclosures (i.e. opponent chamber) in a quasi-randomised fashion. Then the test mouse was returned to the apparatus and allowed to explore all three chambers for 10 min. Finally, test animals were observed in a 10 min social recognition test. For this, a second, unfamiliar same-sex A/J mouse was placed in the previously empty chamber so that the test mouse had the choice to explore either the familiar A/J mouse (from the previous trial) or the novel, unfamiliar mouse. AnyMaze™ tracking software was used to determine the time spent in the different chambers, number of entries and distance travelled by the test mice in each trial.

2.2.5. Y-maze test (YM)

The Y-maze assessed short term spatial working memory and consisted of three grey acrylic arms (10 cm × 30 cm × 17 cm) placed at 120° with respect to each other and a number of external cues were provided around the YM apparatus. The YM consisted of two trials (training and test), with a 1 h inter-trial interval (ITI). The trial duration for training and test was 10 and 5 min respectively (Duffy et al., 2010; Chesworth et al., 2012). During training, one arm was blocked off (novel arm); mice were placed facing the end of one of the other two accessible arms (start arm). In the test trial, all arms were accessible. Mice were placed facing the end of the start arm then allowed to explore the apparatus freely. The apparatus was cleaned thoroughly with 70% ethanol in between each trial. Time, entries and distance travelled in arms were recorded using Any-Maze™ tracking software. An arm entry was scored whenever the centre of the animal (as defined by Any-Maze™) was inside an arm. The percentage of novel arm time was calculated using [(novel arm time / total arm time) * 100]. The corresponding calculations were performed for novel arm distance travelled and novel arm entries.

2.2.6. Fear conditioning (FC)

Fear conditioning assesses associative learning whereby a previously neutral stimulus elicits a fear response after it has been paired with an aversive stimulus. On conditioning day, mice were placed into the test chamber (Model H10-11R-TC, Coulbourn Instruments, USA) for 2 min. Then an 80 dB conditioned stimulus (CS) was presented for 30 s with a co-terminating 0.4 mA 2 s foot shock (unconditioned stimulus; US) twice with an inter-pairing interval of 2 min). The test concluded 2 min later. The next day (context test), mice were returned to the apparatus for 7 min. On day 3 (cue test), animals were placed in an altered context for 9 min. After 2 min (pre-CS/baseline), the CS was presented continuously for 5 min. The test concluded after another 2 min with the absence of the CS. Time spent *freezing* was measured using Any-Maze™ software (Duffy et al., 2010; Cheng et al., 2013).

2.3. Statistical analysis

Analysis of the behavioural parameters was performed using repeated measures (RM) analysis of variance (ANOVA) to investigate main effects of 'genotype' and RM effects of 'chamber' (SPT), '1 min block' (FC), and '5 min block' (OF) as published previously (Cheng et al., 2013; Cheng et al., 2014). Furthermore, the performance in the YM was also assessed using one sample t-tests to determine whether mice show a preference for the novel arm (i.e. exploration is greater than chance, i.e. 33.3%). Data were pooled across sex as no main effects of 'sex' were found (N = 12–17 per genotype). Differences were regarded as significant if $p < .05$. F-values and degrees of freedom are presented and significant one-way ANOVA effects are shown in figures and tables as '*' for 'genotype' (*$p < .05$, **$p < .01$, and ***$p < .001$). Data are shown

as means ± standard error of means (SEM). Analyses were conducted using Statview software Version 5.0.

3. Results

All mice regardless of genotype showed normal motor functions in the pole and the wire hang tests. This was true for averaged latencies to climb down the pole and hang onto the wire (Table 1) as well as for motor learning (i.e. latencies across trials) data not shown.

