Moderate ethanol consumption interacts with Aβ to exacerbate Alzheimer's-like pathological, behavioral, and metabolic deficits in mice

Stephen C. Gironda*[1,2], Stephen M. Day*[1], Caitlin W. Clarke[1], J. Andy Snipes[1], Noelle I. Nicol[3], Hana Kamran[3], Warner Vaughn[3], Shannon L. Macauley[†1,3], Jeff Weiner[†1]

*Indicates co-first-author; [†]Indicates co-last author

[1]Department of Physiology & Pharmacology, Wake Forest School of Medicine, Winston Salem NC, United States, [2]Department of Neurobiology & Anatomy, Wake Forest School of Medicine, Winston Salem NC, United States, [3]Section on Gerontology & Geriatric Medicine, Department of Internal Medicine, Wake Forest School of Medicine, Winston Salem NC, United States

Abstract

Alcohol use disorder is a risk factor for Alzheimer's disease (AD), yet the mechanisms underlying this relationship are poorly understood. Here, we employed the APP/PS1 mouse model of Aβ pathology to examine the effects of a 10 week moderate ethanol drinking paradigm on AD-related phenotypes. Ethanol drinking led to a circadian shift in feeding behavior and glucose intolerance in APPswe/PSEN1dE9 (APP/PS1), but not wild-type or ethanol-naïve APP/PS1 mice. Ethanol exposure also selectively disrupted nest building, a metric of self-care, and decreased brain mass in APP/PS1 mice. While there was a trend towards increased cortical Aβ deposition in APP/PS1 mice, ethanol exposure shifted the distribution of plaque size and numbers, increasing the number of smaller plaques. Lastly, we used in vivo microdialysis to determine if acute ethanol directly affected hippocampal interstitial fluid (ISF) Aβ levels in unanesthetized APP/PS1 mice. Aβ levels were bidirectionally modulated by acute ethanol - decreasing as brain ethanol levels rose and increasing above baseline during withdrawal. Collectively, these studies provide the first evidence that acute ethanol alters ISF Aβ levels and suggest that even modest levels of chronic ethanol consumption interact with Aβ to exacerbate AD-related pathology, behavioral dysfunction, and metabolic deficits.

Introduction

Alzheimer's disease (AD) is responsible for 60-80% of dementia cases and is the most common form of dementia. In the US, there are approximately 6 million people diagnosed with AD, which is expected to increase to 14 million by 2050 [1]. According to the A/T/N framework, AD pathology is characterized by the aggregation of extracellular amyloid-β (Aβ) into amyloid plaques, the intracellular accumulation of tau into neurofibrillary tangles, and neurodegeneration [2]. Aβ aggregation and other pathological events precede the onset of cognitive decline and clinical diagnosis by ~10-20 years [3], making it important to identify risk factors that accelerate the onset, hasten the development, or exacerbate the pathological changes in AD. Epidemiological studies have identified alcohol use disorder (AUD) as a significant risk factor for AD [4-7], yet there is conflicting evidence on how alcohol use directly impacts AD pathogenesis. Moreover, some studies have suggested that lower levels of alcohol (ethanol) consumption may actually reduce AD risk [8]. Preclinical studies suggest that chronic administration of low, moderate, and high levels of ethanol can affect amyloid plaque pathology and amyloidogenic processing in multiple models of Aβ overexpression [9, 10], yet differences in methodology make the results difficult to interpret. Therefore, questions remain as to whether ethanol exposure directly modulates Aβ levels, resulting in amyloid plaque pathology and its associated behavioral and metabolic deficits.

Recognizing this significant gap in knowledge and a critical need to better understand the relationship between AUD and AD, we sought to investigate how chronic ethanol exposure alters behavioral and metabolic disturbances associated with AD pathogenesis. Here, we used a well-validated mouse model of Aβ overexpression (APPswe/PSENdE9; APP/PS1) [11] to examine how long-term consumption of moderate amounts of alcohol alters AD-related pathology, emotion-like and cognitive behaviors, and metabolism. Long-term consumption of moderate amounts of ethanol via a two-bottle choice drinking paradigm led to increased Aβ pathology and exacerbated behavioral and metabolic disturbances in APP/PS1 mice. Using in vivo microdialysis, we also directly measured how acute systemic ethanol administration impacts the concentrations of ethanol, Aβ, and glucose in the

hippocampus of live APP/SP1 mice. We found that chronic, moderate ethanol exposure with at 2-bottle choice paradigm led to increased Aβ pathology and exacerbated behavioral and metabolic disturbances in APP/PS1 mice. Using in vivo microdialysis, we also found that the acute, systemic delivery of ethanol led to a rapid rise in interstitial fluid (ISF) ethanol levels in the hippocampus that lasted for 6 hours. While ISF ethanol was high, ISF Aβ levels decreased. Conversely, as ISF ethanol was cleared from the brain during withdrawal, ISF Aβ levels increased by nearly 20%. This demonstrates that ethanol exposure can bidirectionally modulate ISF Aβ during acute exposure and withdrawal. Collectively, this study provides the first evidence that acute ethanol modulates Aβ levels and lends additional support for the notion that chronic consumption of even moderate amounts of ethanol may exacerbate the development of AD-related symptoms.

Results

APP/PS1 mice consume more ethanol than control mice

We first sought to characterize ethanol drinking behavior in APP/PS1 mice, since an earlier study reported an initial increase in ethanol consumption in 3xTg-AD mice [10]. First, we evaluated cumulative differences in ethanol consumption and preference in APP/PS1 and WT mice. Over the course of the 10 week study, APP/PS1 mice consumed more ethanol than wild-type mice (Figure 1b, $p=0.0047$). APP/PS1 mice consumed greater amounts of ethanol early in the study, which may have contributed to the differences in overall consumption (Figure 1c). Interestingly, APP/PS1 and wild-type mice displayed a similar ethanol preference over the course of the study (Figure 1d-e). Ethanol-exposed wild-type and APP/PS1 mice consumed similar amounts of water throughout the course of the study (Figure 1f-g). Conversely, ethanol-naïve APP/PS1 mice consumed greater amounts of H_2O each week, resulting in a cumulative increase in water consumption (Figure 1h&i, $p < 0.0001$). Together, these data

indicate that APP/PS1 mice exhibit a modest and transient increase in ethanol intake relative to wild-type mice.

