Fingolimod mitigates synaptic deficits and psychosis-like behavior in APP/PSEN1 mice

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Abstract
Introduction: Current treatments for psychosis in Alzheimer’s disease (AD), a syndrome characterized by more rapid deterioration and reduced synaptic protein abundance relative to non-psychotic AD, are inadequate. Fingolimod, a currently US Food and Drug Administration (FDA)–approved pharmacotherapy for multiple sclerosis, alters synaptic protein expression and warrants preclinical appraisal as a candidate pharmacotherapy for psychosis in AD.

Methods: Presenilin and amyloid precursor protein transgenic mice (APPswe/PSEN1dE9) and wild-type mice were randomized to fingolimod or saline for 7 days. Psychosis-associated behaviors were quantified by open field testing, pre-pulse inhibition of the acoustic startle response testing, and habituation of the acoustic startle response testing. Synaptic proteins were quantified by liquid chromatography/mass spectrometry in homogenate and postsynaptic density fractions.

Results: Fingolimod treatment increased the synaptic protein abundance in cortical homogenates and normalized psychosis-associated behaviors in APPswe/PSEN1dE9 mice relative to saline. Mitochondrial-related proteins were preferentially altered by fingolimod treatment and correlated with improvements in psychosis-associated behaviors.

Discussion: Preclinical studies employing complementary psychosis-associated behavioral assessments and proteomic evaluations across multiple AD-related models are warranted to replicate the current study and further investigate fingolimod as a candidate treatment for psychosis in AD.

KEYWORDS
Alzheimer’s disease, fingolimod, proteomics, psychosis, synapse

1 BACKGROUND

Psychosis is a common symptom in Alzheimer’s disease (AD).1 When present, psychosis identifies an AD phenotype with more rapid cognitive and functional decline, greater risk of institutionalization, and earlier death relative to AD without psychosis (reviewed in2). The occurrence of psychosis in AD is highly heritable, suggesting that psychosis in AD arises from a distinct underlying biology.2 Postmortem

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Fingolimod enters the nucleus and acts as a histone deacetylase inhibitor, broadly altering the transcription of genes such as Rap2 and GRIA1 involved in synaptic transmission and formation.20–22 Given the broad loss of synaptic proteins observed in psychotic subjects with AD,3 these in vitro findings evoke a mechanistic foundation on which to test fingolimod’s effects in preclinical models of psychosis in AD. Although prior reports have demonstrated fingolimod’s beneficial effects on learning and memory in rodent models of cerebral amyloidosis,19 none to our knowledge have been designed to test its effects on psychosis-associated behaviors.

We therefore undertook to determine the effects of fingolimod treatment on psychosis-associated behavioral deficits and the synaptic proteome in the APPswe/PSEN1dE9 (APP/PSEN1) mouse model of amyloid beta overproduction. Twelve-month-old APP/PSEN1 mice demonstrated elevated psychosis-associated behaviors relative to wild-type (WT) mice. Intrapertoneal fingolimod administered for 7 days to APP/PSEN1 mice rescued psychosis-associated behavioral deficits relative to APP/PSEN1 littermates treated with saline. After behavioral assessment, WT and APP/PSEN1 mice were killed for evaluation of fingolimod’s effects on the synaptic proteome in homogenate and postsynaptic density (PSD) fractions. We detected an increased
overall abundance of synaptic proteins in cellular homogenate from APP/PSEN1 mice treated with fingolimod relative to saline-treated APP/PSEN1 mice. Functional annotation analysis of the most significantly altered proteins by fingolimod treatment in homogenates from APP/PSEN1 mice identified significant enrichment for a cluster of mitochondrial-related proteins whose abundance correlated with improvement in psychosis-associated behaviors.

2 | METHODS

2.1 | Animals

The Institutional Animal Care and Use Committee at University of Pittsburgh approved all experiments with adherence to National Institutes of Health guidelines for laboratory animal care. APP/PSEN1 mice were developed by Dr. Borchelt, McKnight Brain Institute, University of Florida and contain transgenes encoding mutant presenilin and amyloid precursor protein (B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax) as described previously.23 APP/PSEN1 and WT littermates were generated from breeding pairs as described previously (Supplemental Appendix) and allowed to age for 11–12 months.24 The experimenter was blinded to mouse genotype during behavioral assessment, tissue harvesting, and sample preparation.

