HHS Public Access

Author manuscript

Genes Brain Behav. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Genes Brain Behav. 2017 July ; 16(6): 592–600. doi:10.1111/gbb.12378.

Magel2 knockout mice manifest altered social phenotypes and a deficit in preference for social novelty

Michael D. Fountain[1,2,3], **Huifang Tao**[2,3], **Chun-An Chen**[2,3], **Jiani Yin**[2,3], and **Christian P. Schaaf**[1,2,3,*]

[1]Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA

[2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[3]Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, USA

Abstract

MAGEL2 is one of five protein-coding, maternally imprinted, paternally expressed genes in the Prader-Willi syndrome-critical domain on chromosome 15q11-q13. Truncating pathogenic variants of *MAGEL2* cause Schaaf-Yang syndrome (OMIM #615547), a neurodevelopmental disorder related to Prader-Willi syndrome. Affected individuals manifest a spectrum of neurocognitive and behavioral phenotypes, including intellectual disability and autism spectrum disorder (ASD).

Magel2 knockout mice carrying a maternally inherited, imprinted wildtype allele and a paternally inherited *Magel2-lacZ* knock-in allele, which abolishes endogenous *Magel2* gene function, exhibit several features reminiscent of the human Prader-Willi phenotypes, including neonatal growth retardation, excessive weight gain after weaning, and increased adiposity in adulthood. They were shown to have altered circadian rhythm, reduced motor activity, and reduced fertility. An extensive assessment for autism-like behaviors in this mouse model was warranted, due to the high prevalence of ASD in human patients. The behavior of *Magel2* knockout mice and their wildtype littermates were assayed via open field, elevated plus maze, tube, three-chamber, and partition tests. Our studies confirm decreased horizontal activity of male and female mice and increased vertical activity of females, in the open field. Both sexes spent more time in the open arm of the elevated plus maze, suggestive of reductions in anxiety. Both sexes displayed a lack of preference for social novelty, via a lack of discrimination between known and novel partners in the partition test.

The in-depth investigation of behavioral profiles caused by *Magel2* loss-of-function helps to elucidate the etiology of behavioral phenotypes both for Schaaf-Yang syndrome and Prader-Willi syndrome in general.

*Corresponding Author: Tel: 832-824-8787, Fax: 713-798-8728, schaaf@bcm.edu.

Introduction

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder characterized by a variety of symptoms, including a complex behavioral profile with temper tantrums, stubbornness, controlling and manipulative behavior, obsessive-compulsive characteristics, and difficulty with changes in routine (Dykens et al. 1999). A subset of individuals with PWS meet criteria for autism spectrum disorder (ASD), as analyzed by Bennett et al. (2015). The prevalence of ASD among molecularly confirmed cases of PWS with loss of the paternal copy of chromosome 15q11-q13 was reported as 26.7%. While the studies included in this analysis reported varied ASD prevalences, the study confirmed previously reported observations of a prevalence of 25.8%, as reported by Veltman et al. (2005). This rate is well above the prevalence of ASD in the general population, which is 1.5% based on current numbers provided by the Center for Disease Control. PWS cases can be stratified by genetic mechanisms causal to the disorder. Most individuals have a deletion (DEL) of the paternal 15q11-q13 (~70% of cases) or maternal uniparental disomy (UPD) (~25% of cases), with imprinting defects and gene mutations comprising the remaining cases (Ramsden et al. 2010; Cassidy & Driscoll 2009). When distinguishing the PWS endophenotypes, individuals with UPD manifest higher rates of ASD than those with DEL, with 35.3% and 18.5% ASD prevalence respectively (Bennett et al. 2015). *MAGEL2* is one of five protein-coding genes in the PWS-critical domain on chromosome 15q11 (Cassidy et al. 2012; Buiting 2010). Four initial individuals with truncating mutations of *MAGEL2* were reported to all carry a clinical diagnosis of ASD based on DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) criteria and clinical assessment by an expert physician (Schaaf et al. 2013). Their overall clinical phenotype was considered similar, yet distinct from Prader-Willi syndrome, and was subsequently renamed Schaaf-Yang syndrome (SHFYNG) (MIM #615547). Since the initial report, an additional 24 individuals have been molecularly confirmed to harbor pathogenic truncating *MAGEL2* mutations (Soden et al. 2014; Mejlachowicz et al. 2015; Fountain et al. 2016). Common clinical phenotypes, present in the majority of affected individuals, include hypotonia, contractures, feeding difficulties, hypogonadism, and developmental delay/intellectual disability (Fountain et al. 2016). Interestingly, among individuals with Schaaf-Yang syndrome reported to date, ASD is diagnosed based on DSM-IV or DSM-V criteria at a 77% prevalence. Moreover, patients manifest a behavior profile including impulsivity, compulsivity, stubbornness, and manipulative behaviors 73% of the time (Fountain et al. 2016). These findings underscore that ASD is a common phenotype among individuals with loss-of-function *MAGEL2* mutations. A better understanding of ASD caused by *MAGEL2* loss-of-function may help us to understand a subset of behavioral phenotypes seen more generally in PWS.

Multiple mouse models of PWS have been generated (Resnick et al. 2013), including mouse models with *Magel2* deficiency (Bischof et al. 2007; Schaller et al. 2010; Fountain et al. 2016). To better understand the role of *MAGEL2* in the overall phenotypic manifestation of PWS, *Magel2* knockout mice were created by replacing *Magel2* with the *lacZ* reporter gene on the murine paternal allele (Bischof et al. 2007). The silencing of the maternal wildtype allele by imprinting, combined with the *Magel2-lacZ* paternal knock-in effectively renders the mice null for *Magel2*. These *Magel2* knockout mice were found to manifest a postnatal

lethality at a prevalence of 10%, exhibit neonatal growth retardation, excessive weight gain after weaning, and increased adiposity with altered metabolism in adulthood, thereby recapitulating some of the core features of the PWS phenotype (Bischof et al. 2007). Moreover, adult *Magel2* knockout mice have reduced fertility in both males and females through extended breeding intervals and early reproductive decline and termination (Mercer & Wevrick 2009). When observing for cognitive and behavioral phenotypes, adult *Magel2* knockout mice display hypoactivity in the open field, but have normal motor and learning abilities (Mercer et al. 2009). More recently, a separate *Magel2* knockout mouse was created by replacing the paternal copy of *Magel2* and its promoter, with a *loxP-hygromycin-loxP* cassette in ES cells (Schaller et al. 2010). The *hygromycin-loxP* cassette was subsequently deleted, resulting in a deletion of 3434 basepairs of *Magel2*, to include the promoter and coding region. Due to altered onset of suckling activity and subsequent impaired feeding, this *Magel2* knockout mouse displayed a higher prevalence of neonatal mortality (prevalence of 50%) (Schaller et al. 2010). The surviving mice display altered sociability, learning, and memory.