Ethanol treatment promotes neurodegeneration in APP/PS1 mice

Neurodegeneration is a major component of AD pathology and AUD [2, 8]. Therefore, we explored the effects of ethanol self-administration on brain atrophy in APP/PS1 and wild-type mice by measuring brain mass after the 10 week ethanol self-administration regimen. APP/PS1 mice had decreased brain mass compared to wild-type mice and this effect was exacerbated by ethanol consumption (Figure 2b). Interestingly, there were no differences in cortical thickness (Figure 2c) or in hippocampal area (Figure 2d), suggesting that other brain regions were subjected to brain atrophy due to ethanol consumption.

Ethanol treatment increases the frequency of smaller amyloid plaques

Previous studies have shown that chronic ethanol consumption, at high levels, exacerbates AD-like pathology [9, 10]. Therefore, we sought to measure how a moderate drinking paradigm affected amyloid pathology. Immunohistochemical staining for Aβ deposition was performed in ethanol-naïve and ethanol-exposed APP/PS1 mice (Figure 2a). Aβ deposition, amyloid plaque size, and amyloid plaque number were quantified by a blinded researcher. Ethanol treatment led to a trend towards increased plaque area fraction in the cortex (Figure 2e, $p=0.0762$), but not in the hippocampus (Figure 2h). Ethanol self-administration also resulted in a trend towards increased plaque number in the cortex (Figure 2f, $p = 0.0992$) and a significant increase in the hippocampus (Figure 2i, $p<0.05$). Because ethanol consumption led to a difference in the number of plaques, we next tested whether there were differences in the distribution of plaque sizes. Ethanol self-administration was associated with a greater amount of smaller plaques in the cortex (Figure 2g, Supplementary Table 2) and hippocampus (Figure 2j Supplementary Table 1). This demonstrates that a moderate ethanol drinking paradigm impacts Aβ pathology by generating smaller plaques. This may represent an intermediate stage of plaque

proliferation that becomes more pronounced with ethanol exposure regimens that promote higher blood ethanol levels [9] or greater amounts of daily ethanol consumption [10]. Additional studies will be needed to assess whether these changes in plaque distribution will lead to increased Aβ pathology over time.

Ethanol exposure dysregulates circadian food consumption in APP/PS1 mice

AD and alcohol use disorder are both characterized by disruptions in circadian rhythms [12, 13]. Thus, we measured food consumption every 12 hours across the circadian cycle on drinking days for four weeks, beginning when mice were 7 months old[Figure 3a-c]. Since mice are nocturnal, they consume the majority of their food during their dark cycle. While food consumption was highest at night for all groups, ethanol-drinking APP/PS1 mice consumed less food during this period than all other groups (Figure 3b). In contrast, ethanol-drinking APP/PS1 mice consumed more food during their light cycle (Figure 3c). Interestingly, this effect was not observed in ethanol-drinking wild-type mice as no effect of ethanol consumption was observed across the circadian day (Figure 3b-c). Food consumption across the circadian day was similarly unchanged in APP/PS1 control mice (Figure 3b-c). This circadian misalignment with food intake suggests that chronic ethanol consumption may disrupt sleep, specifically in mice with Aβ overexpression.

Chronic ethanol exposure alters glucose metabolism in APP/PS1 mice

Because AUD leads to metabolic impairment and peripheral hyperglycemia leads to increased brain ISF Aβ levels [14], we performed a glucose tolerance test in 7.5 month-old mice after 9 weeks of ethanol exposure. Fasted blood glucose levels were lower in ethanol naïve APP/PS1 mice compared to wild-type mice (Figure 3d), however this trend was reversed with ethanol exposure. Interestingly, chronic ethanol consumption led to glucose intolerance, a marker of metabolic dysfunction and insulin resistance, in EtOH-exposed APP/PS1 mice. This effect was not observed in wild-type mice. Given these differences in peripheral glucose metabolism, we investigated how ethanol exposure affected

body weights at the beginning and end of the study. Ethanol-naïve wild-type mice began the study with higher body weights, however these differences were no longer significant at the end of the study (Figure 3f-g). Taken together these data indicate that moderate levels of chronic ethanol drinking induces metabolic dysfunction in APP/PS1 mice, independent of changes in body weight or elevated fasted blood glucose.

Chronic ethanol consumption alters anxiety- and dementia related behaviors in APP/PS1 mice

Lower CSF Aβ42 was associated with anxiety scores in individuals with mild cognitive impairment as well as cognitively normal individuals over the age of 50 [15]. Chronic ethanol exposure can also lead to increased anxiety-related behaviors and deficits in cognition. Anxiety-related behaviors were measured using open field analysis (OFA) and the light/dark test (LD) before and after ethanol exposure, while marble-burying, OLM, and nest building were only performed after ethanol exposure. At baseline, APP/PS1 mice showed a trend towards increased locomotor activity during the OFA (Figure 4a, $p = 0.0668$), but not in the percent time spent in the center zone (Figure 4b). After 3 weeks of ethanol exposure there was increased locomotor activity in ethanol-exposed APP/PS1 mice (Figure 4a), as well as a difference between groups in the time they spent in the center zone (Figure 4b). Post-hoc tests showed that ethanol-drinking APP/PS1 mice spent more time in the central zone than wild-type mice. No differences in behavior were observed in LD at baseline, or after 4 weeks of ethanol self-administration (Figure 4c). After 5 weeks of ethanol drinking, mice were tested using the marble burying test, where increased marble burying is used as a measure of anxiety-like behavior. In this test, mice are individually housed in novel cages with 12 marbles. Control APP/PS1 mice buried fewer marbles than wild-type mice, while ethanol-drinking APP/PS1 mice did not (Figure 4d). This suggests that APP/PS1 mice may be modestly more sensitive to the anxiolytic effects of ethanol. After 7 weeks of ethanol treatment, the OLM task evaluated the effects of ethanol treatment on memory. As expected, ethanol-naïve wild-type mice spent more time interacting with the relocated object than with the object in the familiar location (Figure 4e, $p = 0.0078$). Conversely, APP/PS1 mice and ethanol-exposed wild-

type mice spent similar amounts of time investigating both objects (Figure 4e). This indicates that impaired memory due to Aβ pathology was not significantly exacerbated by ethanol exposure, however this may be due to a ceiling effect as APP/PS1 control mice exhibit maximal memory impairment. Lastly, after the final night of ethanol treatment in week 10, mice were provided with fresh bedding material to build a new test. 12 hours later, nests were scored on a 5-point scale to determine each group's degree of self-care related behaviors. APP/PS1 mice showed deficits in nest building, which was exacerbated by ethanol exposure (Figure 4f). Conversely, ethanol treatment had no effect on nest building behavior in wild-type mice. This further demonstrates that ethanol exposure exacerbates anxiety and AD-related behavioral deficits.