2.2 | Fingolimod administration

Fingolimod-HCl (Sigma-Aldrich, St. Louis, MO) was dissolved in pharmaceutical grade normal saline at 1 mg/mL. A dose of 5 mg/kg was determined to be a tolerable daily dose over a 7-day period, capable of inducing effects in the brain without inducing non-specific behavioral effects in 3-month-old C57/B16J WT mice (Supplemental Appendix, Figure S1, Table SF) and was chosen, therefore, as the study dose. A treatment duration of 7 days was informed from a prior report indicating that administration of fingolimod for 7 days is sufficient for preferential accumulation in the CNS.16 Injections were administered as described in the Supplemental Appendix. All animals were included for behavioral and proteomics data with the following n per group: saline-treated WT: 10; fingolimod-treated WT: 9; saline-treated APP/PSEN1: 9; fingolimod-treated APP/PSEN1: 10.

2.3 | Behavioral assessment

Behavioral assessments were conducted by one experimenter at one facility between the hours of 9 am and 12 pm (Supplemental Appendix). The timeline of acclimation to testing conditions and behavioral and proteomic assessments is summarized in Figure 1A and 1B. On the fifth injection day, mice were randomly assigned to one of four rectangular open field testing chambers (Med-Associates Inc., Fairfax, VT) for 30 minutes of testing as described previously.24 Data are reported for the first 10 minutes. On the seventh injection day, mice were randomly assigned to one of four startle testing chambers (StartleMonitor, Kinder Scientific, Poway, CA) in which they were first acclimated to 65 dB of background white noise for 5 minutes and exposed to a pseudo-randomized series of trials as described previously.24 Pre-pulse inhibition of the acoustic startle response (ASR) was calculated as the mean startle response across pre-pulse intensities, expressed as a percent of the mean maximum startle response without pre-pulse. Habituation was calculated as the mean startle response of the last five trials relative to the mean startle response of the first five trials, expressed as a percent.

2.4 | Tissue preparation and liquid chromatography/mass spectrometry

Mice were anesthetized with a lethal 150 mg/kg dose of pentobarbital and perfused transcardially with 10 mL ice-cold normal saline before rapid decapitation and brain extraction. The entire right cerebral cortex was isolated from underlying structures by gross dissection and immediately frozen on dry ice in preparation for proteomic studies.

Samples containing the entire right cerebral cortex were organized in a balanced block distribution throughout tissue processing and liquid chromatography/mass spectrometry (LC-MS/MS) with selected reaction monitoring. Each block included samples from all four groups (WT and APP/PSEN1 mice, saline and fingolimod treatment). Samples were prepared for isolation of homogenate and PSD fractions with minor modifications from that which was described previously and assayed by LC-MS/MS (Supplemental Appendix).

Mouse samples were used to generate pooled control samples, which were assayed in tandem with mouse samples to evaluate for variability in sample preparation as described previously.25 A total of 780 peptides were detected in homogenate and PSD fractions. Precision of the assay was high, with a mean peptide quantification coefficient of variation (CV) of 14.7% in homogenate and 18.0% in PSD in pooled control samples. A median of 2 peptides (range = 1–7) was detected for a given protein. For proteins with more than one peptide, the peptide with the lowest CV in the pooled control samples was selected to estimate protein abundance for subsequent analysis. Peptides with a mean CV of >0.3 in the pooled control samples were eliminated from the analysis, rendering a total of 359 proteins in homogenate and 343 proteins in the PSD.

