BRIEF REPORT

Neuronal subset-specific Pten-deficient mice do not exhibit deficits in sensorimotor gating processes [version 1; peer review: 1 not approved]

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Abstract

Background: Deficits in sensorimotor gating have been reported in individuals with autism spectrum disorder (ASD), as well as in ASD murine models. However, this behavior has been rarely examined in the neuronal subset-specific (NS)-Pten knockout (KO) model of ASD. NS-Pten KO mice exhibit hyperactivity of the PI3K/AKT/mTOR signaling pathway which is implicated in the onset of autistic deficits. This study investigates the potential relationship between PI3K/AKT/mTOR signaling and deficits in sensorimotor gating.

Methods: To assess sensorimotor gating in NS-Pten KO mice we utilized a three-day paradigm. On day 1 (habituation) the mice were administered 80 repetitions of a 120-dB startle stimulus. On day 2, prepulse inhibition was measured with 90 trials of the startle stimulus that was paired with a smaller (70, 75, or 80 dB) prepulse stimulus. Day 3 was assessed one week later, consisting of randomized startle trials and trials with no stimulus and was used to determine the startle threshold.

Results: No significant difference between NS-Pten KO or wildtype (WT) mice was found for habituation (p > 0.05). No significant differences were found between groups when assessing the percentage of prepulse inhibition at 70, 75, and 80 dB (p > 0.05). There was also no difference in startle threshold between groups (p > 0.05).

Conclusion: Our study found that the NS-Pten KO model does not display significant deficits in sensorimotor gating processes. The present findings help to elucidate the relationship between PI3K/AKT/mTOR hyperactivation and sensory reactivity.

Keywords

autism, pten, macrocephaly, ASD, sensorimotor
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Introduction
Sensorimotor gating is the ability of a sensory stimulus to suppress a motor response. It can be measured by assessing prepulse inhibition (PPI), wherein a weak auditory stimulus inhibits a startle response that is induced by the following presentation of a loud sound. Deficits in PPI have been widely reported in various neurological conditions, including autism spectrum disorder (ASD). Similar to humans, impairments in PPI have been reported in ASD models such as Fmr1 and Cntnap2-knockout (KO) mice; however, the underlying mechanism is unknown. Pten mutant mice are another model of autism and can be used to investigate the connection between a cell signaling pathway commonly implicated in ASD, the PI3K/AKT/mTOR pathway, and specific autistic-like deficits. In the present study, we use neuronal subset-specific (NS)-Pten KO mice that exhibit hyperactivation of the PI3K/AKT/mTOR pathway in the cortex, hippocampus, and cerebellum, and assess PPI in order to further elucidate the potential relationship between PI3K/AKT/mTOR signaling and deficits in sensorimotor gating.

Methods
Subjects
Male and female mice on a FVB based mixed background were obtained from Baylor College of Medicine and have been bred for more than 10 generations at Baylor University. Heterozygous NS-Pten males (n=6) and females (n=12) were used to breed NS-Pten wildtype (WT) and KO pups (RRID: MGI:3714016). The housing for the breeders consisted of two females housed with one male. Genotype was determined from toe clippings taken on postnatal day (PD) 10 (performed by Mouse Genotype, Escondido, CA, USA). On PD 21, animals were weaned and housed with mixed genotype littermates in groups of n=3–5 in cages (Allentown Caging PC7115HT, Allentown, PA, USA) filled with suki-chip bedding (7090 Teklad, Envigo, Somerset, NJ, USA) kept in a room on a 12-hr light/dark diurnal cycle held at 22°C. Mice had ad libitum access to food and water. All animals were tested at 9–10 weeks of age between the hours of 10:00 and 11:30 a.m. A total of 29 male mice were assessed, 17 NS-Pten KO and 12 WT mice. The target sample size was determined by, and is in accordance with, the PPI literature. The final sample sizes were as follows: day 1: n=12 WT, n=17 KO, day 2: n=12 WT, n=13 KO, day 3: n=9 WT, n=9 KO. All test procedures were carried out in compliance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by Baylor University’s Institutional Animal Care and Use Committee. Once the experiment concluded, mice were placed into a CO2 chamber and euthanized.