Acute ethanol exposure directly affects Alzheimer's-related pathology and brain metabolism

Having demonstrated that a chronic ethanol drinking paradigm was sufficient to alter AD-like pathology and peripheral metabolism, we used in vivo microdialysis to determine whether ethanol directly modulates Aβ and brain metabolism in unrestrained, unanesthetized APP/PS1 mice. An acute dose of ethanol (3.0 g/kg, ip) led to a rapid increase in ISF ethanol levels (14.79±1.73 mmol/L), which declined over the next 6 hours (Figure 5b-c, supplementary Table 3). Interestingly, ethanol exposure caused bidirectional fluctuations in ISF Aβ levels (Figure 5b). Following the acute ethanol exposure, ISF Aβ levels dropped well below baseline (-15.82% ± 1.58% below baseline), then rose rapidly and peaked at 6 hours post-ethanol (20.07% ± 6.83% above baseline; Figure 5b, supplementary table 3). Conversely, ethanol exposure led to an increase in ISF glucose levels in the first hour, which returned to baseline over the next 6 hours (Figure 5d, supplementary table 3). ISF ethanol and ISF glucose levels were significantly correlated during the 6 hour time period that ethanol was present in the brain (Figure 5e, $r = 0.6921$, $p = 0.0068$). This data has two important implications. First, it demonstrates that when ISF ethanol is elevated, ISF glucose is elevated as well. This could explain, in part, the glucose intolerance phenotype seen in the chronic study, where ethanol causes increased extracellular levels of glucose (Figure 3d). Second, this data also suggests that when the hippocampus is exposed to

ethanol, less ISF Aβ is released. However, when ethanol is cleared from the brain, the brain's response during withdrawal drives ISF Aβ release and increases the extracellular pool of Aβ. Thus, acute ethanol is sufficient to raise ISF Aβ levels and concurrently dysregulate brain metabolism.

Discussion

In this study, we found that long-term moderate ethanol self-administration increases AD-related pathology, alters peripheral metabolism, and exacerbates behavioral deficits in APP/PS1 mice. Whether it is related to alterations in pathology, metabolism, or behavior, we consistently found that ethanol exposure exacerbated affected phenotypes in APP/PS1 mice compared to wild-type controls. This suggests that early changes in Aβ pathology synergize with ethanol exposure to potentiate damage to the brain. We also found with acute ethanol experiments that ethanol exposure and withdrawal can bidirectionally modulate Aβ levels within the hippocampal ISF in vivo, providing the first evidence that even a single exposure to ethanol can modulate a protein in vivo known to play an integral role in the pathology related to AD and other forms of dementia. These findings build upon existing studies demonstrating that ethanol exposure, even at moderate doses, is sufficient to modulate AD-related pathology and behavioral phenotypes.

In humans, amyloid pathology begins to accumulate ~10-20 years before the onset of clinical symptoms [3]. In APP/PS1 mice, Aβ begins to aggregate into plaque from 6-9 months of age. Previous studies consistently show that chronic ethanol exposure is sufficient to increase amyloid burden in mouse models of cerebral amyloidosis [9, 10]. Here we found that APP/PS1 mice showed signs of increased neurodegeneration, which was exacerbated by chronic consumption of moderate amounts of ethanol (Figure 2b). Interestingly, ethanol drinking significantly decreased brain mass in APP/PS1 mice, but not in wild-type mice. This phenotype could be due to an interaction with the formation of amyloid plaques or entirely independent of AD-like pathology. It is well-documented that AUD can lead to different forms of dementia, including vascular dementia, Parkinson's disease, Korsakoff syndrome, or alcohol-related dementia – all of which display alcohol-related brain damage, neurodegeneration,

and brain atrophy [16]. Future studies will need to tease apart whether the brain atrophy observed in this study was dependent on changes in the Alzheimer's cascade. While changes in amyloid pathology were mixed in this study, there was a clear increase in the number of plaques in ethanol-exposed APP/PS1 mice (Figure 2f, i). Further data analysis revealed that ethanol drinking led to changes in the distribution of plaque sizes, where ethanol exposure increased the number of smaller plaques (Figure 2g, j). These changes could be viewed in two different ways. First, chronic ethanol may exacerbate amyloid pathology by increasing the number of smaller plaques at an age that corresponds to presymptomatic AD. A greater number of smaller plaques could create multiple aggregation sites that attract extracellular Aβ and ultimately result in greater plaque proliferation later in life. Alternatively, the emergence of smaller amyloid plaques at low-to-moderate levels of chronic ethanol consumption could suggest that ethanol restricts plaque growth, leading to smaller plaques. Additional studies are needed to clarify whether the change in plaque size and plaque number is a harmful or protective response to moderate alcohol consumption. However when considering the beneficial effects of moderate ethanol consumption, future studies will need to account for the ethanol-associated reduction in brain mass.

Lastly, we used in vivo microdialysis to measure how ethanol modulates biomarkers of AD-like pathology and brain metabolism in unrestrained and unanaesthetized APP/PS1 mice. Ethanol altered ISF Aβ levels in 3 month-old APP/PS1 mice – decreasing ISF Aβ immediately following an exposure, then increasing ISF Aβ during ethanol clearance or withdrawal (Figure 5b). Ethanol is known to directly modulate neuronal activity, increasing inhibition during exposure and increasing excitation during withdrawal [17-19]. Since Aβ is released from neurons in an activity-dependent manner [20-22], the changes in ISF Aβ may be due to ethanol-induced changes in neuronal activity, specifically hyperexcitability associated with withdrawal. Alternatively, hyperglycemia can also directly increase ISF Aβ levels [14]. Given that acute ethanol exposure leads to a rapid increase in ISF glucose levels (Figure 5d), the changes observed in ISF Aβ levels may be in direct response to brain hyperglycemia, and not changes in neuronal activity. Thus, further studies are needed to elucidate the mechanisms by which ethanol drives these changes in Aβ.

Chronic ethanol consumption can lead to increased anxiety- and depression-related behaviors, which can be further exacerbated by changes in glucose metabolism [23]. Here we found that, during weekly withdrawal periods, ethanol drinking APP/PS1 mice exhibited changes in locomotor activity and central zone exploration in the OFA (Figure 4a-b), but not in LD exploration (Figure 4c). Ethanol did not induce these behavioral changes in wild-type mice, which may indicate that APP/PS1 mice are especially sensitive to anxiety-related behaviors. Alternatively, ethanol drinking may exacerbate the hyperactive, impulsive or compulsive phenotype observed in APP/PS1 mice [24]. Conversely, ethanol treatment did not aggravate the deficits in the marble-burying task observed in APP/PS1 controls (Figure 4d). Thus, further tests should be conducted to better understand the behavioral phenotypes reported here.