3.1. Locomotion and anxiety

3.1.1. Open field

One-way ANOVA revealed wild type-like locomotion (i.e. total distance travelled; Fig. 1A) and exploration (i.e. vertical activity; Fig. 1B) as tested in the OF for *Snord116* deficient mice (all *p*'s > .05). Furthermore, all mice regardless of genotype habituated to the OF arena as the locomotive response to the novel arena reduced over the 30 min test session ['5 min block': $F(5,135) = 134.0$, $p < .0001$ — no '5 min block' × 'genotype' interaction; Fig. 1C]. However, SNORD KO mice exhibited significantly less small motor movements [$F(1,27) = 6.3$, $p = .02$; Table 1]. These mice also displayed an increased percentage of locomotion in the centre of the OF [$F(1,27) = 6.6$, $p = .02$; Fig. 2A] and spent more time in that centre [strong trend; $F(1,27) = 3.8$, $p = .06$; Fig. 2B]. These findings suggest that *Snord116* deficiency induces an anxiolytic-like phenotype in the OF.

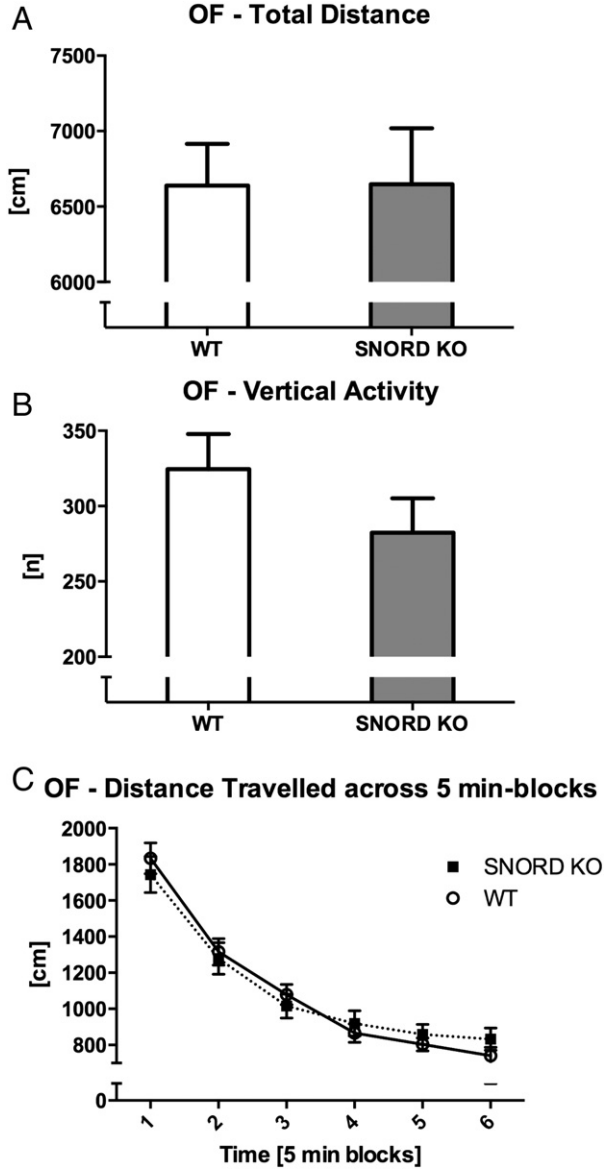

Fig. 1. A–C: locomotion and exploration in the open field (OF): A) total distance travelled [cm], B) vertical activity (i.e. frequency of *rearing*) [n], and C) total distance travelled across time (i.e. in 5 min blocks) [cm]. Data for control (WT) and *Snord116* knockout mice (SNORD KO) are shown as mean + SEM (males and females combined: N = 12–17 per genotype).

3.1.2. Elevated plus maze

There was no effect of 'genotype' on explorative behaviours (i.e. total number of *rearings* and *head dips*; all *p*'s > .05; Table 1). However, SNORD KO mice were more anxious than WT mice in the EPM as measured by percentage entries into open arms [$F(1,27) = 7.8$, $p = .009$; Fig. 2C] and time spent in open arm [strong trend; $F(1,27) = 4.0$, $p = .06$; Fig. 2D]. Furthermore, *Snord116* deficient mice displayed a hyperlocomotive phenotype in the enclosed arms for distance travelled [$F(1,27) = 4.7$, $p = .04$] but not arm entries [$F(1,27) = 1.9$, $p > .05$] (Table 1).