Based on the high prevalence of ASD observed in individuals with Schaaf-Yang syndrome, we hypothesized that the loss of functional *MAGEL2* predisposes to autism-like phenotypes in mice. Up to now, an assessment for behaviors for autism-like phenotypes has not been performed, warranting further investigation. After performing a battery of behavioral assays to test for exploration, anxiety, and social interaction, we report that young mice null for *Magel2* display altered exploratory activity, altered social interaction in female mice, and a lack of preference for social novelty.

Materials and Methods

Animals

Mice were maintained on a 14 hour light/10 hour dark cycle, with access to regular mouse chow and water. Wildtype (WT) mice (C57BL/6J) and mice deficient for *Magel2* (C57BL/6 background, *Magel2*^{tm1Stw}/J, stock number 009062) were purchased from Jackson Laboratory (Bar harbor, ME). Mice deficient for *Magel2* harbor a maternally inherited imprinted wildtype allele and a paternally inherited *lacZ* knock-in allele abolishing endogenous *Magel2* expression (Kozlov et al. 2007). As described previously (Mercer et al. 2009), male mice containing the *Magel2-lacZ* allele were housed and paired with female wildtype mice to produce both wildtype mice and mice expressing the paternally inherited *Magel2-lacZ* knock-in. Identification of mutant offspring was performed by PCR genotyping with *Magel2* and *LacZ* oligonucleotide primers (forward, RW3430, 5′-ATGGCTCCATCAGGAGAAC; *Magel2* reverse, RW4400, 5′-GATGGAAAGACCCTTGAGGT; and *LacZ* reverse, RW4237, 5′-GGGATAGGTCACGTTGGTGT), with a *Magel2* wildtype band at 300bp and mutant bands at 300bp and 400bp. *Magel2* knockout mice and their wildtype littermates were then delivered to our facility at Baylor College of Medicine for housing and analysis. 20 WT (♂), 20 *Magel2* knockout (♂), 20 WT (♀), and 20 *Magel2* knockout (♀) mice were all subject to the described battery of experiments. The sample size of 20 animals per genotype per gender was based on the statistical assumptions of: 1) a difference of 25% in average value between

test groups, 2) a standard deviation of 20% of the average value for each group, and 3) an alpha error of 5%. Utilizing a two-tailed test design, this achieves a power of 93.7%. Experiments were performed during the light cycle and the experimenter remained blinded to the genotype status until completion of behavior battery. All research and animal care procedures were approved by the Baylor College of Medicine Animal Care and Use Committee.

Open field assay

Locomotor activity and anxiety level of *Magel2* knockout and wildtype mice were assessed at 8-9 weeks of age, using the open field assay as described previously with a few modifications (Spencer et al. 2011). Briefly, all mice assayed were placed into an open chamber and allowed to traverse freely throughout the chamber. Open field activity was recorded over a 30 minute period, using the Fusion software. Among the data analyzed, time spent in the chamber zones and the distance traveled (defined as the centimeters traveled determined from a progressive beam interruption along a horizontal path) in those zones, were collected. As well, the number of horizontal or vertical beam breaks (defined as the total number of beam interruptions, as detected by the horizontal or vertical sensor, respectively, within a given sample period) were collected.

Elevated plus maze

Anxiety levels of *Magel2* knockout and wildtype mice were assessed using elevated plus maze, as described previously with a few modifications (Lacaria et al. 2012). The test was performed at 700-750 lux illumination, and background white noise at approximately 60 dB. Mice at 8-9 weeks of age were put at the intersection of the open and closed arms, facing to the open arm and allowed to traverse freely between the open and closed arms. Activity data over a 10 minute period was collected by the Fusion software (AccuScan Instruments, Columbus, OH, USA).

Tube test

The tube test was used to evaluate alterations in social dominance. After an initial habituation pass, test *Magel2* knockout or wildtype mice at 12 weeks of age, and gender and age-matched novel C57BL/6 partner mice were placed head first at opposite ends of a clear plastic tube (3.1 cm inner diameter for males, 2.6 cm inner diameter for females, 30.5 cm in length) and released simultaneously. The match ended when one mouse completely retreated from the tube or after a total time of 2 minutes had elapsed. The mouse remaining in the tube was designated as winner (score = 1), while the mouse that retreated from the tube was designated as loser (score = 0). Each test and partner mouse was subjected to three matches, each time with a novel congener partner (Lacaria et al. 2012). The proportion of wins were calculated for wildtype (versus novel partner) and *Magel2* knockout (versus novel partner) male and female mice.

Three-chamber test

The three-chamber test was used to assess sociability of *Magel2* knockout and wildtype mice at 10 weeks of age as described previously with a few modifications (Spencer et al.

2005). After a 10 minute habituation period, a gender and age-matched C57BL/6 mouse was placed into one wire cup, and a Lego object of similar size and color was placed into the wire cup on the opposite compartment. Test mice were then allowed to explore for another 10 minutes. Data of activity in each compartment and amount of time interacting with each wire cup in the two phases were calculated using ANY-Maze. Novel partner mouse and novel object placement was randomized for all testing cohorts to prevent a chamber bias.

Partition test

Social interaction and social novelty was assessed on *Magel2* knockout and wildtype mice via the partition test as described previously with a few modifications (Spencer et al. 2005). At 11 weeks of age, each test mouse was allowed to habituate overnight, alone in a novel cage separated into two compartments by a perforated plastic partition. On day two, each test mouse was housed overnight with a novel, age and gender matched C57BL/6 partner mouse (baseline partner) separated by the partition, allowing for only sight, sound, and smell interaction. On day three, activity at the partition board with the known partner was assayed for five minutes. The baseline partner mouse was removed and replaced by a novel, unfamiliar partner (novel partner). Interest at the partition was assayed for five minutes. The novel partner mouse was then replaced by the original, familiar partner (original partner) and interest was assayed for five minutes. Interest at the partition was manually scored and calculated using a Psion Handheld Computer and Observer XT (Noldus Information Technology, Netherlands).