Ethanol's effects on cognition are well-documented [25] and our results indicate that chronic ethanol drinking may be detrimental to long-term memory in wild-type mice. In the object location memory task (OLM), ethanol exposure disrupted memory in wild-type mice, but had no effect on APP/PS1 mice (Figure 4e). Future studies should employ more sensitive cognitive tasks to identify the degree to which ethanol affects cognition in APP/PS1 mice. In addition to these findings, we also observed a major impact of ethanol exposure on self-care-related behaviors, as measured by nest building [26]. One major deficits commonly observed in patients with mild cognitive impairment is a disruption in self-care behaviors (i.e. cleaning one's room, showering, etc.) [27]. Ethanol naïve APP/PS1 mice displayed reduced nest-building behaviors, and this deficit was exacerbated in ethanol-exposed APP/PS1 mice (Figure 4f). This outcome further suggests that ethanol-drinking APP/PS1 mice are experiencing earlier onset of negative affective behaviors associated with AD progression. Interestingly, these differences in nest-building behavior were similar to changes in brain mass at the end of the study. While ethanol exposure had no effect on brain mass in wild-type mice, this measure was reduced in control APP/PS1 mice, and further reduced in ethanol-drinking APP/PS1 subjects (Figure 2b).

In this study, ethanol-exposed APP/PS1 mice developed an interesting metabolic phenotype. These mice consumed less food during their dark cycle (Figure 3b), more food during their light cycle (Figure 3c), and displayed aspects of glucose intolerance (Figure 3d) – all without displaying an overt metabolic phenotype (e.g. hyperglycemia, obesity). These changes were not observed in ethanol-exposed wild-type mice, suggesting that Aβ interacts with ethanol to exacerbate changes in metabolism in an AD-specific manner. While metabolic diseases like type-2 diabetes are known to put the brain at risk for AD [28, 29], a growing body of evidence suggests that AD can also exacerbate metabolic dysfunction, glucose intolerance, or insulin resistance. This circadian mismatch in feeding behavior also suggests that APP/PS1 mice have altered sleep/wake cycles. A bidirectional relationship exists between AD and sleep impairment, where disrupted sleep increases AD risk, and increased Aβ and tau aggregation further disrupts sleep [12]. Alcohol use disorder is similarly sufficient to disrupt sleep [13]. Thus alterations in sleep and circadian rhythms, like those observed with feeding behavior, may provide one explanation for why AUD increases AD risk. Interestingly, a recent study also demonstrated that nest-building increases during proximity to sleep in mice [30]. Although further studies will be needed, collectively these findings may suggest that chronic ethanol drinking exacerbates disruptions in sleep that are frequently observed in AD.

Several groups have demonstrated that APP/PS1 mice exhibit increased impulsivity and compulsivity [24], which may explain the initial increase in ethanol consumption of APP/PS1 mice. Alternatively, the differences in ethanol consumption could be driven by the metabolic disturbances commonly reported in APP/PS1 mice. In APP/PS1 mice peripheral hyperglycemia leads to increased brain ISF Aβ levels [14, 31]. In addition to dysregulated glucose metabolism, the differences in food consumption may also reflect disturbances in metabolism typically observed in mouse models of AD-like pathology. Thus, ethanol may be replacing the caloric needs being met by ethanol-naïve APP/PS1 mice. It should be noted, however, that this data only reflects food consumption as it coincides with days during which mice had access to ethanol. Follow-up studies will expand upon these data by including food consumption during the withdrawal period, as well as during the ethanol treatment period.

Together, these findings suggest that chronic ethanol consumption may exacerbate the metabolic disturbances associated with AD-like pathology.

Taken together, our findings demonstrate that, contrary to some prior clinical and preclinical findings, chronic intake of moderate amounts of ethanol may actually exacerbate some of the behavioral and neurobiological AD-like phenotype in APP/PS1 mice. This study also demonstrates for the first time that a single ethanol exposure bidirectionally alters ISF Aβ levels, possibly reflecting the biphasic effects of acute ethanol on neuronal excitability. Collectively, these findings add to the growing body of evidence suggesting that chronic alcohol consumption represents an important, modifiable risk factor for AD. Future studies will be needed to further characterize the biological mechanisms linking alcohol drinking and AD-related pathology.

Materials & Methods

Animals: We used 5.5 month-old male APPswe/PS1dE9 mice [11] (APP/PS1; The Jackson Laboratory; n=17) age-matched wild-type B6C3 control mice (n=17). All animals were housed individually in standard mouse cages under a 12-h artificial light–dark cycle. Room temperature and humidity were kept constant (temperature: 22 ± 1 °C; relative humidity: 55 ± 5%). Standard laboratory rodent chow and tap water were provided ad libitum throughout the experimental period. Mice underwent a battery of behavioral tests at baseline, and at various stages during ethanol exposure. We used a separate cohort of 3 month-old male APP/PS1 mice (n = 4-6) for acute ethanol exposure experiments. All experimental procedures were approved by the Committee on Animal Care and Use at Wake Forest School of Medicine.

Experimental Design: At 4 months of age, APP/PS1 and control mice were run through a battery of behavioral tests to identify baseline differences in anxiety- and depression-related behavior. Following completion of this behavioral battery at 5.5 months of age, APP/PS1 and control mice were randomly assigned to either an EtOH exposure or water alone condition. Ethanol exposure was provided via a 10 week two-bottle choice paradigm [32]. Mice were weighed before and after each

drinking session. Throughout the 10 week drinking period, mice were assessed for changes in anxiety and AD-related behaviors. All behavioral assays were conducted during the three-day abstinence period. At the end of the study, mice were euthanized within 24-48 hours after the final ethanol exposure period (see figure 1a for experimental timeline).

Two Bottle Choice Procedure (2BC): Mice were individually housed and treated with a modified two bottle-choice drinking paradigm for 10 weeks [32]. Briefly, home-cage water bottles were replaced with two 50 mL bottles containing H_2O or a 20% ethanol solution (ethanol-naïve group received only H_2O in both bottles). Mice were given access to ethanol during their dark cycle for 12 hrs/day for 4 days/week, and the position of the bottles were alternated daily to avoid side preference. Mice were habituated to 20% EtOH over a two week period by incrementally increasing EtOH concentration in the water from 5% to 20% (v/v). Fluid consumption was measured by weighing bottles before and after each drinking session. Ethanol is presented as g of ethanol per kg of mouse (g/kg). EtOH preference was calculated as a percent of ethanol intake to total liquid intake.