3.2. Cognition

3.2.1. Social Preference Test

All mice regardless of genotype demonstrated sociability in the 3-chamber social preference test. RM ANOVA detected a significant effect of 'chamber' for all mice for total time spent in test chambers where mice spent more time in the chamber of the opponent mouse than the empty chamber [$F(1,27) = 20.0$, $p < .0001$ — no 'chamber' × 'genotype' interaction; Fig. 3A)]. One-way ANOVA for percentage time in opponent chamber confirmed that there were no effects of 'genotype' on sociability [$F(1,27) = 1.1$, $p > .05$].

Similarly, RM ANOVA revealed a significant effect of 'chamber' in the social preference trial with all mice spending more time with the novel mouse than the familiar mouse [$F(1,27) = 9.1$, $p = .005$ — no 'chamber' × 'genotype' interaction; Fig. 3B]. Also, there was no 'genotype' effect on percentage time in the chamber with the novel mouse suggesting intact social recognition memory of SNORD KO mice [$F(1,27) = 1.9$, $p > .05$; data not shown].

3.2.2. Y-maze

One-way ANOVA showed that the genotype had no effect on the percentage exploration of the novel arm regardless of the parameter investigated (all *p*'s > .05; Table 1). Furthermore, t-test confirmed that all mice recognised the novel arm and explored this unfamiliar environment more than the other two familiar, previously visited arms [one sample t-tests for percentage novel arm distance travelled: WT: $t(11) = 3.9$, $p = .002$; SNORD KO: $t(16) = 5.1$, $p = .0001$ — for percentage novel entries: WT: $t(11) = 4.3$, $p = .001$; SNORD KO: $t(16) = 4.7$, $p = .0002$ — for percentage novel arm time: WT: $t(11) = 2.1$, $p = .06$; SNORD KO: $t(16) = 4.2$, $p < .001$; Table 1].

3.2.3. Fear conditioning

All mice responded to the electric foot shocks delivered during the conditioning phase (i.e. vocalisation). Furthermore, the baseline *freezing* response in the first 2 min of the conditioning trial was similar across genotypes ($p > .05$; data not shown). Contextual fear conditioning (i.e. total time spent *freezing* during context test) of SNORD KO mice was WT-like [$F(1,25) = .3$, $p > .05$; Fig. 4A] as was the *freezing* response over time in the cue test [RM ANOVA: no '1 min block' × 'genotype' interaction: $F(8,200) = 1.0$, $p > .05$; Fig. 4B].

4. Discussion

Here we present a behavioural characterisation of male and female test mice of a homozygous (biallelic) model for *Snord116*. Some of the key features of human PWS are altered motoric function, reduced