Behavioral data analysis and statistics

Statistical analysis of all behavioral studies was performed utilizing a two-tailed, unpaired t-test or a two-way analysis of variance (ANOVA), for each comparison where appropriate (GraphPad Prism 6.0e, La Jolla, CA, USA). If an ANOVA was significant, a Šídák's method *post hoc* test was performed for multiple comparisons. P values of $p<0.05$ were considered to be statistically significant. All data were presented as mean ± SEM.

Results

Magel2 knockout mice show alterations in exploration and anxiety

To assess whether mutations in *MAGEL2* have an effect on exploratory activity, anxiety-like behaviors, and restricted interests, we assayed mice deficient for *Magel2* (maternally imprinted, paternally expressed *lacZ* knock-in) and their wildtype littermates in an open field assay and elevated plus maze. Neither male nor female *Magel2* knockout mice showed any significant differences in the total distance covered and the amount of time spent in the center of the open field assay (Figure 1a, 1b). Male *Magel2* knockout mice spent significantly less time in the center of the open field at the 30 minute time point (Fig. 1c). Female *Magel2* knockout mice spent significantly more time in the center of the open field at the 20 minute timepoint (Fig. 1d). However, both male and female *Magel2* knockout mice showed significant decreases in open field horizontal activity at 20 and 30 minute timepoints, and males also showing a significant decrease in the first ten minutes (based on the number of horizontal beam breaks) (Fig. 1e, 1f). Overall, there is a significant effect of genotype on horizontal activity in the open field assay ($F(1,39) = 10$, $P < 0.003$). *Magel2*

knockout female mice showed significant increases ($F(1,19) = 13$, $P < 0.002$) in the amount of vertical activity in the open field. However, male *Magel2* knockout mice showed no difference in vertical activity (based on the number of vertical beam breaks) (Fig. 1g, 1h). Analyzing for vertical exploration in the center versus the wall of the open field showed that male *Magel2* knockout mice showed reduced vertical activity in the center of the open field at the 30 minute timepoint, while female *Magel2* knockout mice tended to have increased vertical activity in both the center and the wall of the open field (Supplemental. fig. 1a, 1b, 1c, 1d). When observing for the amount of time spent in either the open or closed arms of the elevated plus maze, male and female *Magel2* knockout mice showed a significant increase in amount of time spent in the open arm, while female *Magel2* knockout mice spent significantly decreased amount of time in the closed arm, compared to their wildtype counterparts (Fig. 2a, 2b). *Magel2* knockout mice showed similar differences when assaying for the amount of distance traveled in either the open or closed arms of the elevated plus maze, with female Magel2 knockout mice traveling more in the open arm and both male and female Magel2 knockout mice traveling less in the closed arm (Supp. fig. 2a, 2b, 2c, 2d). Further, female *Magel2* knockout mice made significantly more entries into the open arm compared to wildtype counterparts, while male *Magel2* knockout mice showed no difference (Fig. 2c, 2d). Taken together, these results suggest that *Magel2* knockout mice display alterations in exploratory behavior and decreased anxiety. Increases in vertical exploration and closed-to-open arm entries may indicate a tendency for impulsive, repetitive behaviors.

Female Magel2 knockout mice show alterations in social interaction

To address whether alterations in *Magel2* have an effect on social interaction, we performed a tube test for social dominance and a three-chamber assay for social approach. When observing for social dominance in the tube test, *Magel2* knockout mice or wildtype mice are paired against novel partner mice, with three novel partner pairings for each mouse. Observing for the proportion of wins for each experimental mouse versus novel partner mouse identified no significant difference between the number of wins of *Magel2* knockout mice and the number of wins of their wildtype counterparts (Fig. 3a, 3b). When assaying for social approach utilizing a three-chamber assay, male *Magel2* knockout mice did not show a significant difference from wildtype mice in the amount of time spent with a novel partner or novel object (Fig. 3c). However, female *Magel2* knockout mice spent significantly more time with novel partner mice when compared to female wildtype mice (Fig. 3d). The results suggest that *Magel2* deficiency causes no deficit in social interest, yet may even lead to increased social interaction.

Magel2 knockout mice show a deficit in preference for social novelty

To further explore the effect of *Magel2* deficiency on social interaction, knockout and wildtype mice were subjected to a partition assay for social interaction and recognition.

When observing for differences between genotypes, male *Magel2* knockout mice showed no difference than wildtype mice, in their amount of time spent with baseline partner mice (Fig. 4a). Female *Magel2* knockout mice spent a significantly increased amount of time with their baseline partner mice compared to wildtype mice (Fig. 4b). Following the introduction of a novel partner mouse, both male and female *Magel2* knockout mice showed no significant

increase in interest in the partner mouse, based on the amount of time spent at the perforated partition (Fig. 4a, 4b). This is in marked contrast to the significantly increased amount of time the wildtype mice spent with novel partner mice (Fig. 4a, 4b). In both male and female mice, there was a significant effect of the type of partner in the results of the partition assay ($F(2,78) = 37$, $P < 0.0001$). While no overall effects of genotype were observed, a significant effect of genotype by partner type interaction was observed ($F(2,78) = 7$, $P < 0.002$). To better appreciate the observed effect of a *Magel2* deficiency, the partition data was further analyzed for the within-group differences. As seen in Fig. 4c and 4d, male and female wildtype mice show a significant increase in the amount of time spent with novel partner mice, when compared to time spent with the baseline partner mouse. Expectedly, wildtype mice show a significant decrease in the amount of time interacting, when the original partner mice were returned (Fig. 4c, 4d). In contrast, *Magel2* knockout mice show no significant difference in the amount of time spent with a novel partner versus the time spent with baseline partner mice, highlighting the deficit in preference for novel partners in a social setting.

Discussion

Pathogenic variants of *MAGEL2* have been shown to cause neurobehavioral phenotypes associated with Schaaf-Yang syndrome and Prader-Willi syndrome (Schaaf et al. 2013; Soden et al. 2014; Fountain et al. 2016). Previous studies in *Magel2* loss-of-function mouse models showed alterations in fat and muscle deposition, neurotransmitter signaling, brain volume, reproduction, and behavior in novel environments (Kozlov et al. 2007; Bischof et al. 2007; Mercer & Wevrick 2009; Tennese & Wevrick 2011; Mercer et al. 2009; Lee et al. 2003). To date, studies of neurobehavioral phenotypes associated with autism-like behaviors in *Magel2* knockout mice younger than 14 weeks of age have been limited. We studied the consequences of *Magel2* deletion in mice via behavior assays relevant to exploration, anxiety, and social interaction. We report that male and female *Magel2* knockout, young mice display neurobehavioral dysfunction, specifically altered exploratory activity, altered social interaction in female mice, and a lack of preference for social novelty.

