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# Neuroanatomical and behavioral deficits in mice haploinsufficient for *Pericentriolar material 1* (*Pcm1*)

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ABSTRACT

The pericentriolar material (PCM) is composed of proteins responsible for microtubule nucleation/anchoring at the centrosome, some of which have been associated with genetic susceptibility to schizophrenia. Here, we show that mice haploinsufficient for *Pericentriolar material 1* (*Pcm1*$^{+/-}$), which encodes a component of the PCM found to bear rare loss of function mutations in patients with psychiatric illness, manifest neuroanatomical phenotypes and behavioral abnormalities. Using *ex vivo* magnetic resonance imaging of the *Pcm1*$^{+/-}$ brain, we detect reduced whole brain volume. *Pcm1* mutant mice show impairment in social interaction, specifically in the social novelty phase, but not in the sociability phase of the three-chamber social interaction test. In contrast, *Pcm1*$^{+/-}$ mice show normal preference for a novel object, suggesting specific impairment in response to novel social stimulus. In addition, *Pcm1*$^{+/-}$ mice display significantly reduced rearing activity in the open field. *Pcm1*$^{+/-}$ mice behave normally in the elevated plus maze, rotarod, prepulse inhibition, and progressive ratio tests. Together, our results suggest that haploinsufficiency at the *Pcm1* locus can induce a range of neuroanatomical and behavioral phenotypes that support the candidacy of this locus in neuropsychiatric disorders.



## 1. Introduction

Schizophrenia is a common disorder of largely obscure etiology. Genetic analyses have highlighted the influence of susceptibility alleles for schizophrenia, which, in turn, raised the expectation that the cloning of such loci will illuminate the key pathways for the understanding of the disease pathology.

One candidate, *PCM1* (*Pericentriolar material 1*), is attractive in molecular psychiatry for two reasons. First, we and others have linked the chromosomal location 8p22 and specifically *PCM1* to schizophrenia in European populations. Family and trio samples showed significant transmission disequilibrium between a marker in the *PCM1* locus and schizophrenia. A case–control sample also found significant association between *PCM1* markers and schizophrenia (Gurling et al., 2006). This was followed by identification of specific non-synonymous mutations in coding and regulatory regions of *PCM1* in schizophrenia (Kamiya et al., 2008; Datta et al., 2010). Of note, a meta-analysis in Japanese failed to find a significant association (Hashimoto et al., 2011). Second, we have reported previously that transient depletion of *Pcm1* by *in utero* gene transfer can affect the neuroarchitecture of the developing cortex (Kamiya et al., 2008). PCM1 is recruited to the centrosome by Disrupted in Schizophrenia-1 (DISC1) and Bardet–Biedl syndrome 4 proteins (Kamiya et al., 2008; Narayan et al., 2013). PCM1 also interacts with Huntingtin, which is critical for regulation of ciliogenesis (Keryer et al., 2011).

Given these observations, we targeted the *Pcm1* locus through homologous recombination. Here we show that mice haploinsufficient for *Pcm1* show a significant reduction in brain volume. *Pcm1*$^{+/-}$ mice were impaired in response to a novel social

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stimulus, but not to a novel object. In addition, they displayed reduced rearing in the open field test, whereas motor function appeared unimpaired.

## 2. Materials and methods

### 2.1. Mouse colony

Mice deficient in *Pcm1* were generated in a previous study from our group (Brodar, 2015). In brief, the *Pcm1* gene was targeted through insertion of a gene-trap cassette between exon 4 and 5. The depletion of Pcm1 protein is demonstrated in the present manuscript (Fig. 1A). Mice heterozygous for *Pcm1* were backcrossed to C57BL/6N nine times. All the experiments were performed with heterozygous males and their wild-type (WT) littermates. All animal experiments were carried out in accordance with the National Institute of health Guide for Care and Use of Laboratory Animal (NIH Publications No. 823) and were approved by the Johns Hopkins IACUC.