2.5 | Statistical analysis and detection of psychosis-associated behavioral deficits

Behavioral and proteomic data were analyzed with SPSS. We analyzed behavioral data a priori to examine genotype and treatment effects on a psychosis-associated behavioral summary score as our primary composite outcome, similar to our previous approach.24 In an effort to create a single behavioral outcome on which to test the effect of fingolimod treatment in APP/PSEN1 mice, we
FIGURE 1  Experimental design, timeline of pharmacological intervention and behavioral assessments, and effects of fingolimod treatment on psychosis-associated behavioral deficits in presenilin and amyloid precursor protein transgenic (APP/PSEN1) mice. Wild-type (WT) and APP/PSEN1 mice were treated with fingolimod or saline over 7 days during which behavioral assessments were conducted. On the 7th day of treatment, mice were sacrificed, transcardially perfused with normal saline, and the right cerebral cortex was harvested on dry ice. The right cerebral cortex of each mouse was then homogenized and biochemically fractionated into homogenate and postsynaptic density (PSD) fractions.
identified behavioral impairments for which there were at least trend-level differences (P < .10) in saline-treated APP/PSEN1 mice relative to saline-treated WT mice using analysis of variance (ANOVA) (Figure S2), and then combined these measures into a single psychosis-associated behavioral summary score for each mouse (see Supplemental Appendix for detection of impaired behaviors). Examination of the psychosis-associated behavior summary score—composed of pre-pulse inhibition of the ASR, habituation of the ASR, total distance traveled in open field, and ambulatory episodes in open field—revealed significant impairment in saline-treated APP/PSEN1 mice relative to saline-treated WT mice (F[1,17] = 11.03, P = .004) (Figure S2E). Summary Z scores using component measures were then calculated for fingolimod-treated APP/PSEN1 mice for comparison with those from saline-treated APP/PSEN1 mice using ANOVA to evaluate for a treatment effect. ANOVA was also used to evaluate for the effects of fingolimod treatment on component measures in APP/PSEN1 mice. Further exploratory analyses with all four groups were conducted using ANOVA and the least significant difference (LSD) post hoc test to identify the non-specific effects of fingolimod treatment in WT mice and to evaluate the degree of behavioral effect in fingolimod-treated APP/PSEN1 mice relative to saline-treated WT mice.

Proteomics data were analyzed as described previously.3 One-sample t-tests of raw protein abundance ratios were conducted to evaluate for shifts in distributions of PSD-enriched protein levels. The PSD-enriched proteome, defined as a ratio of protein abundance in PSD/homogenate >1.5 (Table SA), was evaluated in APP/PSEN1 mice for fixed effects of treatment, cellular compartment, and treatment x cellular compartment interaction using ANOVA. Corrected false discovery rate (FDR) values (Q values) were generated for PSD-enriched protein levels in homogenate and PSD fractions using the Benjamini-Hochberg method,26 with a significance threshold of Q < 0.05 for comparison between saline-treated WT and APP/PSEN1 groups (Table SB) and between saline and fingolimod treatment groups in APP/PSEN1 mice (Table SC). DAVID 6.8 Functional Annotation Tool27,28 was used to evaluate for functional enrichment of the most altered proteins by fingolimod treatment in APP/PSEN1 mice, defined as those proteins in fingolimod-treated APP/PSEN1 mice relative to saline-treated APP/PSEN1 mice with a liberalized Q value <0.2 using the Benjamini-Hochberg method (Table SD). To increase stringency, using DAVID 6.8 default settings, functional annotation analysis was conducted against a background of the 359 proteins assayed in homogenate rather than against the entire genome.

3 | RESULTS

3.1 | Psychosis-associated behaviors

Fingolimod treatment in APP/PSEN1 mice significantly attenuated psychosis-associated behavioral deficits relative to saline treatment (F[1,17] = 8.54, P = .010) (Figure 1C). For component measures, there were significant effects of fingolimod treatment in APP/PSEN1 mice on pre-pulse inhibition (F[1,17] = 16.49, P = .001) (Figure 1D) and habituation of the ASR (F[1,17] = 6.11, P = .024) (Figure 1E). There were no effects of fingolimod treatment on total distance traveled (F[1,17] = 2.10, P = .101) or on number of ambulatory episodes (F[1,17] = 0.20, P = .660) (Figure 1F,G, respectively). Post hoc comparisons revealed that fingolimod treatment in APP/PSEN1 mice attenuated psychosis-associated behavioral deficits to a level that did not differ from saline-treated WT mice (mean difference = 0.26, confidence interval [CI] = −0.39, 0.91; P = .425). There were no effects of fingolimod treatment in WT mice, either on the psychosis-associated behavior summary score or any component behavioral measure (data not shown), suggesting fingolimod’s disease specificity.