The lack of preference for social novelty is most evident based on timed interaction with either a novel partner mouse or a baseline partner mouse. Mice deficient for *Magel2* appear to display an indiscriminate interest in either partner mouse. Interestingly, female *Magel2* knockout mice spent more time with their baseline partner mice, when compared to their wildtype counterparts. This provides additional evidence for increased social interest by female *Magel2* knockout mice, similar to the observations made in the three-chamber assay. Male *Magel2* knockout mice displayed no discernable difference in the interest exhibited towards baseline or novel partner mice, based on the amount of time spent at the partition, when comparing within-group differences of each genotype. Indeed, female *Magel2* knockout mice showed no difference in interest towards either a baseline partner or novel partner mouse, when comparing within-group differences. However, when reintroducing the original partner mouse, interest decreased even further. The data from the partition assay serve as an extension of the findings of the three-chamber assay, highlighting that *Magel2* knockout mice do not show a preference for social novelty even though they do not show a

deficit in social interaction. The tube test data indicate that there are no alterations in social dominance or aggressiveness.

The data presented here suggest that mice deficient for *Magel2* manifest a modest reduction in anxiety, as these null mice display increases in vertical activity and exploration into the open arms of the elevated plus maze. While the open field assay shows no differences in distance covered in the chamber, male and female *Magel2* knockout mice show a progressively significant reduction in horizontal activity. The reduction in horizontal activity would indicate a reduced number of ambulatory movements, specifically tail, head, and limb movements that would register a beam interruption. The reduction in these ambulatory movements may suggest a reduction in anxiety, hypoactivity, or a lack of interest towards the novel environment over time. Most recently, this *Magel2* knockout mouse was shown to have poor muscle tone due aberrancies in muscle autophagy (Kamaludin et al. 2016). This finding may account for some of the observed hypoactivity. Interestingly, while male *Magel2* knockout mice showed minimal difference in vertical activity, female *Magel2* knockout mice manifested an increased interest in vertical exploration, further suggestive of a reduction in anxiety.

Mercer et al. previously reported alterations in response to novel stimuli in *Magel2* knockout adult mice (Mercer et al. 2009). An important point to note is that Mercer et al. described neophobia to novel environments and novel foods. In their assessment, female, but not male, *Magel2* knockout mice had no preference for a novel over a familiar object when introduced after five minutes. Similarly, female *Magel2* knockout mice buried fewer marbles in a marble-burying assay. Further, they observed that *Magel2* knockout mice consumed significantly less novel food than their wildtype littermates. Their findings were interpreted as an avoidance of novel objects and anxiety in novel environments. We now expand on this neophobia by providing data to support the idea of *Magel2* knockout mice also lacking preference for social novelty.

The present study of the role of *Magel2* loss-of-function made a similar conclusions to previously reported studies of behavior phenotypes. However, it is interesting that the observed phenotype of each assay was not necessarily recapitulated. While *Magel2* knockout mice show an increase in time in the open arm of the elevated plus maze in our studies, no difference was observed by Mercer et al. While both studies identified a reduction in horizontal activity over time, Mercer et al. describe a decrease in vertical activity and potentially an increase in anxiety, which is in contrast to the increase in vertical activity reported in our study. Interestingly, Mercer et al. highlighted an increased off-wall rearing of *Magel2* knockout adult male mice (Mercer et al. 2009). While this was not assayed for in the present study, it is possible to have been accounted for by the vertical activity described. The differences described are likely attributable to a number of factors. First, the mice presented in this study were assayed between 8-12 weeks of age, compared to the previous report of 14-26 weeks of age. Second, we report cohorts of 20 mice per sex, per genotype, compared to the previous analysis of 5-6 mice per genotype. Third, we analyzed both males and females compared to an all male analysis by Mercer et al. (with the exception of a novel object recognition, marble burying, and grooming assays on both male and female mice).

More recently, Meziane et al. reported altered social behavior in another *Magel2* knockout mouse model (Schaller et al. 2010; Meziane et al. 2014). Male *Magel2* knockout mice spent significantly less time exploring novel partner mice in a social recognition task. Further, male *Magel2* knockout mice spent significantly less time exploring novel partner mice in a social interaction assay in the open field. However, these mice displayed no difference in non-social behaviors, such as grooming, during the same assay (Meziane et al. 2014). Strikingly, these mice manifested a 50% neonatal lethality due to suckling defects in both male and female mice (Schaller et al. 2010). The reason for the observed differences between the two mouse models is not yet clear. It is conceivable that differences in the construction of the knockout of *Magel2* lead to phenotypic variability, as Meziane et al.'s model deletes both the *Magel2* promoter and coding region versus Mercer et al.'s model keeping the promoter to drive *lacZ*. Of considerable importance, Meziane et al. were able to rescue the phenotypes associated with *Magel2* loss-of-function by early oxytocin injections (Meziane et al. 2014). It would be interesting to assess the effects of oxytocin treatment on the phenotypes seen in the *Magel2* knockout mouse described in this study.

Individuals with truncating mutations in *MAGEL2* have a high prevalence of ASD and behavioral anomalies (Fountain et al. 2016; Schaaf et al. 2013). . It is possible that this phenotype is recapitulated in this *Magel2* knockout mouse model, with no deficits of social interaction at baseline, yet lack of increased social interest following the introduction of a novel partner. Diagnostic criteria for ASD include aberrancies or deficits in the maintenance of social interactions (Fakhoury 2015; Lobar 2015). While increased interaction with partner mice may seem to speak against autism-like phenotypes, altered and increased sociability is observed in various mouse models of autism, and may reflect a risk-taking, impulsive behavior (Chao et al. 2010; Feyder et al. 2010; Schaevitz et al. 2010). When analyzing data associated with behavior analysis, there can be a tendency for observers to interject an anthropomorphic bias on these behaviors. Moreover, differences in each behavior assay could be subtle manifestations of an associated phenotype, not easily discerned and accounted for. As an example, the increase in vertical activity observed with *Magel2* null mice is typically associated with decreased anxiety and increased exploratory behavior. Because this is an assay of beam breaks in vertical direction, it is also conceivable that this could be a subtle manifestation of a stereotypy or hyperactivity. More sensitive assays may help to elucidate these subtle differences.

In summary, we investigated the behavioral and autism-related phenotypes in a mouse model of SHFYNG and PWS. Further investigations are necessary to explore the robustness of this mouse model, especially prior to embarking on preclinical investigations relevant to the human disease phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Joan and Stanford Alexander Family, the Foundation for Prader-Willi Research, and the Intellectual and Developmental Disabilities Research Center (1U54 HD083092) Neurobehavioral Core for

use of facilities. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development or the National Institutes of Health. The authors declare no conflict of interest.