Open Field Assay (OFA): The open field assay was performed as described previously [33]. Briefly, mice were placed in the center of a plexiglass chamber (40cm x 40cm x 30cm) equipped with Omnitech Superflex Sensors (Omnitech Electronics, Inc). This box uses arrays of infrared photodectors located at regular intervals along each way of the chamber. The chamber walls were solid and were contained within sound-attenuating boxes with a 15-watt light bulb to illuminate the arena. Exploratory activity was measured for 15 minutes and quantified as locomotor activity and % time spent in the central zone. OFA activity was assessed at baseline (mice were ~3 months-old), and after 3 weeks ethanol drinking (mice were ~6 months-old)

Light/Dark Assay (LD): The light/dark box test was conducted as previously described [34]. Control and APP/PS1 mice were placed into a polycarbonate box (40 cm x 40 cm) with two equally sized regions. One region was dark and concealed, while the other was open and light. A 10 cm opening allowed free movement between both regions. Mice were monitored for five minutes. Latency to enter the light side, number of light-side entries, and total time spent in the light-side of the box were recorded

with EthoVision XT tracking software. Increased reluctance to venture into the light, uncovered, side was interpreted as anxiety-related behavior. LD activity was assessed at baseline (mice were ~3 months-old), and after 4 weeks ethanol drinking (mice were ~6 months-old)

Marble Burying: The marble burying test was performed as previously described [35]. Control and APP/PS1 mice were brought into a novel environment and habituated for one hour before behavioral testing. Mice were placed in a cage (19.56 cm x 30.91 cm x 13.34 cm) containing 12 marbles (13 mm diameter) on corncob bedding (5 cm depth). Mice were allowed to freely move within the cage for 30 minutes. Following the 30-minute period, mice were removed from the cage and returned to their respective home cages. Images of each cage was recorded, and the number of marbles were counted. A marble was considered buried when >75% of the object was covered by bedding.

*Object location memory task (OLM)***:** Object location memory task was conducted as previously described [36]. Mice were habituated to an opaque plastic chamber (40cm x 40cm) with visible spatial cues for 10 min. After 24 h, mice were returned to the chamber with two identical objects in the chamber and were allowed to freely explore and interact with the objects for 10 min. Twenty-four hours later, mice were returned to the chamber again, where one of the two objects had been relocated to an adjacent position. Objects and changes in object location were randomly determined and counterbalanced. Time spent with each object was measured and calculated as a percentage of the total object interaction time. Relocated object preference of ~50% indicates memory impairments. Time with objects was measured both manually and with EthoVision XT tracking software. Mice with a total object interaction time of <5 s were excluded from analysis. Data collection and analysis were performed blinded to condition.

Nest Building: Nest building behavior was assessed as previously described [37]. 24 hours following the last day of EtOH treatment during the dark cycle, control and APP/PS1 mice were provided fresh nesting material (a paper Bed-r'Nest (TheAndersons) and cotton nestlet (Ancare)) in their home cages. At the beginning of the light cycle, photos of the nests were recorded and rated on a 1-5 scale

by two blinded analysts. A score of 1 was considered a completely unconstructed nest, while a 5 was considered a completed nest that integrated all available materials.

Glucose tolerance test (GTT): Glucose tolerance test was performed as previously described [36]. Briefly, mice were fasted for 4 h and 2.0 g/kg glucose was administered via i.p injection. Blood samples were taken from tail veins and blood glucose was measured at baseline, 15-, 30-, 45-, 60-, 90-, and 120 minutes from glucose injection using a glucometer (Bound Tree Medical Precision XTRA Glucometer; Fisher).

Brain mass and Aβ immunohistochemistry: 8-month-old wild-type and APP/PS1 mice (n=7-10 per group) were anesthetized with an overdose of sodium pentobarbital and transcardially perfused with ice-cold 0.3% heparin in DPBS. The brains were weighed and fixed in 4% paraformaldehyde at 4°C. Prior to sectioning, brains were cyroprotected in 30% sucrose and then sectioned on a freezing microtome at 50 μm. 3 serial sections (300 μm apart) through the anterior-posterior aspect of the hippocampus were immunostained for Aβ deposition using a biotinylated, HJ3.4 antibody (anti-$A\beta_{1-13}$, a generous gift from Dr. David Holtzman, Washington University), and developed using a vectastain ABC kit and DAB reaction. The brain sections were imaged using a NanoZoomer slide scanner and the percent area occupied by HJ2 was quantified using ImageJ software (National Institutes of Health) by a blind researcher as previously described [21, 38] [berro 2011-roh 2012]. Statistical significance was determined using a two-tailed, unpaired t-test. Additionally, we ran a histogram analysis to quantify the frequency of each plaque by pixel size, excluding any plaques smaller than 10 pixels. Statistical significance was determined using a two-tailed, unpaired t-test (percent area) and a 2-way ANOVA with Šídák's multiple comparisons post-hoc tests (size x frequency). Data is represented by means ±SEM.

In vivo microdialysis: To determine how ethanol directly affects ISF Aβ, we exposed a separate cohort of 3 month-old APP/PS1 mice to a single intoxicating dose of ethanol (3.0 g/kg, 15% w/v) and continuously collected hippocampal ISF, using in vivo microdialysis. In vivo microdialysis was performed as previously described [14]. Five days prior to acute ethanol exposure, guide cannulas (BASi) were stereotaxically implanted into the hippocampus (from bregma, A/P: -3.1 mm; M/L: -2.5 mm;

D/V: -1.2 mm; at 12° angle) and secured into place with dental cement. One day prior to ethanol, 3 month-old APP/PS1 mice were transferred to sampling cages (Bioanalytical Systems) and microdialysis probes (2 mm; 38 kDa molecular weight cut off; BR-style; BASi) inserted into the guide cannula, connected to a syringe pump and infused with artificial cerebrospinal fluid (aCSF; 1.3mM CaCl2, 1.2mM MgSO4, 3mM KCl, 0.4mM KH2PO4, 25mM NaHCO3 and 122mM NaCl; pH=7.35) at a flow rate of 1 μL/min. Hourly collection of hippocampal ISF began in the afternoon. The following afternoon, mice were administered 3.0 g/kg ethanol via i.p. injection from a 15% ethanol (w/v; in 0.9% saline).