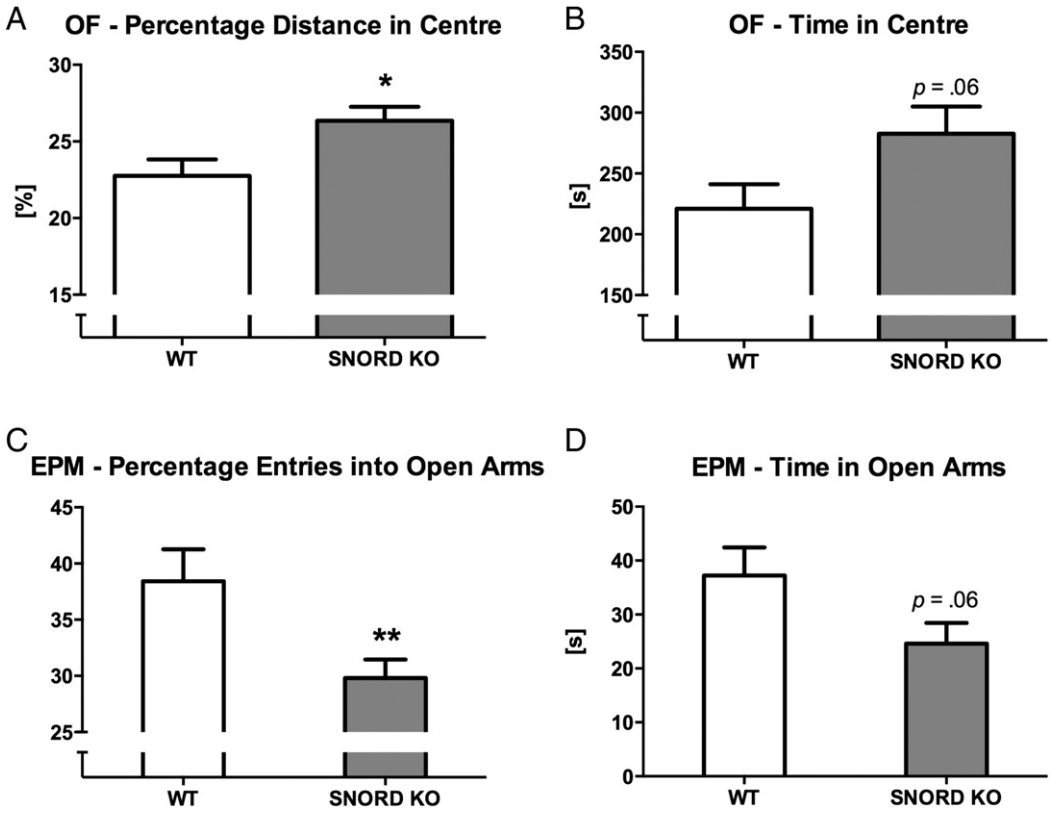

Fig. 2. A–D: anxiety-related behaviours in the open field (OF) and the elevated plus maze (EPM): A) percentage locomotion in the OF centre [%], B) time spent in the OF centre [s], C) percentage of open arm entries in the EPM [%], and D) time spent in open arms of the EPM [s]. Data for control (WT) and *Snord116* knockout mice (SNORD KO) are shown as mean + SEM (males and females combined: N = 12–17 per genotype). Significant genotype effects versus WT mice are indicated with '*' ($^{*}p < .05$ and $^{*}p < .01$).

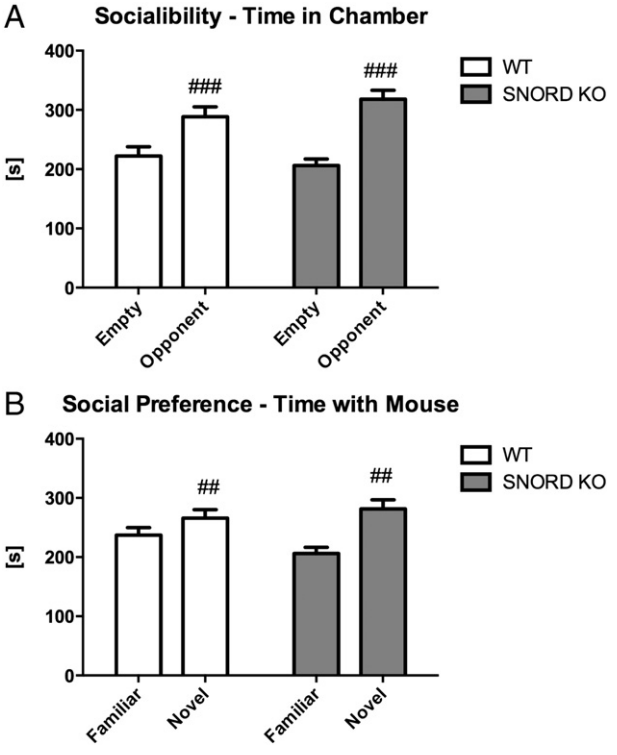

Fig. 3. A–B: sociability and social preference (i.e. social recognition memory): A) time spent in an empty chamber versus a chamber containing another mouse [s] and B) time spent in a chamber with a familiar or a novel mouse [s]. Data for control (WT) and *Snord116* knockout mice (SNORD KO) are shown as mean + SEM (males and females combined: N = 12–17 per genotype). Significant RM ANOVA effects of 'chamber' are indicated with '#' ($^{\#\#}p < .01$ and $^{\#\#\#}p < .001$).