Bibliography

Bennett JA, et al. Autism spectrum disorder in Prader-Willi syndrome: A systematic review. American Journal of Medical Genetics Part A. 2015; 167A:2936–2944. Available at: http://doi.wiley.com/10.1002/ajmg.a.37286. [PubMed: 26331980]

Bischof JM, Stewart CL, Wevrick R. Inactivation of the mouse Magel2 gene results in growth abnormalities similar to Prader-Willi syndrome. Human Molecular Genetics. 2007; 16(22):2713–2719. [PubMed: 17728320]

Buiting K. Prader-Willi syndrome and Angelman syndrome. American Journal of Medical Genetics Part C: Seminars in Medical Genetics. 2010; 154C(3):365–376. Available at: http://doi.wiley.com/10.1002/ajmg.c.30273.

Cassidy SB, et al. Prader-Willi syndrome. Genetics in Medicine. 2012; 14(1):10–26. [PubMed: 22237428]

Cassidy SB, Driscoll DJ. Prader–Willi syndrome. European Journal of Human Genetics. 2009; 17(1):3–13. Available at: http://www.nature.com/ejhg/journal/v17/n1/full/ejhg2008165a.html%5Cnhttp://www.nature.com/ejhg/journal/v17/n1/pdf/ejhg2008165a.pdf. [PubMed: 18781185]

Chao HT, et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature. 2010; 468(7321):263–269. Available at: http://dx.doi.org/10.1038/nature09582. [PubMed: 21068835]

Dykens E, Cassidy SB, King B. Maladaptive behavior differences in Prader-Willi syndrome due to paternal deletion versus maternal uniparental disomy. Am J Ment Retard. 1999; 104(1):67–77. [PubMed: 9972835]

Fakhoury, M. Autistic spectrum disorders: A review of clinical features, theories and diagnosis; International Journal of Developmental Neuroscience. 2015. p. 1-8.Available at: http://linkinghub.elsevier.com/retrieve/pii/S0736574815000519

Feyder M, et al. Association of mouse Dlg4 (PSD-95) gene deletion and human DLG4 gene variation with phenotypes relevant to autism spectrum disorders and Williams' syndrome. American Journal of Psychiatry. 2010; 167(12):1508–1517. [PubMed: 20952458]

Fountain, MD., et al. The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families; Genetics in Medicine. 2016. p. 1-8.Available at: http://www.nature.com/doifinder/10.1038/gim.2016.53

Kamaludin, AA., et al. Muscle dysfunction caused by loss of Magel2 in a mouse model of Prader-Willi and Schaaf-Yang syndromes; Human molecular genetics. 2016. p. 1-12.Available at: http://www.ncbi.nlm.nih.gov/pubmed/27436578

Kozlov SV, et al. The imprinted gene Magel2 regulates normal circadian output. Nature genetics. 2007; 39(10):1266–1272. [PubMed: 17893678]

Lacaria M, et al. Enriched rearing improves behavioral responses of an animal model for CNV-based autistic-like traits. Human Molecular Genetics. 2012; 21(14):3083–3096. [PubMed: 22492990]

Lee S, Walker CL, Wevrick R. Prader-Willi syndrome transcripts are expressed in phenotypically significant regions of the developing mouse brain. Gene Expression Patterns. 2003; 3:599–609. [PubMed: 12971993]

Lobar, SL. DSM-V Changes for Autism Spectrum Disorder (ASD): Implications for Diagnosis, Management, and Care Coordination for Children With ASDs; Journal of Pediatric Health Care. 2015. p. 1-7.Available at: http://dx.doi.org/10.1016/j.pedhc.2015.09.005

Mejlachowicz, D., et al. Truncating Mutations of MAGEL2, a Gene within the Prader-Willi Locus, Are Responsible for Severe Arthrogryposis; The American Journal of Human Genetics. 2015. p. 1-5.Available at: http://linkinghub.elsevier.com/retrieve/pii/S0002929715003328

Mercer RE, et al. Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene Magel2. American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics. 2009; 150(February):1085–1099.

Mercer RE, Wevrick R. Loss of Magel2, a candidate gene for features of Prader- Willi syndrome, impairs reproductive function in mice. PLoS ONE. 2009; 4(1)

Meziane H, et al. An Early Postnatal Oxytocin Treatment Prevents Social and Learning Deficits in Adult Mice Deficient for Magel2, a Gene Involved in Prader-Willi Syndrome and Autism. Biological Psychiatry. 2014; 78:85–94. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0006322314008877. [PubMed: 25599930]

Ramsden SC, et al. Practice guidelines for the molecular analysis of Prader-Willi and Angelman syndromes. BMC medical genetics. 2010; 11:70. [PubMed: 20459762]

Resnick JL, Nicholls RD, Wevrick R. Recommendations for the investigation of animal models of Prader-Willi syndrome. Mammalian Genome. 2013; 24:165–178. [PubMed: 23609791]

Schaaf CP, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. Nature genetics. 2013; 45(11):1405–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3819162&tool=pmcentrez&rendertype=abstract. [PubMed: 24076603]

Schaevitz LR, et al. Cognitive and social functions and growth factors in a mouse model of Rett syndrome. Physiology & Behavior. 2010; 100(3):255–263. Available at: http://dx.doi.org/10.1016/j.physbeh.2009.12.025. [PubMed: 20045424]

Schaller F, et al. A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Human Molecular Genetics. 2010; 19(24):4895–4905. [PubMed: 20876615]

Soden SE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Science Translational Medicine. 2014; 6(265):1–14.

Spencer CM, et al. Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes, Brain and Behavior. 2005; 4(7):420–430.