3.2 | Synaptic proteome

We next sought to determine the effect of fingolimod treatment on the synaptic proteome in homogenate and PSD fractions from APP/PSEN1 and WT mice. Given our prior data indicating that resilience to psychosis in AD is associated with an increased abundance of synaptic proteins, particularly those that are normally enriched within the PSD,3 we first examined the abundance of PSD-enriched proteins. No individual PSD-enriched protein was significantly altered in saline-treated APP/PSEN1 mice compared with saline-treated WT mice after adjustment for multiple comparisons (Table SB). However, there was an overall significant reduction in the abundance of synaptic proteins, both in homogenate (mean [SD] = 0.96[0.05], P = < .001) (Figure 2A) before conducting liquid chromatography/mass spectrometry (LC-MS/MS) with selected reaction monitoring of a previously described panel25,49 of synthetically localized proteins (A). WT and APP/PSEN1 mice were given intraperitoneal injections of fingolimod or saline on days 1 to 7. On day 4, mice were exposed to the open field test (OFT) room prior to undergoing the OFT on day 5. Following the OFT on day 5, mice were exposed to the room in which acoustic startle response (ASR) testing occurred. On day 6, mice were exposed to the ASR restraint apparatus prior to ASR testing on day 7. After ASR testing on day 7, mice were sacrificed and perfused with normal saline immediately prior to brain extraction and subsequent proteomics evaluation (B). Fingolimod treatment in 12-month-old APP/PSEN1 mice reduces psychosis-associated behaviors relative to saline treatment in APP/PSEN1 mice of the same age (C). Component measures of the psychosis-associated behavioral summary score, the selection of which is informed by behaviors that were found to be significantly impaired in saline-treated APP/PSEN1 mice relative to saline-treated WT mice (Figure S2) are contained in (D–G). Fingolimod treatment in APP/PSEN1 mice significantly enhanced pre-pulse inhibition of the ASR (D) and habituation of the ASR (E) relative to saline treatment. There was no effect of fingolimod treatment relative to saline treatment in APP/PSEN1 mice on measures of locomotor hyperactivity in the OFT, including total distance traveled (F) and number of ambulatory episodes (G). Error bars denote standard error of the mean.
FIGURE 2 Relative distributions of postsynaptic density (PSD)–enriched protein levels from wild-type (WT) and APP/PSEN1 mice treated with saline or fingolimod. Saline-treated APP/PSEN1 mice contain lower levels of PSD-enriched proteins in homogenate (A) and in the PSD (B) relative to saline-treated WT mice. Direct comparisons of treatments in APP/PSEN1 mice revealed that, relative to saline treatment, fingolimod treatment conferred a significant increase in PSD-enriched proteins in homogenate (C), but no effect on abundance of these proteins in the PSD (D). Fingolimod-treated APP/PSEN1 mice contain increased overall abundance of PSD-enriched proteins in homogenate (E), but lower levels of PSD-enriched proteins in the PSD (F) compared with saline-treated WT mice.

and in the PSD (mean [SD] = 0.89[0.10], P = <.001) (Figure 2B) of saline-treated APP/PSEN1 mice compared with saline-treated WT mice. A comparison of PSD-enriched protein levels between fingolimod and saline treatment in APP/PSEN1 mice (Table SC) revealed broadly elevated levels of PSD-enriched proteins with fingolimod treatment in homogenate (mean [SD] = 1.07[0.07], P = <.001) (Figure 2C), but no treatment effect in the PSD (mean [SD] = 1.04[0.21], P = .095) (Figure 2D). Relative to saline-treated WT mice, fingolimod treatment in APP/PSEN1 mice increased levels of PSD-enriched proteins in homogenate (mean [SD] = 1.02[0.09], P = .003) (Figure 2E). However, there still existed within the PSD a relative reduction in the abundance of PSD-enriched proteins in fingolimod-treated APP/PSEN1 mice relative to saline-treated WT mice (mean [SD] = 0.91[0.15], P = <.001) (Figure 2F). The differential effect of fingolimod on the mean abundance of PSD-enriched proteins in homogenate and PSD fractions was significant (treatment x compartment interaction: F[1,388] = 4.44, P = .036; Figure 3).