### 2.2. Western blot

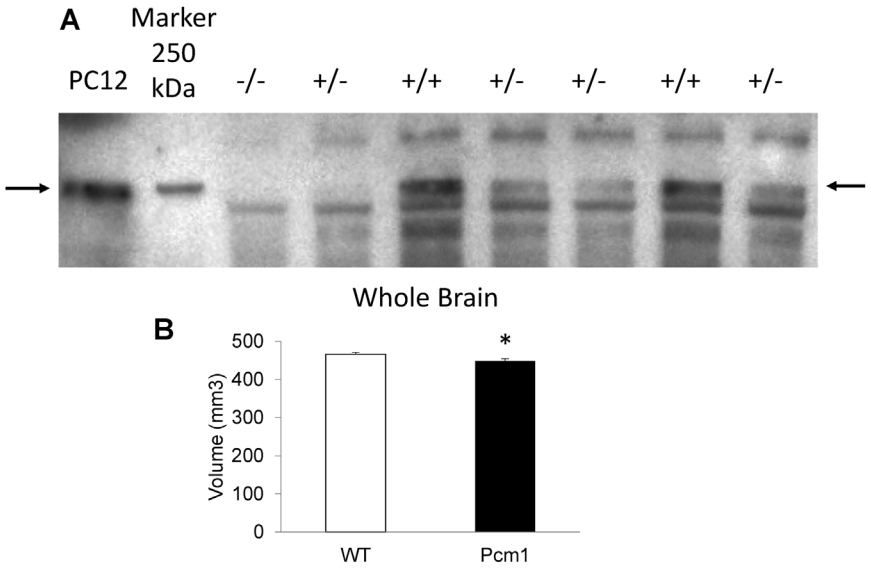
Lysates from PC12 cells as a reference and from the cortex of 10 weeks old $Pcm1^{+/-}$ mice were subjected to immunoblot with Rabbit anti-Pcm1 (1:500) (Kamiya et al., 2008).

### 2.3. Ex vivo *MRI*

High resolution T2-weighted MRI of postmortem specimens was performed on an 11.7 T vertical bore NMR spectrometer (Bruker Biospec, Inc., Billerica, MA, USA) using a 15 mm diameter volume coil as the radiofrequency transmitter and receiver. A three-dimensional (3D) rapid acquisition with refocused echoes (RARE) sequence was used with the following parameters: TE of 45 ms, TR of 1500 ms, RARE factor of 8, and 4 signal averages. The imaging field of view and matrix size were 16.0 mm × 12.0 mm × 10.0 mm and 200 × 152 × 128 respectively, and the native resolution was approximately 80 μm × 80 μm × 80 μm. The total imaging time was 4 h for each specimen. We imaged 4 WT mice and 5 $Pcm1^{+/-}$ mice at the age of 4 months.

### 2.4. Behavioral testing

Behavioral testing was performed starting at the age of three months. In order to minimize inter-trial interference the tests were performed with about a week between the tests and from less stressful to more stressful trials in the following order: open field test, elevated plus maze, three chamber social interaction, rotarod, and prepulse inhibition of acoustic startle. The above series of tests was performed on 11 WT and 16 $Pcm1^{+/-}$ mice. Testing was done as published (Hikida et al., 2007; Jaaro-Peled et al., 2013), except that the open field test was analyzed in bins of 10 min. Novel object recognition test was performed with a second cohort of 14 WT and 8 $Pcm1^{+/-}$ mice. First the mice were habituated to the testing box over 3 days. On the fourth day the mice were exposed to two identical objects for 10 min. After 1 h one of the familiar objects was replaced by a novel object and the mice were allowed to explore them for 5 min. Progressive ratio was performed on a third cohort of 11 WT and 8 $Pcm1^{+/-}$ mice as published (Johnson et al., 2013).




| Volume (% of whole brain) | WT | | Pcm1 | | Comparison | |
|---|---|---|---|---|---|---|
| | Avg | SE | Avg | SE | Pcm1/WT | t-test |
| Caudate-putamen | 4.38 | 0.09 | 4.43 | 0.09 | 1.01 | 0.67 |
| Cerebellum | 13.67 | 0.41 | 13.64 | 0.41 | 1.00 | 0.97 |
| Hippocampus | 5.10 | 0.19 | 5.28 | 0.19 | 1.03 | 0.42 |
| Hypothalamus | 2.04 | 0.19 | 2.09 | 0.19 | 1.02 | 0.90 |
| Neocortex | 21.07 | 0.82 | 21.38 | 0.82 | 1.01 | 0.77 |
| Thalamus | 4.67 | 0.20 | 4.52 | 0.20 | 0.97 | 0.63 |
| Ventricles | 0.99 | 0.12 | 1.24 | 0.12 | 1.26 | 0.35 |

**Fig. 1.** Immunoblot to demonstrate haploinsufficiency in $Pcm1^{+/-}$ mice (A) and gross brain anatomy by structural magnetic resonance imaging at 4 months of age (B). Whole brain volume of $Pcm1^{+/-}$ mice was reduced significantly compared to WT mice, $^{*}p < 0.05$. No significant differences in the relative volumes (out of whole brain volume) of the other regions of interest were observed in $Pcm1^{+/-}$ brains.