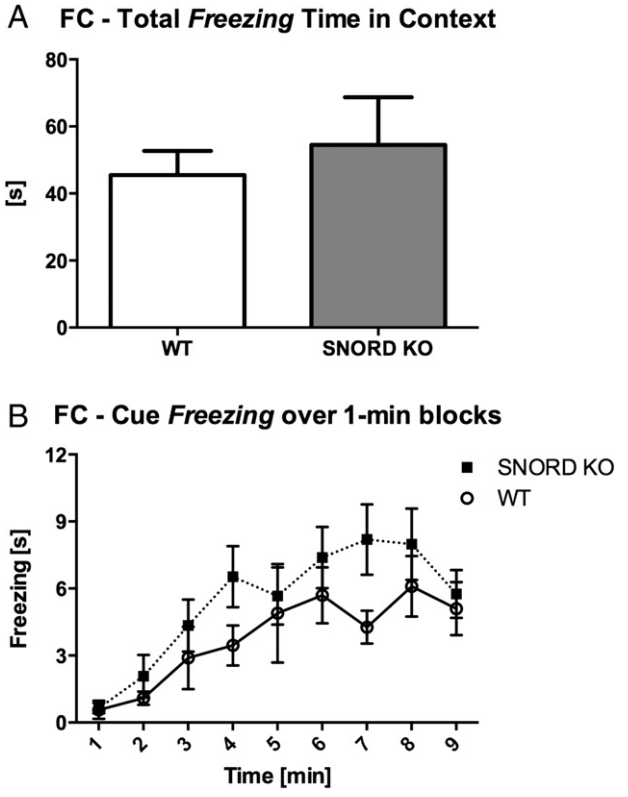

Fig. 4. A–B: fear-associated memory in the fear conditioning task (FC): A) total time spent *freezing* in the context test [s], and B) time spent *freezing* per 1 min block in the cue test [s]. Data for control (WT) and *Snord116* knockout mice (SNORD KO) are shown as mean + SEM (males and females combined: N = 12–17 per genotype).

activity, learning disabilities, and can include mood instability, temper outbursts and, in some cases, psychotic episodes (reviewed in Relkovic and Isles, 2013). In our study, male and female *Snord116* knockout mice displayed normal motor behaviours and exploration. *Snord116* deficiency resulted in a task-dependent impact on locomotion and anxiety-related behaviours where deficient mice where only hyper-locomotive in the elevated plus maze (but not the open field test). Furthermore, the same two paradigms revealed either an anxious-like response (elevated plus maze) or an anxiolytic-like phenotype (open field). Sociability was well developed in all SNORD KO mice. Finally, *Snord116* deficiency had no impact on any cognitive parameters investigated including social recognition memory, spatial working memory, and fear-associated behaviours.

Our experiments revealed no changes to the motor behaviour (i.e. muscle strength and motor coordination) and exploration of SNORD KO mice. This initial characterisation is important to exclude that impaired motor functions results in false positive or negative findings in other tests, for which motor functions are essential (reviewed in Crawley and Paylor, 1997; Karl et al., 2003). Only one other mouse model for *Snord116* has been characterised behaviourally to date (Ding et al., 2008). This model is based on parentally inherited *Snord116* deletions and only male mice were investigated in a neurobehavioural test battery. Those knockout mice developed impairments in motor learning (i.e. accelerod performance across days), which was not investigated in our current study. However, motor functions as measured in the wire hang were unaltered in both our biallelic as well as the parentally inherited *Snord116* deficient mouse model by Ding and co-workers when body weight differences were also considered.

PWS patients exhibit increased anxiety levels (Feurer et al., 1998; Reddy and Pfeiffer, 2007). Thus, we analysed anxiety behaviour of our mouse model in two well-established tasks for anxiety. Interestingly, *Snord116* deficient mice displayed a task-dependent anxiety phenotype probably based on the different anxiogenic properties of open field and elevated plus maze (Carola et al., 2002). SNORD KO mice were more anxious in the plus maze test but showed a moderately decreased anxiety level in the open field. This is interesting in the context of proven associations between exploratory values in these two tests (Lalonde and Strazielle, 2008) although only the plus maze measures fear of height (Carola et al., 2002; Lalonde and Strazielle, 2008). The discrepancies between our findings and what has been reported for open field-plus maze correlations might be due to our experimental protocol as test duration differed significantly between the two paradigms (i.e. OF for 30 min versus EPM for 5 min) suggesting that the longer habituation period to the novelty of the OF tasks might have overridden the anxiety phenotype detected in the EPM. Our findings are in line with Ding and co-workers as their *Snord116* deficient mice exhibited increased anxiety levels only in the plus maze but not the open field test (Ding et al., 2008). Thus, both studies suggest a task-dependent anxiety phenotype to be a consistent feature of *Snord116* knockout models. This is also in line with a number of studies investigating fear reactivity in other mouse models for PWS (*Magel 2* knockout mice and *Necdin* knockout mice, *PWS-IC*$^{+/-}$ mice), which revealed no anxiety phenotype for any of these models in the open field test (reviewed in Relkovic and Isles, 2013).