Tennese, Aa, Wevrick, R. Impaired hypothalamic regulation of endocrine function and delayed counterregulatory response to hypoglycemia in Magel2-null mice. Endocrinology. 2011; 152(October):967–978. [PubMed: 21248145]

Veltman MWM, Craig EE, Bolton PF. Autism spectrum disorders in Prader-Willi and Angelman syndromes: a systematic review. Psychiatric genetics. 2005; 15:243–254. [PubMed: 16314754]

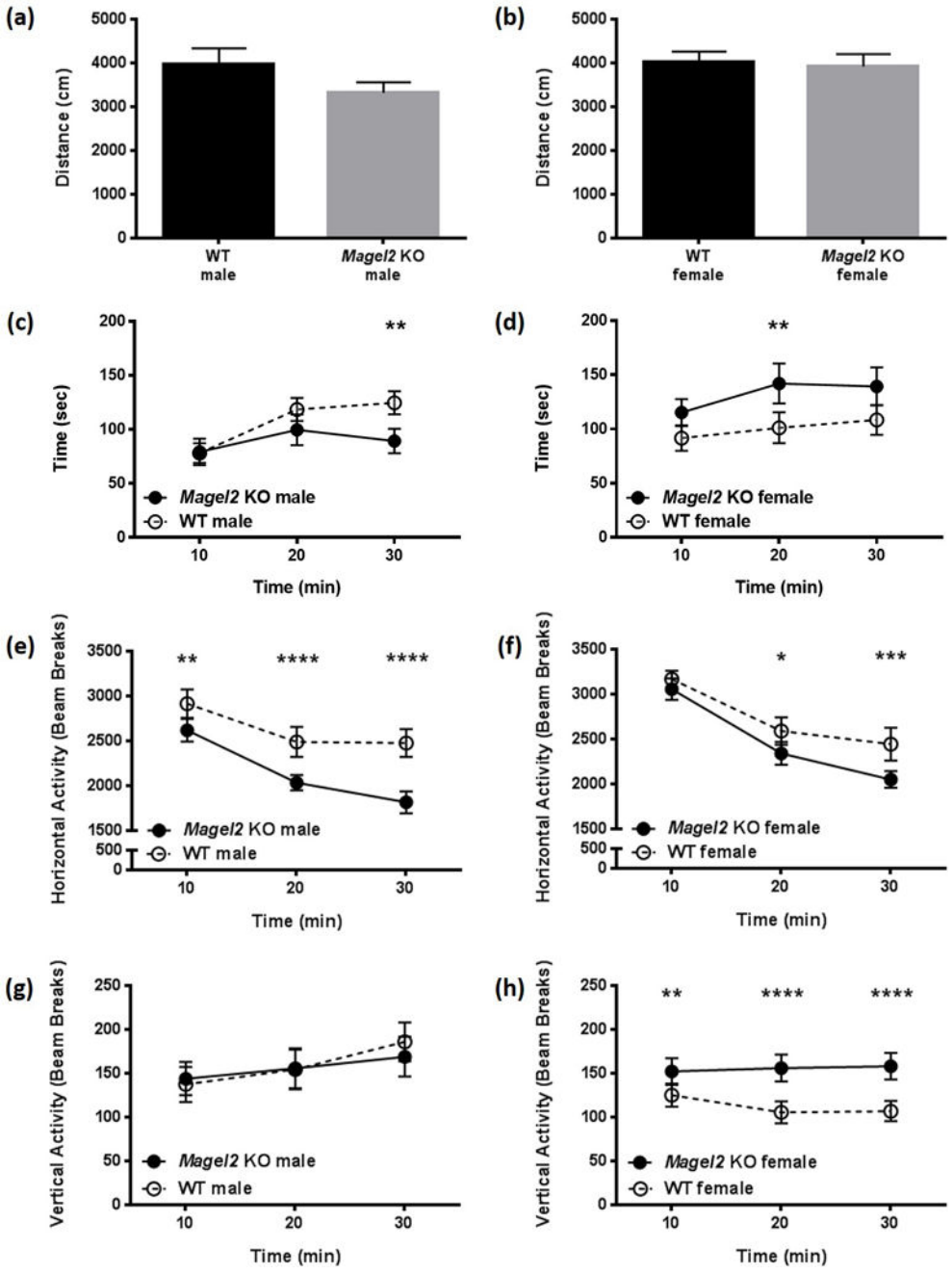

Figure 1. Comparisons between *Magel2* knockout mice and wildtype littermates in an open field assay

(a,c) No differences in the total distance covered or the first 20 minutes of time spent in the center of the chamber, between male wildtype (black bars) and male *Magel2* knockout mice (grey bars), were observed in the open field assay. *Magel2* knockout male mice did showed significantly reduced time in the center of the chamber, in the last 10 minutes of the assay (unpaired t-test). **(b,d)** No differences in the total distance covered or the first and last 10 minutes of time spent in the center of the chamber, between female wildtype (black bars) and female *Magel2* knockout mice (grey bars), were observed in the open field assay.

Magel2 knockout female mice did showed significantly increased time in the center of the chamber, in the second 10 minute bin of the assay (unpaired t-test). **(e,f)** Male and female *Magel2* knockout mice (solid line) showed a decreased horizontal activity when compared to wildtype (dashed line) counterparts, with male *Magel2* knockout mice significantly different at 20 and 30 minute time points (2-way ANOVA). **(g)** When assaying for vertical activity, male *Magel2* knockout mice show no difference when compared to wildtype littermates (2-way ANOVA). **(h)** When assaying for vertical activity, female *Magel2* knockout mice show significantly increased vertical activity when compared to wildtype littermates (2-way ANOVA). Total assay time = 30 minutes. n=20 per genotype per sex. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