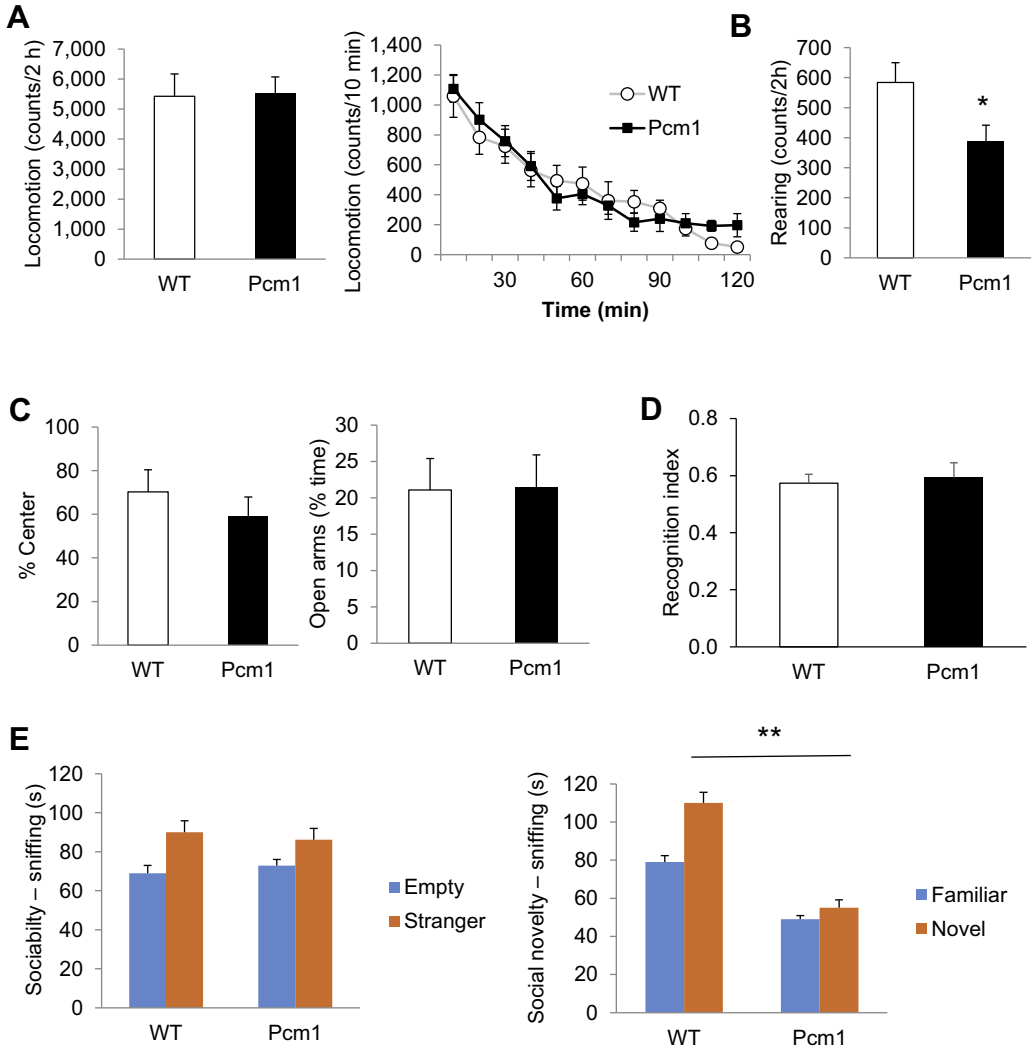



**Fig. 2.** *Pcm1*$^{+/-}$ mice were impaired in recognition of a novel social stimulus, but not a novel object. (A) No difference in locomotion in the open field (left). *Pcm1*$^{+/-}$ mice stopped habituating at 80 min, *t*-test comparing 80 min *vs.* 120 min: $p < 0.01$ for WT, $p = 0.85$ for *Pcm1*$^{+/-}$ mice (right). (B) *Pcm1*$^{+/-}$ mice reared less in the open field, $^{*}p < 0.05$. (C) No change in measurements of anxiety based on % center activity in the open field (left) and % time in the open arms of the elevated plus (right). (D) In the novel object recognition test, *Pcm1*$^{+/-}$ mice showed normal preference for the novel object after a 1 h inter-trial interval. Presented as interaction with novel object/total interaction time. (E) The three-chamber social interaction test. In the sociability phase only WT mice interacted more with the mouse than with an empty enclosure ($p < 0.05$), but ANOVA did not detect a significant effect of *Pcm1*$^{+/-}$ on sociability (left). In the social novelty phase the *Pcm1*$^{+/-}$ mice were impaired, $F(1,25) = 12.38$, $^{**}p < 0.01$ (right). WT mice interacted both with the familiar and with the novel mouse significantly longer than the *Pcm1*$^{+/-}$ mice interacted with each (Bonferroni post-test analysis, $p < 0.001$).