We also analysed cognitive performance of the *Snord116* mouse model comprehensively as we evaluated a variety of cognitive domains including spatial memory, recognition memory, and fear-associated memory. *Snord116* appears to play no role in these domains, a result which extends the finding by Ding and colleagues of unaltered spatial memory and spatial alternation of their *Snord116* mouse model (Ding et al., 2008). This is interesting considering reports of cognitive impairments in PWS patients (Cassidy and McCandless, 2005). Indeed, neuropsychological studies suggest that deficits in 'frontal' cognitive processes (e.g. attention and executive functioning) may underlie the learning disabilities of PWS patients (reviewed in Relkovic and Isles, 2013). Thus, it is possible that running more complex cognitive tasks than the tests used in our study (e.g. by using the 5-choice serial reaction task: (Higgins and Breysse, 2008)) or that adding a developmental component to the evaluation of the model's face validity (i.e. testing mice beyond the 6 months mark) would reveal learning or memory impairments.

The *Snord116* deficient mouse models are not the only genetic mouse models for PWS. Other mouse models can be classified as full genetic models (e.g. *PWS-IC*$^{+/-}$ and *TgPWS*) and smaller deletion models (e.g. knockout mice for *Mrkn3*, *Magel2* and *Necdin*). These models exhibit a diverse range of behavioural abnormalities including reduced attentional functioning (as measured by the 5-choice serial reaction task), improved spatial memory (in the Morris water maze), hypoactivity, and deficient prepulse inhibition but predominantly unaltered open field behaviour (reviewed in Bervini and Herzog, 2013; Relkovic and Isles, 2013). These findings suggest that animal models for PWS have not only shed light on a number of aspects of this disorder but also identified the relevance of its genetic locus for neuropsychiatric diseases such as autism and psychosis (reviewed in Relkovic and Isles, 2013). In conclusion, the human PWS phenotype is a consequence of the loss of expression of multiple, rather than single genes, which explains why none of the PWS models to date reproduce the biphasic course of the disease or show all of the cardinal symptoms of PWS (reviewed in Bervini and Herzog, 2013). Interestingly, however, the biallelic *Snord116* deficiency mouse model exhibits some similarities to human PWS, which gives evidence for partial face validity of this model for particular endophenotypes of the syndrome. Thus, this novel mouse model represents a valid tool for studying aspects of the complex PWS aetiology and for novel therapeutic strategies. More generally, PWS mouse models will enable us to examine the neural and molecular correlates of PWS-related behaviours in great detail and will increase our understanding of the neurodevelopmental pathways and neurotransmitter systems involved. Importantly, the high postnatal lethality rate of some PWS mouse models, which seems to be at least partially related to the genetic background of the mouse strains utilised, demand a careful selection of the best suitable mouse model to allow research into PWS using adult mice. Finally, it should be mentioned here that three genes located in the PWS critical region, *C15ORF2*, *SNORD109A* and *SNORD109B* are found exclusively in humans. Thus, their contributions to the PWS phenotype cannot be assessed in mouse models (Bervini and Herzog, 2013).

Acknowledgements

TK is supported by the Schizophrenia Research Institute utilising infrastructure funding from the NSW Ministry of Health, and a career development award (1045643) from the National Health and Medical Research Council (NHMRC). We thank Jerry Tanda for critical comments on the manuscript and the staff of the Australian BioResources and the Garvan Biological Testing Facility for taking care of our mice.

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