Sensorimotor gating assessment
Sensorimotor gating was assessed via the SR-LAB system, which consists of a 15 x 14 x 18 inch isolation cabinet, a plexiglass cylinder (3.2-cm diameter) mounted on a sensor platform, a standard speaker used to generate white noise, and a high-frequency speaker used to generate stimuli (San Diego Instruments, San Diego, CA, USA). The paradigm consisted of three separate testing days: habituation, prepulse inhibition, and startle response, and was conducted as previously described. Each test day is further detailed in the Figure 1 legend. To eliminate potential confounds during testing, background sound levels were maintained at 68 dB and the experimenter was not present.

Statistical analysis
GraphPad Prism 7 software (La Jolla, CA) or SPSS 21.0 (IBM, USA) were used to analyze the data. Repeated-measure ANOVAs were run for habituation, prepulse inhibition, and startle threshold. No post-hoc tests were performed. A total of n=4 KO mice were excluded from the day 2 analysis and n=11 mice (3 WT and 8 KO) were excluded from the day 3 analysis due to protocol malfunction or death as a result of the severity of the knockout. A value of p < 0.05 was considered significant for each statistical test.

Results
When assessing the sensorimotor gating paradigm, no main effects were found for habituation (F[1,27] = 0.17, p >0.05), prepulse inhibition (F[1,23] = 2.65, p >0.05) or startle threshold (F[1,16] = 2.33, p >0.05). There were also no interactions for habituation (F[7,189] = 0.91, p >0.05), prepulse inhibition (F[2,46] = 0.71, p >0.05), or startle threshold (F[10,160] = 1.94, p >0.05) (Figure 1a–c). Raw results for each procedure on each day for every animal are available as Underlying data.

Discussion
The NS-Pten KO mice did not exhibit significantly different sensorimotor gating from WT mice. A previous study by Kwon et al. (2006) assessed neuron-specific enolase (Nse)-Pten KO mice in a variation of the PPI protocol and reported a decrease in percent inhibition at 4 dB but no differences at 8 or 16 dB. Our study assessed percent inhibition at 70, 75, and 80 dB, per established protocol, and found no differences at these intensity levels. Therefore, this indicates that there may only be changes in percent inhibition in Pten mutant mice when the prepulse is comparatively quiet, as no impairments are reported for dB levels higher than 4 dB. Additionally, in accordance with our study, no differences in prepulse inhibition have been reported in the BTBR and Shank1 mouse models of autism. This indicates that alterations in sensory reactivity may be a less sensitive measure of an autistic-like phenotype and may also be present in particular ASD models.

Overall, the current study found that hyperactivity of the PI3K/AKT/mTOR pathway does not result in sensorimotor gating deficits in NS-Pten KO mice, suggesting that the pathway may not directly affect prepulse inhibition. This conclusion is supported by a prior study that assessed PPI in a transgenic mouse model of tuberous sclerosis complex, another model of ASD and mTOR hyperactivation, which similarly reported no deficits in prepulse inhibition between WT and KO mice. Taken together, these studies indicate that despite mTOR’s contribution to an autistic-like phenotype, it does not significantly contribute to the onset of sensorimotor gating deficits in...
**Figure 1. Habituation, prepulse inhibition, and startle threshold in NS-Pten KO mice.**

(a) On the first day of testing, the animal was acclimated to the room for 30 minutes then was placed inside the cylinder for a 5-minute habituation period, which was followed by 80 startle stimuli delivered at a fixed interval of 15 seconds. The startle stimulus was a 40-ms, 120-dB noise burst, with a rise/fall time of less than 1 ms. We found that there was no significant difference in habituation between KO and WT mice (p > 0.05).

(b) Day 2 of testing occurred 24 hours after day 1 and tested prepulse inhibition. Once the mice were in the apparatus, there was a 5-minute habituation phase that was followed by 20 presentations of a 40-ms, 120-dB noise burst that had a fall time of less than 1 ms. In the prepulse phase, mice were presented with 90 trials consisting of three prepulse intensities, 70, 75, and 80 dB. Each prepulse was 20 ms in duration with a rise/fall time of less than 1 ms and were spaced 15 seconds apart. We found no difference in the percentage of prepulse inhibition between groups following prepulses of 70, 75, or 80 dB (p > 0.05).