### 2.5. Statistics

Statistical analysis was done with student *t*-test, except for social interaction and prepulse inhibition tests for which we used two-way repeated measures ANOVA (RM ANOVA). $p < 0.05$ was considered significant.

## 3. Results

To achieve a homogeneous genetic background, *Pcm1*$^{+/-}$ mice were backcrossed nine generations to a C57BL/6N (Charles River) background. *Pcm1*$^{-/-}$ animals were produced at a low frequency from *Pcm1*$^{+/-}$ mating, suggesting early lethality. Gross histological analyses of *Pcm1*$^{+/-}$ and wild-type littermates showed no overt pathology; therefore this genotype was used for subsequent experiments to address key significant questions in molecular psychiatry.

First we confirmed by immunoblot that the mice were indeed haploinsufficient for Pcm1 (Fig. 1A). To detect potential neuroanatomical abnormalities in the *Pcm1*$^{-/+}$ mice, we performed *ex vivo* MRI and measured the volume of the following brain regions: caudate-putamen; cerebellum; hippocampus; hypothalamus; neocortex; thalamus; and lateral ventricles. A significant reduction in total brain volume of *Pcm1*$^{+/-}$ samples was observed and a similar trend in discrete brain regions was annotated (Fig. 1B). To control for overall lower whole brain volume, we also compared the relative volume of the brain regions out of the whole brain volume, but did not detect any differences.

Next we tested the *Pcm1* mice in a series of behavioral tests. *Pcm1*$^{+/-}$ mice did not differ from WT controls in total horizontal locomotion in the open field test (Fig. 2A, left). However, when we analyzed activity over time, it became evident that although WT mice continued habituating till the end of the 2 h testing period and reached a low activity level of 50 counts/10 min, *Pcm1*$^{+/-}$ mice stopped habituating at 80 min and kept that level of activity (~200 counts/10 min) until the end of the 2 h testing (Fig. 2A, right). Analysis of the vertical activity showed that the *Pcm1*$^{+/-}$ mice reared significantly less than WT mice (Fig. 2B). *Pcm1*$^{+/-}$ mice did not show signs of anxiety based on the time spent in the center of the open field (Fig. 2C, left) and performance in the elevated plus maze (Fig. 2C, right).

