PNAS LETTER

Inner ear deficits complicate interpretation of depression-like behaviors in *Gpr156* mutant mice

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The article by Miller et al., "*A rare variant in GPR156 associated with depression in a Mennonite pedigree causes habenula hyperactivity and stress sensitivity in mice*" (1), proposes an interesting link between GPR156 and depression-related phenotypes. However, well-established prior results demonstrating auditory and vestibular roles of GPR156 in mice are directly relevant to the interpretation of the behavioral assays reported in this study but were not considered by the authors.

Importantly, the mouse models examined by Miller et al. are either homozygous for knockout of *Gpr156* or homozygous for the depression-associated E533D allele, whereas the human subjects in the study are all heterozygous carriers of E533D. This difference in genetic context is critical given the known inner ear abnormalities due to recessive loss of function of *Gpr156.*

The knockout strain is presented as newly generated but corresponds to a model generated by the KOMP consortium based on a Velocigene design (RRID:MMRRC_047937-UCD). This strain has been available at the MMRRC repository and is the same strain we used for inner ear studies. The strain used is consequential for interpreting the behavioral data in Miller et al.

First, defective swimming behavior (figure 5) is interpreted as depression-like behavior. However, knockout mice carrying the same *Gpr156* null allele are known to exhibit severe vestibular dysfunction that impairs their ability to maintain head orientation and stability in water and also impairs the vestibulo-ocular reflex (2, 3). In this context, erratic swimming behavior in both knockout and E533D homozygous mice could arise from vestibular impairment rather than an altered affective state. Similarly, interpretation of the acoustic startle response (*SI Appendix*, figure S9) is problematic, as the knockout strain is known to exhibit hearing loss associated with abnormal hair cell orientation (4). Notably, heterozygous mice of these strains do not exhibit hearing loss, vestibular problems, or abnormal behavioral features, consistent with the recessive nature of these deficits. The omission of these well-established results raises the question of whether other behavioral phenotypes in the Miller study may reflect sensory deficits rather than depression-related behaviors.

Since our original discovery of *Gpr156* as a gene important for auditory function in mice (4), multiple damaging variants of GPR156 have been identified and associated with congenital hearing loss in humans (5–7). These studies were not considered by Miller et al. and further validate the relevance of GPR156 to inner ear function, reinforcing the significance of the sensory phenotypes to the behavioral assays in mice.

Finally, we have generated a hair cell-specific conditional *Gpr156* knockout strain and demonstrated that hearing and vestibular deficits arise specifically from misoriented sensory hair cells in the inner ear (8). Together with prior work, these findings indicate that inner ear dysfunction complicates the interpretation of several behavioral assays of homozygous mouse models used by Miller et al. We suggest that these sensory phenotypes should be carefully considered when evaluating the behavioral effects attributed to *Gpr156* mutations.

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The authors declare no competing interest.

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