(c) One week after the prepulse session, the startle threshold was assessed. Following the 5-minute habituation period, the mice were presented with 99 trials of 11 trial types. These include a no stimulus trial and 10 startle stimuli trials ranging from 75–120 dB at 5 dB intervals. The startle stimuli are 40 ms noise bursts with a rise/fall of less than 1 ms. We found no difference in startle threshold between NS-Pten KO and WT mice (p > 0.05). Data are presented as the mean ± standard error of the mean (SEM).
Data availability

Underlying data


This project contains the following underlying data:
- PPI Day 1 Pten Raw Data 9-6.xlsx (raw data from all experiments performed for all animals; day 1).
- PPI Day 2 Pten Raw Data 9-6.xlsx (raw data from all experiments performed for all animals; day 2).
- PPI day 3 Pten Raw Data 9-6.xlsx (raw data from all experiments performed for all animals; day 3).

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain). 

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References


Page 5 of 8
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Whether sensorimotor gating, as evaluated by the prepulse inhibition (PPI) of the acoustic startle reflex paradigm, is attenuated or exaggerated in ASD is still controversial. The present study attempted to investigate this using a mutant mouse model. Specifically, neuronal deletion of Pten in the mouse is expected to result in P13K/AKT/mTOR hyperactivity implicated in ASD onset. The study may potentially clarify whether this genetic manipulation would be sufficient to modify PPI expression. No difference between mutants and wild type (WT) mice was reported. Indeed, the magnitude, habituation and threshold of the startle response as such were reported to be highly comparable between genotypes. The null results led the authors to conclude that the contribution of the mTOR pathway to ASD-related PPI deficits is limited. Closer examination of the methods and data reveal significant concerns that undermine confidence in the reliability and robustness of the reported findings.

1. No attempt was made to examine sex difference, while it is highly relevant to ASD.

2. Methodology details were not sufficient. Essential test parameters such as ITI and SOA in prepulse-pulse trials were not reported. Wide of response window was not reported, although it could be discerned from the raw data file.

3. Apparently, 8 (out of 17) mutant mice died from Experiment 1 to Experiment 3. This led one to suspect that the mutant mice had serious and widespread physiological defects, which could undermine any meaningful comparison. One would like to see body weights reported at least. Were the mutants significantly lighter?

4. Statistical results are poorly reported. Only “main effects” (supposedly the genotype effects) were considered. Statistics towards ascertaining the presence of startle habituation (e.g., Trials or blocks of 10 trials effects), and prepulse inhibition (the effect of prepulse intensities) etc. are not provided. To report that “no main effects were found for habituation F(1,27)=…” is inappropriate, because the comparison of habituation between genotypes could only be meaningfully evaluated by reference to the Genotype x Blocks of 10 trials interaction. Reporting the main effect of Genotype does not allow an effective assessment of the habituation profile, merely the overall
magnitude of startles.

5. The plot shown in Figure 1a cannot be reproduced from the raw data provided.

6. It is also observed that the first trial of Day 1 data were all very low (in all mice). This is highly unusual and may indicate a protocol failure, or misalignment of data.

7. Examination of Day 2 data for PPI assessment also reveals another anomaly. At least 4 mice (ID: 2081, 2084, 2085, 2072) exhibited very weak startle values (well under 100) in all “120startle” trials – substantially lower than the startle magnitude obtained on the previous startle habituation test. The change is massive and inexplicable. The problem may be more extensive and include other mice. The authors should exercise due diligence in examining their data before analysis.

8. In their discussion of Kwon et al.’s (2006) reported findings of a PPI deficit (Nse)-Pten KO mice, the authors mistook the prepulses at 4dBm 8dB and 16dB as the actual magnitude of the prepulses used by Kwon et al. In fact, these refer to prepulse of intensity at 4, 6 and 18 decibels units above background. The use of 70, 75 and 80dB prepulses here were presented against a background noise level of 68dB, and thus effectively be +2, +7 and +12 decibel units above background. Hence, it is incorrect to conclude (by comparison between the present study and Kwon et al.) that “there may only be changes in percent inhibition in Pten mutant mice when the prepulse in comparatively quiet”. If anything, the +2 (or 70 dB) condition here was even lower than the weakest prepulse used by Kwon et al.

9. The authors evaluated the startle reactivity curve as a function of increasing pulse intensity – as a means to examine the “startle threshold”. Yet no attempt was made to measure individual startle threshold for comparison between genotypes. Otherwise, it is misleading to conclude that threshold did not differ when only the group’s average profile was presented.

Hence, although the available data tend to support the overall lack of an effect of the gene KO on PPI, the methods, presentation, data analysis are clearly inadequate.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly
**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Prepulse inhibition, Behavioural phenotyping of mutant mice, animal models of schizophrenia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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