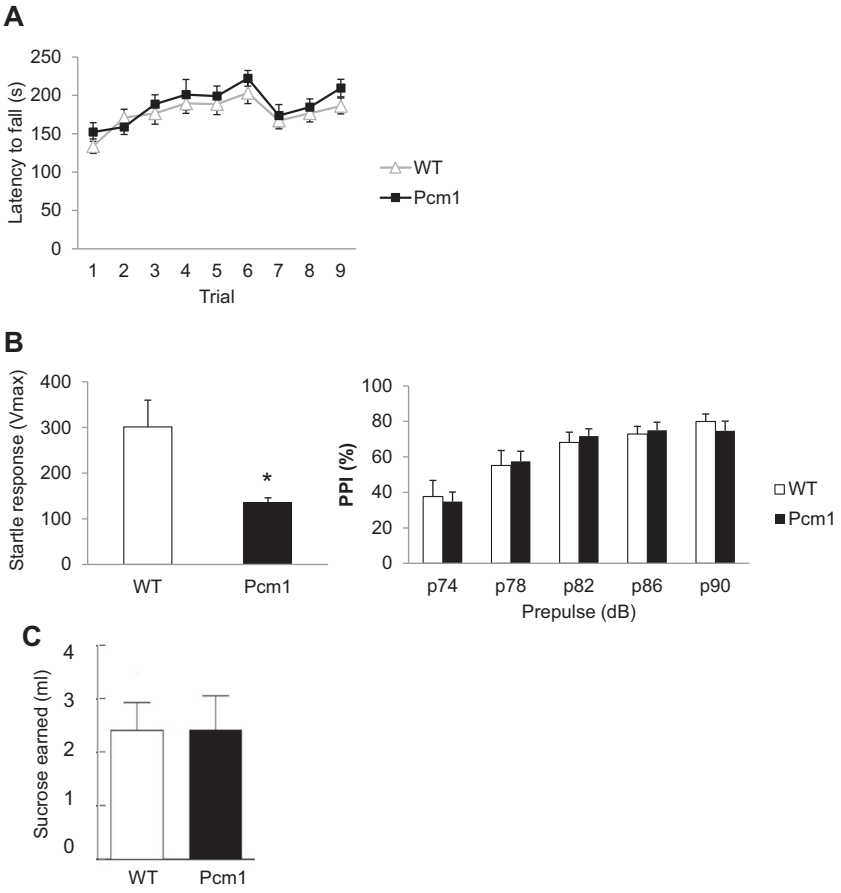



**Fig. 3.** Normal motor and reinforcement behavioral responses in *Pcm1*$^{+/-}$ mice. (A) Similar rotarod performance was observed between *Pcm1*$^{+/-}$ and WT littermates. (B) Sensorimotor gating of acoustic startle: *Pcm1*$^{+/-}$ mice had reduced startle response (left, $^{*}p < 0.05$), but normal prepulse inhibition (right). (C) Progressive ratio test: *Pcm1*$^{+/-}$ mice earned the same amount of sucrose indicating that they were as willing as WT mice to work for a reward.

Given the candidacy of *Pcm1* for contributing mutations to psychiatric illness, we tested the mice for cognitive function using the novel object recognition test. The *Pcm1*$^{+/-}$ mice behaved normally in this test and preferred the novel object over the familiar object (Fig. 2D). Next we assessed social function using the three-chamber social interaction test. Although only WT mice interacted more with the mouse than with an empty enclosure, ANOVA did not detect a significant effect of the genotype on sociability (Fig. 2E, left). In the social novelty phase of the test, however, the social impairment was clear; *Pcm1*$^{+/-}$ mice did not interact more with a novel stranger than with the by-then familiar mouse and RM ANOVA detected a significant genotype x chamber interaction (Fig. 2E, right). Bonferroni post-test analysis indicated that WT mice interacted both with the familiar and the novel mouse significantly longer than the *Pcm1*$^{+/-}$ mice interacted with each.

*Pcm1*$^{+/-}$ mice performed normally in the rotarod test for motor function and learning (Fig. 3A). They displayed a lower startle response to an auditory stimulus of 120 dB, but normal prepulse inhibition, suggesting normal sensorimotor gating (Fig. 3B). *Pcm1*$^{+/-}$ mice also behaved normally in the progressive ratio test in which motivation is assessed by gradually increasing the number of licks required to get a reward (Fig. 3C).

## 4. Discussion

The significance of the present study is to report brain and psychiatry-associated phenotypes (gross anatomical changes and unique set of behavioral alteration) in *Pcm1*$^{+/-}$ mice that our group originally developed (Brodar, 2015).

The two most prominent behavioral phenotypes of *Pcm1*$^{+/-}$ mice were reduced rearing in the open field and reduced social interaction in the three-chamber social interaction test. Rearing is a motor function aimed at the visual scanning of the (novel) environment. Reduced rearing was not due to a motor dysfunction, since horizontal activity (locomotion) and rotarod performance were normal. *Pcm1*$^{+/-}$ mice did not show any preference for a novel social stimulus *vs.* a familiar social stimulus. This was not due to anxiety based on both open field and elevated plus maze measures. Interestingly, this deficit was specific to the social context, as the mice behaved normally in a test of novel object recognition. The phenotype of *Pcm1*$^{+/-}$ mice was relatively mild as expected for heterozygotes. The mild phenotype provides a good background for combining this model of a genetic risk factor with environmental risk factors to study gene–environment interactions in schizophrenia.

Since PCM1 is an interactor of DISC1, it is of interest to compare the behavioral profile of the *Pcm1*$^{+/-}$ mice with the many published DISC1 mouse models (Jaaro-Peled, 2009; Brandon and Sawa, 2011). Impaired social interaction is shared by many of the *Disc1* mutant mouse lines and the *Pcm1*$^{+/-}$ mice. Whether the DISC1–PCM1 pathway mediates social function is an interesting question for future studies.

The gray matter volume of the orbitofrontal cortex is reduced in patients with schizophrenia who carry a disease-associated risk allele of the *PCM1* gene (Gurling et al., 2006). This may potentially be relevant to the social novelty deficit we found in *Pcm1*$^{+/-}$ mice, as the orbitofrontal cortex can underlie, at least in part, flexible decision making in face of changing environment, especially

in social context (Rushworth et al., 2007; Nelson and Guyer, 2011).

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## Conflict of interest

There are no known conflicts of interest for any author.

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