ISF ethanol & glucose measurements: ISF glucose and ISF ethanol concentrations were measured in each ISF sample from 3-month-old APP/PS1 mice (n=4) using the YSI 2900 analyzer (YSI incorporated) per the manufacturer's instructions. Data is represented by means ± SEM.

Aβ40 ELISA: ISF Samples from 3-month-old APP/PS1 mice (n=5) collected from microdialysis experiments were analyzed for Aβ40 using sandwich ELISAs as previously described [14, 21, 38]. Briefly, Aβ40 was quantified using monoclonal capture antibodies (a generous gift from Dr. David Holtzman, Washington University) targeted against amino acids 45-50 (HJ2). For detection, we then used a biotinylated monoclonal antibody against the central domain (HJ5.1B), followed by streptavidin-poly-HRP-40. The assay was developed using Super Slow TMB (Sigma) and the plates were read on a Bio-Tek Synergy 2 plate reader at 650 nm.

Statistical Analysis: Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). Data were expressed as means ± SEM. Two-way-repeated-measures ANOVAs were used to analyze differences between-subject factors (genotype and time or genotype and alcohol exposure). Following a significant F value, *post hoc* analyses (Šídák's multiple comparisons) were performed for assessing specific group comparisons. One-way ANOVAs were employed for all other statistical analyses using Tukey's HSD test for all post hoc analyses. The level of statistical significance was set at $p \leq 0.05$. See supplemental table 1 for expanded listing of statistical results.

ACKNOWLEDGEMENTS

We would like to acknowledge the following grants: P50AA26117 (JLW), K01AG050719 (SLM), R01AG068330 (SLM), T32AA007565 (SMD, SCG), and Wake Forest Translational Alcohol Research Center (WF-TARC) Pilot Grant (SMD, SLM). We would also like to acknowledge the Draelos Foundation and Draelos Science Scholars Program through the Center for Precision Medicine for supporting this work (WV, HK). We would also like to thank Dr. David Holtzman for the generous contribution of Aβ antibodies for our post-mortem analyses.

COMPETING INTERESTS

The authors have nothing to disclose.

REFERENCES:

1. Long, J.M. and D.M. Holtzman, *Alzheimer Disease: An Update on Pathobiology and Treatment Strategies.* Cell, 2019. **179**(2): p. 312-339.
2. Jack, C.R., et al., *A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers.* Neurology, 2016. **87**(5): p. 539-547.
3. Jack, C.R., Jr., et al., *Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade.* The Lancet Neurology, 2010. **9**(1): p. 119-128.
4. Harwood, D.G., et al., *The effect of alcohol and tobacco consumption, and apolipoprotein E genotype, on the age of onset in Alzheimer's disease.* Int J Geriatr Psychiatry, 2010. **25**(5): p. 511-8.
5. Xu, W., et al., *Alcohol consumption and dementia risk: a dose–response meta-analysis of prospective studies.* European Journal of Epidemiology, 2017. **32**(1): p. 31-42.
6. Schwarzinger, M., et al., *Contribution of alcohol use disorders to the burden of dementia in France 2008-13: a nationwide retrospective cohort study.* Lancet Public Health, 2018. **3**(3): p. e124-e132.
7. Zhornitsky, S., et al., *Cognitive dysfunction and cerebral volumetric deficits in individuals with Alzheimer's disease, alcohol use disorder, and dual diagnosis.* Psychiatry Res Neuroimaging, 2021. **317**: p. 111380.
8. Rehm, J., et al., *Alcohol use and dementia: a systematic scoping review.* Alzheimers Res Ther, 2019. **11**(1): p. 1.
9. Huang, D., et al., *Ethanol Alters APP Processing and Aggravates Alzheimer-Associated Phenotypes.* Mol Neurobiol, 2018. **55**(6): p. 5006-5018.
10. Hoffman, J.L., et al., *Alcohol drinking exacerbates neural and behavioral pathology in the 3xTg-AD mouse model of Alzheimer's disease.* Int Rev Neurobiol, 2019. **148**: p. 169-230.

11. Jankowsky, J.L., et al., *Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase.* Hum Mol Genet, 2004. **13**(2): p. 159-70.

12. Carroll, C.M. and S.L. Macauley, *The Interaction Between Sleep and Metabolism in Alzheimer's Disease: Cause or Consequence of Disease?* Frontiers in Aging Neuroscience, 2019. **11**.

13. Koob, G.F. and I.M. Colrain, *Alcohol use disorder and sleep disturbances: a feed-forward allostatic framework.* Neuropsychopharmacology, 2020. **45**(1): p. 141-165.

14. Macauley, S.L., et al., *Hyperglycemia modulates extracellular amyloid-β concentrations and neuronal activity in vivo.* J Clin Invest, 2015. **125**(6): p. 2463-7.

15. Krell-Roesch, J., et al., *Association between CSF biomarkers of Alzheimer's disease and neuropsychiatric symptoms: Mayo Clinic Study of Aging.* Alzheimer's & Dementia, 2022. **n/a**(n/a).

16. Hakon, J., et al., *Multisensory stimulation improves functional recovery and resting-state functional connectivity in the mouse brain after stroke.* Neuroimage Clin, 2018. **17**: p. 717-730.

17. Weiner, J.L. and C.F. Valenzuela, *Ethanol modulation of GABAergic transmission: the view from the slice.* Pharmacol Ther, 2006. **111**(3): p. 533-54.

18. Ramachandran, B., et al., *Ethanol inhibits long-term potentiation in hippocampal CA1 neurons, irrespective of lamina and stimulus strength, through neurosteroidogenesis.* Hippocampus, 2015. **25**(1): p. 106-18.

19. Roberto, M. and F.P. Varodayan, *Synaptic targets: Chronic alcohol actions.* Neuropharmacology, 2017. **122**: p. 85-99.

20. Cirrito, J.R., et al., *Synaptic activity regulates interstitial fluid amyloid-β levels in vivo.* Neuron, 2005. **48**(6): p. 913-22.

21. Bero, A.W., et al., *Neuronal activity regulates the regional vulnerability to amyloid-beta deposition.* Nat Neurosci, 2011. **14**(6): p. 750-6.

22. Verges, D.K., et al., *Opposing synaptic regulation of amyloid-beta metabolism by NMDA receptors in vivo.* J Neurosci, 2011. **31**(31): p. 11328-37.

23. Bouwman, V., et al., *Depression, anxiety and glucose metabolism in the general dutch population: the new Hoorn study.* PloS One, 2010. **5**(4): p. e9971.

24. Shepherd, A., et al., *Evaluation of attention in APP/PS1 mice shows impulsive and compulsive behaviours.* Genes, Brain, and Behavior, 2021. **20**(1): p. e12594.