Given that fingolimod appeared to exert its effects in homogenate rather than in the PSD, we expanded our analysis of homogenate proteins to include those that were not PSD-enriched to uncover the functional character of proteins whose levels were increased by fingolimod treatment. We identified proteins whose mean levels
Mitochondria-related proteins were most increased by fingolimod relative to saline treatment in APP/PSEN1 mice with an FDR <0.2 (Table SD Supplemental Figure S3). We then conducted functional annotation analysis against a background of all 359 proteins assayed in homogenate (Table 1), and detected significant enrichment for a set of 39 proteins involved in mitochondrial function (see Table SE for the entire functional annotation chart of enrichment terms with $P \leq 0.05$). Finally, to explore whether this cluster of mitochondria-related proteins was associated with the severity of psychosis-associated behavior in APP/PSEN1 mice, we calculated for each mouse the mean abundance level of the 39 mitochondria-related proteins for correlation with the psychosis-associated behavior summary score of each mouse. Greater abundance of mitochondria-related proteins levels correlated with reduced expression of psychosis-associated behavior ($r = -0.57$, $N = 19$, $P = .011$; Figure 4).

## 4 | DISCUSSION

Fingolimod is a structural mimetic of sphingosine, which is generated from the cleavage of sphingosylphosphorylcholine by ENPP6, a phosphodiesterase, the gene of which was one of only two genes that contained single nucleotide polymorphisms (SNPs) of genome-wide significance to AD with psychosis in a recent genome-wide meta-analysis. Within brain, ENPP6 is expressed mainly in oligodendrocytes and the abundance of its corresponding RNA is at its maximum during early differentiation of mature oligodendrocytes from oligodendrocyte precursor cells (OPCs), a process that is reportedly impaired in schizophrenia and in neurodegenerative diseases including AD (reviewed in 31). Although there is no evidence from our current study that fingolimod exerted effects on OPCs or oligodendrocytes, other in vitro studies have demonstrated that treatment with either sphingosine or low-dose fingolimod enhances differentiation from OPCs to mature oligodendrocytes. One potential mechanism by which sphingosine receptor signaling may support OPC differentiation may involve regulation of mitochondrial function, as OPC differentiation is a process of high metabolic demand and high vulnerability to oxidative stress, during which mitochondrial DNA relative to nuclear DNA increases 4-fold and the OPC proteome becomes enriched with proteins involved in metabolism. Although our discovery that fingolimod increased the abundance of mitochondrial-related proteins may be relevant to previous studies that highlight the importance of sphingosine receptor signaling during oxidative stress, future studies designed to test fingolimod’s direct effects on oligodendrocytes and their precursors are required prior to considering enhanced conversion of OPCs to oligodendrocytes as a potential mechanism for fingolimod’s effects on psychosis-associated behaviors and synaptic protein abundance in APP/PSEN1 mice.

Our findings suggest that alterations in mitochondrial-related proteins may be a potential mechanism by which fingolimod reduced psychosis-associated behavioral deficits in APP/PSEN1 mice. Other studies have provided more downstream evidence of fingolimod’s effects on mitochondrial function in rodent models of other CNS diseases. For example, one study using a two-vessel occlusion rat model for chronic cerebral hypoperfusion demonstrated that hippocampi from rats treated with 7 weeks of 1 mg/kg fingolimod relative to saline contained reduced malondialdehyde levels concurrent with increased adenosine triphosphate (ATP) content and ATP-synthase activity, during which mitochondrial DNA relative to nuclear DNA increases 4-fold and the OPC proteome becomes enriched with proteins involved in metabolism. Perturbations in mitochondrial function are well appreciated in AD and in primary psychotic disorders such as schizophrenia, although, to our knowledge, specific alterations in mitochondrial function have not been explored previously as a potential biologic correlate to AD with psychosis. However, recent evidence suggests that phosphorylated tau at paired helical filament-1 (PHF-1) sites accumulates in synaptic mitochondria and induces mitochondrial dysfunction, a finding of particular relevance to psychosis in AD given that increased abundance of fibrillar tau in cerebral cortex is perhaps the most well-replicated neuropathologic correlate of AD with psychosis relative to AD without psychosis. Furthermore, given the known contribution of impaired mitochondrial function to altered proteostasis in subjects with psychiatric symptoms in other neurodegenerative disorders (reviewed in 32), as well as our previous findings that AD subjects with psychosis lack the compensatory increase in synaptic protein abundance that is present in AD subjects without psychosis, altered mitochondrial dynamics may represent an upstream source for synaptic alterations in AD with psychosis.