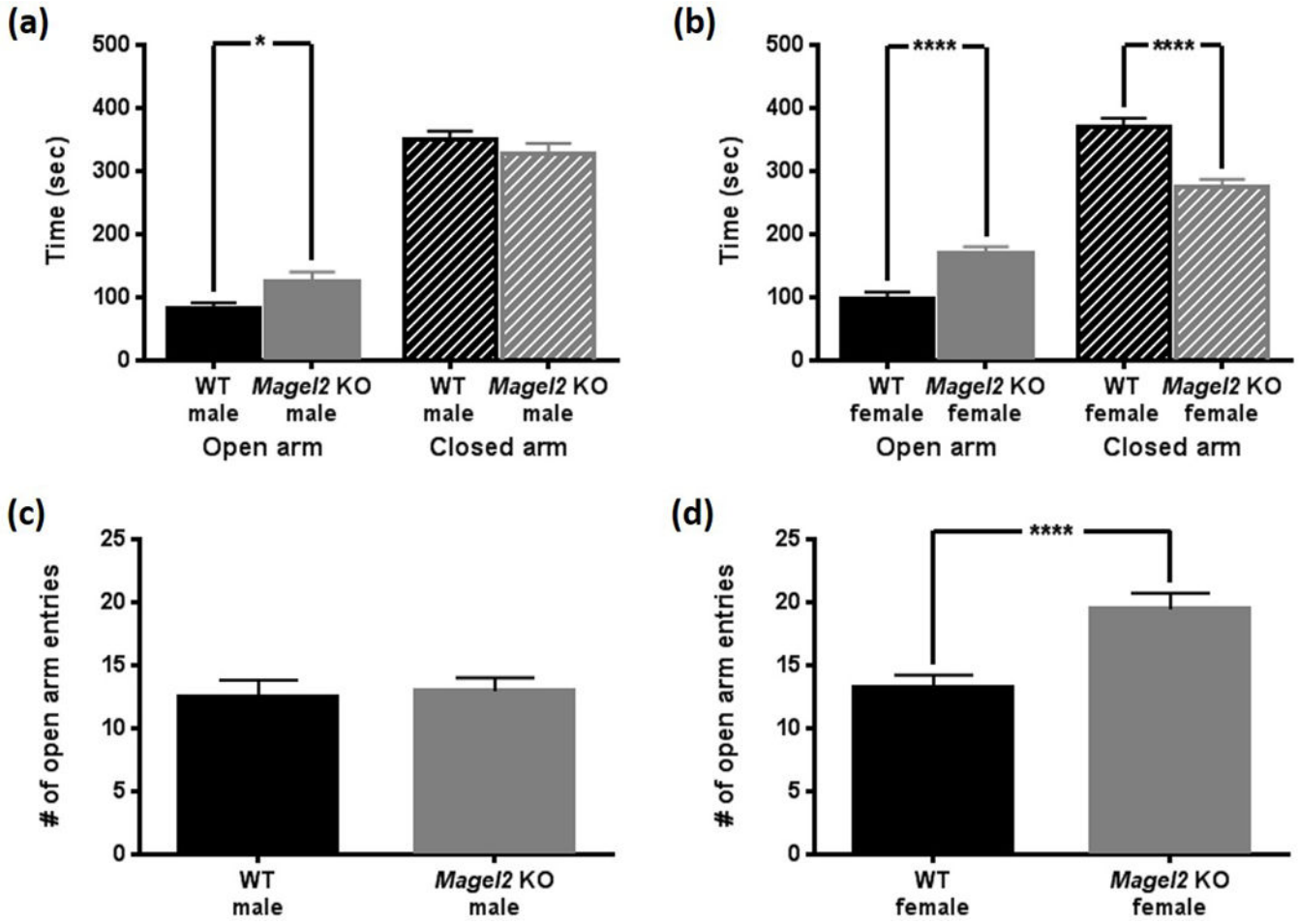

Figure 2. Comparisons between *Magel2* knockout mice and wildtype littermates in the elevated plus maze

(a) Male *Magel2* knockout mice (grey bar) show a significantly increased amount of time spent in the open arm when compared to wildtype littermate mice (black bar) (unpaired t-test). *Magel2* knockout mice (hashed grey bar) showed no difference in amount of time spent in the closed arm, when compared to wildtype littermates (hashed black bar) (unpaired t-test). **(b)** Female *Magel2* knockout mice (grey bar) show a significantly increased amount of time spent in the open arm when compared to wildtype littermate mice (black bar) (unpaired t-test). Female *Magel2* knockout mice (hashed grey bar) showed a significantly reduced amount of time spent in the closed arm, when compared to wildtype littermates (hashed black bar) (unpaired t-test). **(c)** Male *Magel2* knockout mice (grey bar) show no difference in the number of entries into the open arm compared to wildtype littermate mice (black bar) (unpaired t-test). **(d)** Female *Magel2* knockout mice (grey bar) show a significantly increased number of entries into the open arm when compared to wildtype littermate mice (black bar) (unpaired t-test). Total assay time = 10 minutes. n=20 per genotype per sex. *, $p<0.05$; ****, $p<0.0001$.