25. Sabia, S., et al., *Alcohol consumption and cognitive decline in early old age.* Neurology, 2014. **82**(4): p. 332-339.

26. Jirkof, P., *Burrowing and nest building behavior as indicators of well-being in mice.* J Neurosci Methods, 2014. **234**: p. 139-46.

27. Hirschfeld, R.M.A., et al., *Social Functioning in Depression: A Review.* The Journal of Clinical Psychiatry, 2000. **61**(4): p. 0-0.

28. Ott, A., et al., *Diabetes mellitus and the risk of dementia: The Rotterdam Study.* Neurology, 1999. **53**(9): p. 1937-42.

29. Arnold, S.E.A., Z.//Macauley-Rambach, S. L.//Koenig, A. M.//Wang, H. Y.//Ahima, R. S.//Craft, S.//Gandy, S.//Buettner, C.//Stoeckel, L. E.//Holtzman, D. M.//Nathan, D. M., *Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums.* Nat Rev Neurol, 2018. **14**(3): p. 168-181.

30. Sotelo, M.I., et al., *Lateral hypothalamic neuronal ensembles regulate pre-sleep nest-building behavior.* Curr Biol, 2022.

31. Zhou, Q., et al., *Metabolic fate of glucose in the brain of APP/PS1 transgenic mice at 10 months of age: a 13C NMR metabolomic study.* Metabolic Brain Disease, 2018. **33**(5): p. 1661-1668.

32. Huynh, N., et al., *Murine Drinking Models in the Development of Pharmacotherapies for Alcoholism: Drinking in the Dark and Two-bottle Choice.* J Vis Exp, 2019(143): p. e57027.

33. Ewin, S.E., et al., *Chronic intermittent ethanol exposure selectively increases synaptic excitability in the ventral domain of the rat hippocampus.* Neuroscience, 2019. **398**: p. 144-157.

34. Miller, S.M., C.C. Piasecki, and J.S. Lonstein, *Use of the light-dark box to compare the anxiety-related behavior of virgin and postpartum female rats.* Pharmacology, biochemistry, and behavior, 2011. **100**(1): p. 130-137.

35. Amodeo, D.A., et al., *Differences in BTBR T+ tf/J and C57BL/6J mice on probabilistic reversal learning and stereotyped behaviors.* Behavioural Brain Research, 2012. **227**(1): p. 64-72.

36. Day, S.M., et al., *Glucagon-Like Peptide-1 Cleavage Product Improves Cognitive Function in a Mouse Model of Down Syndrome.* eNeuro, 2019. **6**(2): p. ENEURO.0031-19.2019.

37. Deacon, R.M., *Assessing nest building in mice.* Nat Protoc, 2006. **1**(3): p. 1117-9.

38. Roh, J.H.H., Y.//Bero, A. W.//Kasten, T.//Stewart, F. R.//Bateman, R. J.//Holtzman, D. M., *Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology.* Sci Transl Med, 2012. **4**(150): p. 150ra122.