There are limitations inherent in the use of the APP/PSEN1 mouse to model the neuropathologic, synaptic, and behavioral deficits present in the psychotic AD phenotype. Our selection of the APP/PSEN1
| Category            | Term                                      | Count | Enrichment Score | Unadjusted P-value | Benjamin-Adjusted P-value |
|---------------------|-------------------------------------------|-------|------------------|---------------------|--------------------------|
| GOTERM_CC_DIRECT    | Mitochondrion                             | 39    | 1.6              | 1.17E-04            | .01                      |
| GOTERM_CC_DIRECT    | Mitochondrial inner membrane              | 16    | 2.2              | 5.24E-04            | .04                      |
| KEGG_PATHWAY        | Parkinson disease                         | 12    | 2.5              | 6.34E-04            | .11                      |
| KEGG_PATHWAY        | Biosynthesis of antibiotics               | 18    | 1.9              | 1.71E-03            | .15                      |
| GOTERM_CC_DIRECT    | Myelin sheath                             | 30    | 1.6              | 2.25E-03            | .14                      |
| GOTERM_BP_DIRECT    | Gluconeogenesis                           | 10    | 2.6              | 3.27E-03            | 1.00                     |
| KEGG_PATHWAY        | HTLV-I infection                          | 8     | 2.8              | 4.54E-03            | .25                      |
| KEGG_PATHWAY        | Carbon metabolism                         | 15    | 1.9              | 5.60E-03            | .25                      |
| KEGG_PATHWAY        | cGMP-PKG signaling pathway                | 13    | 2.0              | 8.67E-03            | .26                      |
| KEGG_PATHWAY        | Metabolic pathways                        | 28    | 1.5              | 8.75E-03            | .26                      |
| KEGG_PATHWAY        | Calcium signaling pathway                 | 14    | 1.8              | .02                 | .45                      |
| GOTERM_CC_DIRECT    | Mitochondrial matrix                      | 12    | 1.9              | .02                 | .8                       |
| KEGG_PATHWAY        | Oxidative phosphorylation                 | 8     | 2.3              | .02                 | .53                      |
| KEGG_PATHWAY        | Pyruvate metabolism                       | 6     | 2.7              | .03                 | .56                      |
| KEGG_PATHWAY        | Cysteine and methionine metabolism        | 5     | 3.2              | .03                 | .56                      |
| KEGG_PATHWAY        | Biosynthesis of amino acids               | 9     | 2.0              | .03                 | .56                      |
| GOTERM_BP_DIRECT    | Tricarboxylic acid cycle                  | 8     | 2.2              | .04                 | 1.00                     |
| KEGG_PATHWAY        | Huntington disease                        | 12    | 1.7              | .04                 | .62                      |
| GOTERM_BP_DIRECT    | Protein stabilization                     | 7     | 2.3              | .04                 | 1.00                     |
| GOTERM_CC_DIRECT    | Extracellular matrix                      | 10    | 1.9              | .05                 | 1.00                     |

Note: Proteins whose abundances were most altered by fingolimod relative to saline treatment in presenilin and amyloid precursor protein transgenic (APP/PSEN1) mice with a false discovery rate (FDR) < 0.2 were identified and evaluated for enrichment of functional annotation terms using the Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 Functional Annotation Chart Tool27,28 with default settings. Evaluation for enrichment was conducted against a background of the 359 proteins assayed in cellular homogenate rather than against a background of the entire genome to increase stringency. Gene ontology (GOTERM) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms with an unadjusted P-value < .05 and with enrichment scores > 1.5 are included in the Table 1. The entire functional annotation chart with additional enrichment categories along with gene accession numbers contributing to enrichment category with unadjusted P-value < .05 is contained in Table SE. Two terms, both related to mitochondrial cellular components, were significantly enriched by fingolimod treatment in APP/PSEN1 mice after adjustment for false discovery.