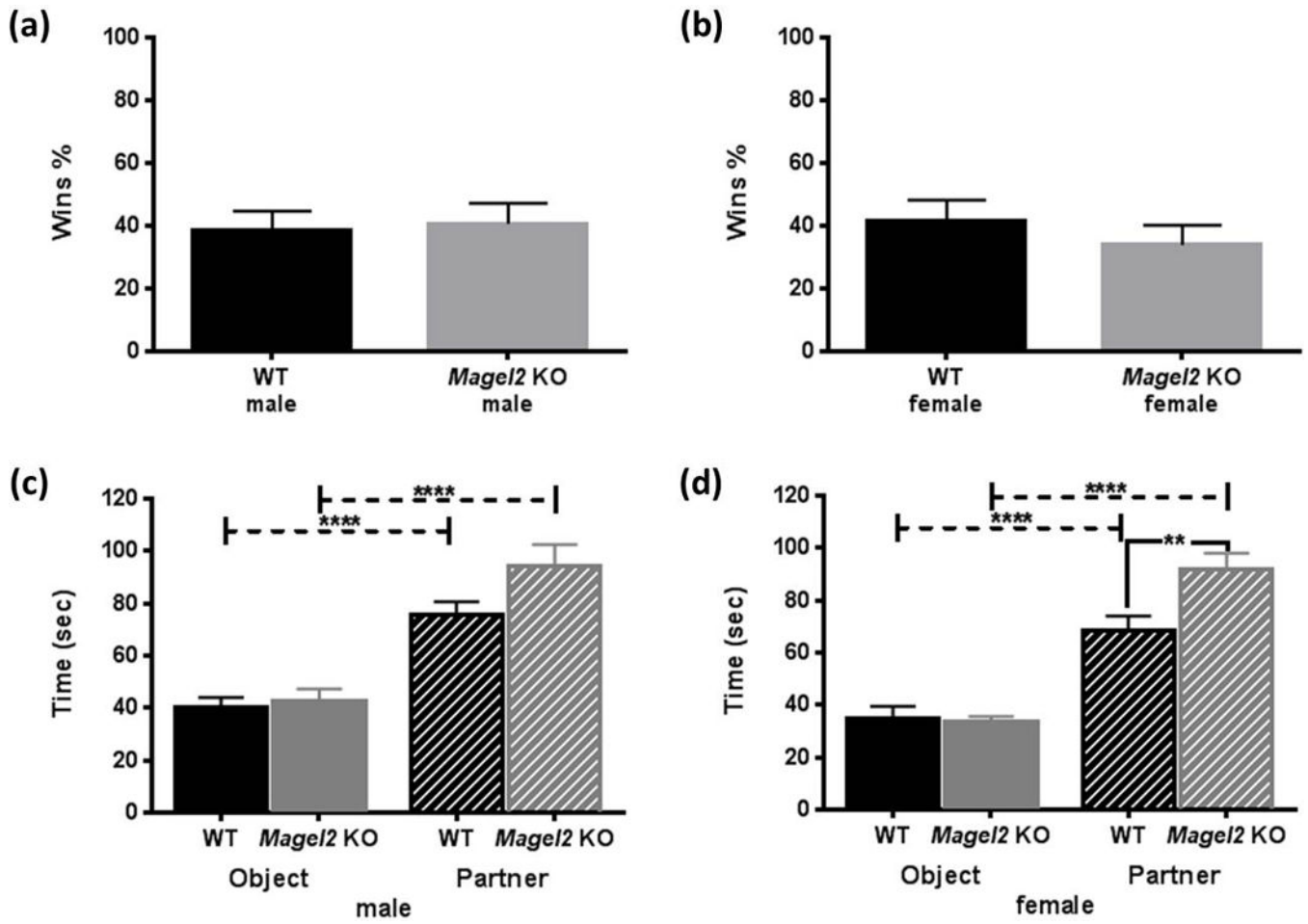

Figure 3. Interactions between wildtype or *Magel2* knockout mice and novel partner mice in a tube test or three-chamber assay

(a,b) Tube test shows no difference for either sex in the percentage of wins between wildtype mice and novel partners or *Magel2* knockout mice and novel partners (three novel partner pairings per experimental mouse) (unpaired t-test). **(c,d)** Both wildtype and Magel2 knockout male and female mice spent more time with novel partners than novel objects (unpaired t-test). **(c)** Male *Magel2* knockout mice showed no difference in time spent with novel partners than did their wildtype counterparts (unpaired t-test). **(d)** Female *Magel2* knockout mice spent more time with novel partner mice than did their wildtype counterparts (unpaired t-test). n=20 per genotype per sex. **, $p<0.01$; ****, $p<0.0001$.

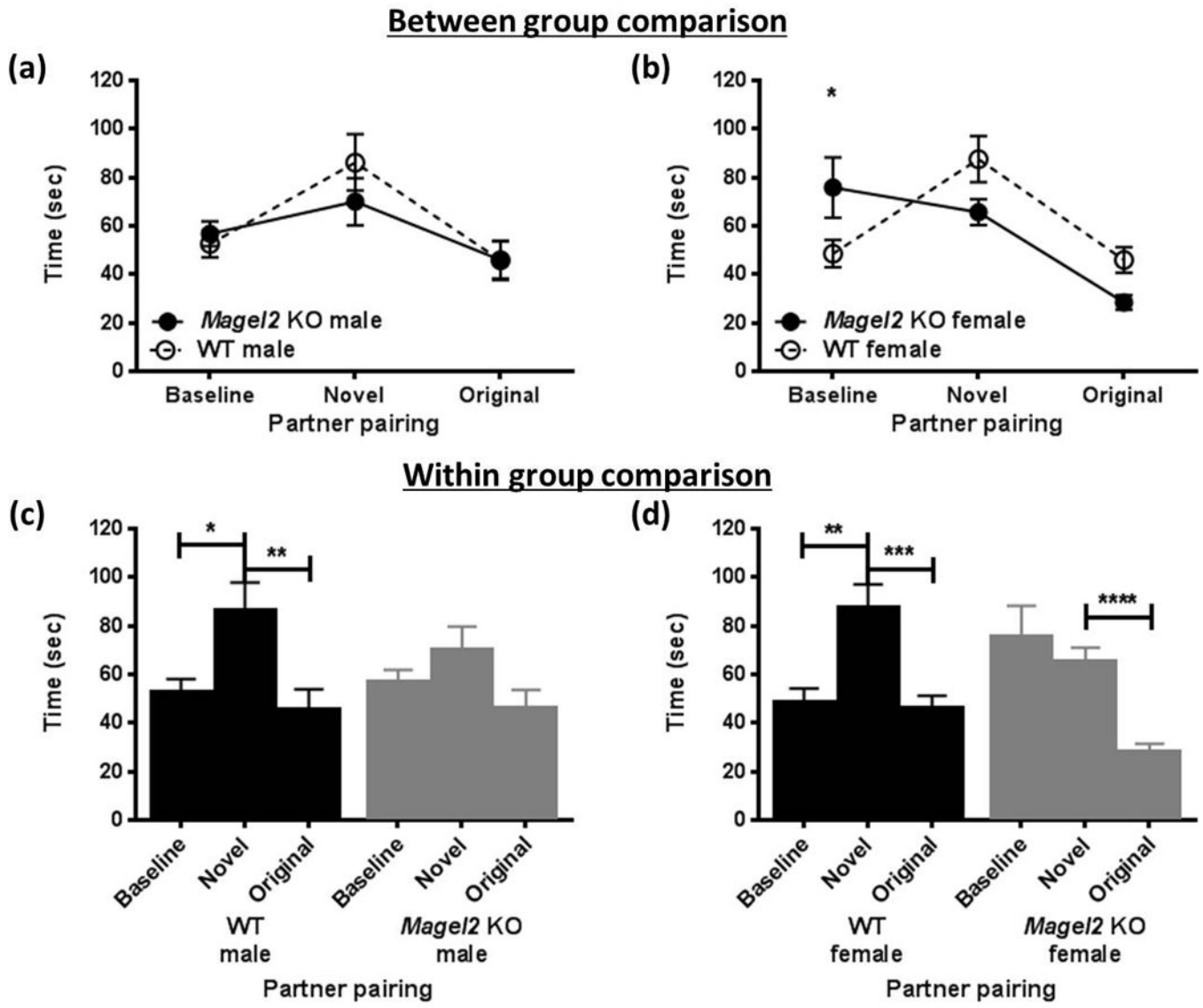

Figure 4. Between genotype/within genotype differences in the amount of time experimental mice spent with partner mice in the partition assay. Between genotype comparison

(a) Male *Magel2* knockout mice (solid line) showed no difference in time spent with either baseline or novel partners when compared to male wildtype counterparts (dash line) (2-way ANOVA). **(b)** Female *Magel2* knockout mice (solid line) spent significantly more time with baseline partner mice, and no difference in time with novel partners, compared to female wildtype mice (dash line) (2-way ANOVA). **Within genotype comparison: (c,d)** Both male and female wildtype mice (black bars) show significant difference between each partner pairing (experimental mouse and baseline partner/novel partner/original baseline partner). Both male and female *Magel2* knockout mice (grey bars) show no significant difference between partner pairings with baseline partners and novel partner mice (unpaired t-test). Female *Magel2* knockout mice show a significant difference between novel partner pairings

and original partner pairings (unpaired t-test). n=20 per genotype per sex. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.