Abbreviations: GOTERM_CC_DIRECT, Gene Ontology Cellular Compartment terms; KEGG_PATHWAY, Kyoto Encyclopedia of Genes and Genomes terms; GOTERM_BP_DIRECT, Gene Ontology Biological Process terms; HTLV-1, Human T-Lymphotropic Virus Type 1; cGMP, Cyclic guanosine 3′, 5′-monophosphate; PKG, Protein Kinase G.

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model was informed by our prior studies, which indicated resemblance between the synaptic proteomes of APP/PSEN1 mice and AD subjects with psychosis,3 and the model’s ability to express high levels of behaviors that are associated with psychotic symptoms.24 However, this model fails to accumulate significant fibrillar tau pathology (reviewed in43), an important neuropathologic marker, the abundance of which is increased in psychotic AD subjects relative to non-psychotic AD subjects.3 In addition, although we have demonstrated that the APP/PSEN1 model exhibits high levels of psychosis-associated behaviors relative to WT mice, the ability of these behaviors to predict human response to treatment is unclear. For example, despite findings from a preclinical study44 that demonstrated significant effects of pimavanserin on locomotor hyperactivity and pre-pulse inhibition of the ASR deficits in a mouse model of cerebral amyloidosis, a large, randomized double-blind placebo-controlled trial found no effect of pimavanserin on psychotic symptoms in AD after 12 weeks of treatment.45 One potential explanation for the limited capacity of preclinical pharmacologic studies in such murine models to predict clinical response in humans derives from data indicating that risk and resilience to behavioral and neuropathologic markers in AD-related mouse models is in part mediated by background genetic variability.46 Ultimately, given the phenotypic variability and multifactorial causality of psychosis in AD, no single mouse model or set of mouse behaviors will likely be capable to entirely represent this uniquely human syndrome.43 We also note that in this study, only mice of male sex were used because our previous study that detected elevated psychosis-associated behaviors at 12 months of age utilized male mice.24 Previous work has indicated considerable sex differences in the
Mitochondrial protein levels correlate with behavioral deficits in APP/PSEN1 mice. The mean abundance of mitochondrial-related proteins identified by pathway analysis was calculated for correlation with the psychosis-associated behavioral summary Z score for each mouse. The dashed line represents the regression trend line for both treatment groups. A significant, moderate strength correlation is present.

In summary, we demonstrated that fingolimod treatment in APP/PSEN1 mice attenuates psychosis-associated behavioral deficits and increases the abundance of synaptic proteins in cortical homogenates relative to saline treatment. Given the lack of high-quality animal models for psychosis in AD and the debated relevance of psychosis-associated behaviors in mice, additional studies using complementary behavioral tasks and molecular measures across multiple models of AD-related pathology are required prior to considering fingolimod as a candidate pharmacotherapy for treatment of psychosis in AD.

ACKNOWLEDGMENTS
The authors would like to thank Dr. Ying Ding for her assistance in providing balanced randomization for mouse samples during tissue processing and proteomic assessment and generating Benjamini-Hochberg adjusted Q values for synaptic protein levels. The University of Pittsburgh holds a Physician-Scientist Institutional Award from the Burroughs Welcome Fund (JMK). This work was supported by National Institutes of Health (NIH) grants MH116046 (RAS) and K01 MH107756 (MLM). This project used the Hillman Biomedical Mass Spectrometry Core, which is supported in part by award P30CA047904.

CONFLICT OF INTEREST
Author disclosures are available in the supporting information.
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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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**How to cite this article:** Krivinko JM, Erickson SL, MacDonald ML, Garver ME, Sweet RA, et al. Fingolimod mitigates synaptic deficits and psychosis-like behavior in APP/PSEN1 mice. *Alzheimer’s Dement.* 2022;8:e12324. https://doi.org/10.1002/trc2.12324