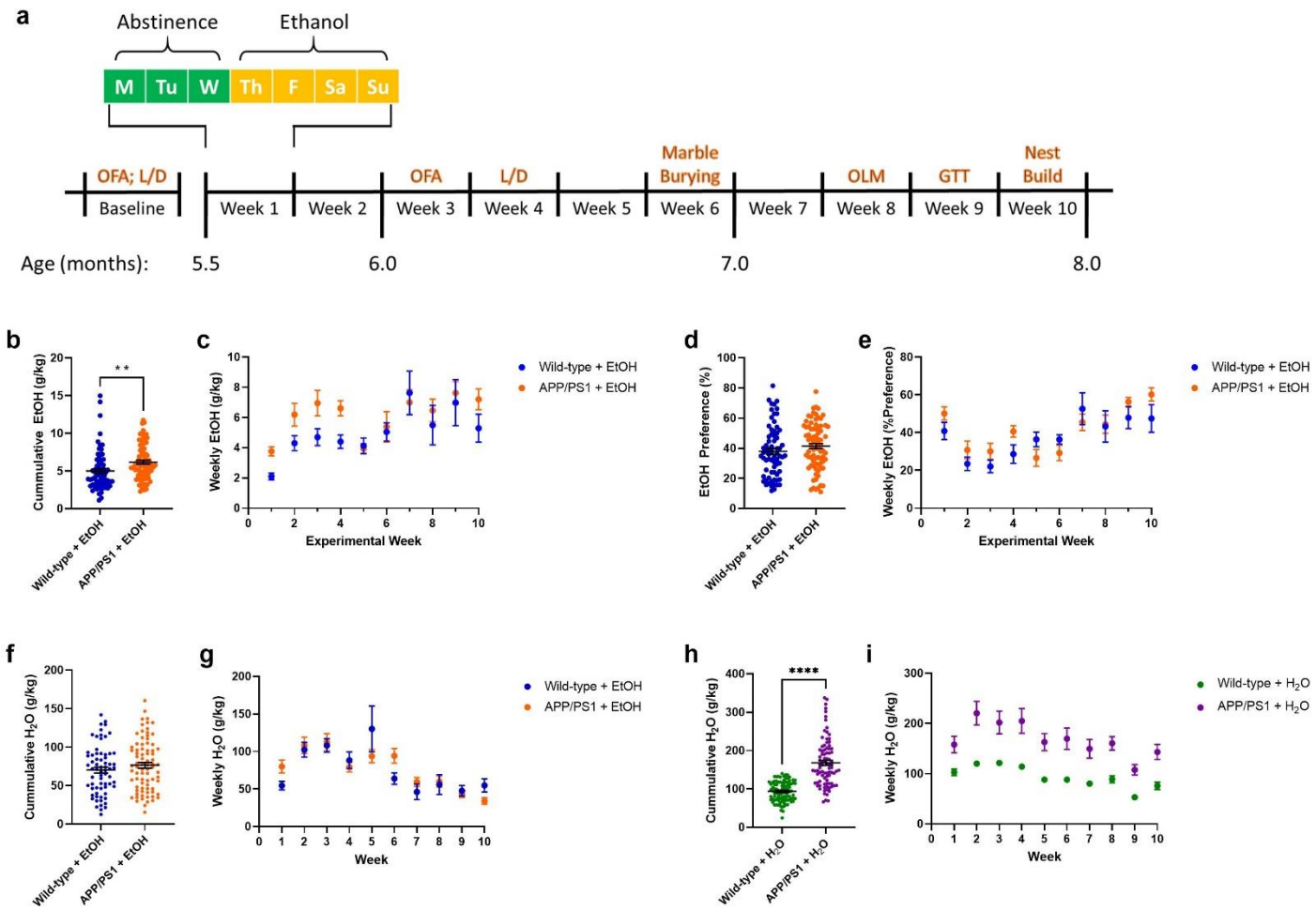
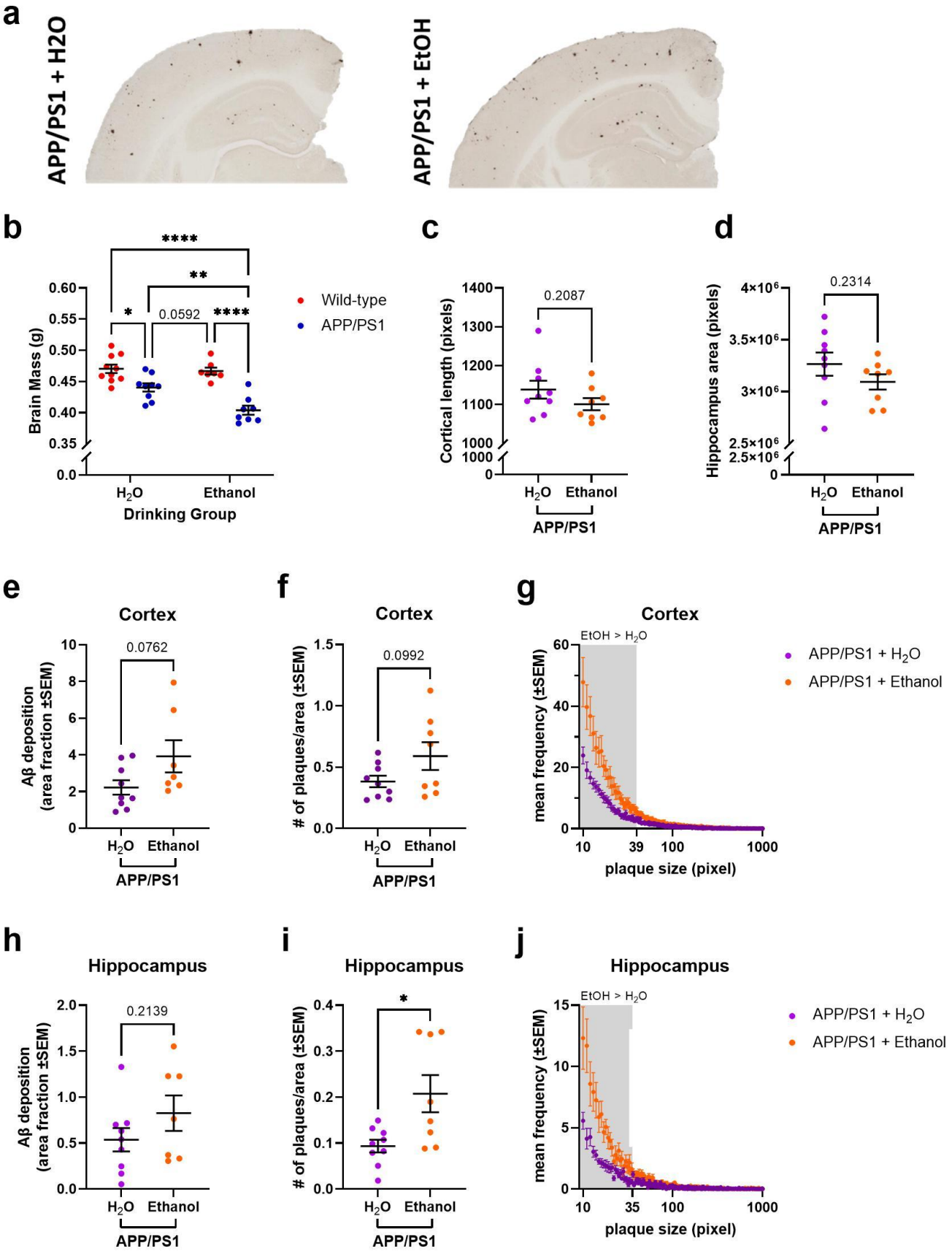


Figure 1: APP/PS1 mice consume more ethanol than control mice.

a) Timeline for the experimental protocol. Full details are provided in the methods. b) Average EtOH preference (% total fluid) from the 10-week exposure period plotted as a function of genotype. c) An unpaired t-test showed no difference between EtOH preference between wild-type and APP/PS1 mice over the course of the experiment. d) Average EtOH intake (g/kg) from the 10-week plotted as a function of genotype. e) An unpaired t-test showed APP/PS1 mice consumed more EtOH than wild-type mice across the (p=.01). f) Average water consumption across the 10-week EtOH exposure in EtOH-treated mice. g) An unpaired t-test showed no difference in weekly water consumption in EtOH-treated wild-type or APP/PS1 mice. h) Average water consumption across the 10-week EtOH exposure period in ethanol-naïve mice. i) Ethanol-naïve APP/PS1 mice consumed more water than ethanol-naïve wild-type mice.


a
APP/PS1 + H2O
APP/PS1 + EtOH
b
Brain Mass (g)
Drinking Group
H2O
Ethanol
Wild-type
APP/PS1
0.0592
c
Cortical length (pixels)
0.2087
APP/PS1
d
Hippocampus area (pixels)
0.2314
APP/PS1
e
Cortex
Aβ deposition (area fraction ±SEM)
0.0762
f
Cortex
# of plaques/area (±SEM)
0.0992
g
Cortex
EtOH > H2O
mean frequency (±SEM)
plaque size (pixel)
APP/PS1 + H2O
APP/PS1 + Ethanol
h
Hippocampus
0.2139
i
Hippocampus
j
Hippocampus

527

Figure 2: Ethanol exposure decreases brain mass and exacerbates amyloid pathology in APP/PS1 mice.

a) Representative images of Aβ deposition in brain sections from EtOH-naïve APP/PS1 mice stained with anti-Aβ antibody to visualize Aβ immunopositive amyloid plaques; b) Brain mass, a metric of brain atrophy, was reduced in EtOH-naïve and EtOH-exposed APP/PS1 c) Unpaired t-test revealed no significant differences in cortical thickness between EtOH-treated and EtOH-naïve APP/PS1 mice; d) Unpaired t-test showed no differences in hippocampus area between EtOH-treated and EtOH-naïve APP/PS1 mice. e) Percentage of cortex occupied by Aβ deposition. Unpaired t-test showed trend ($p = 0.0762$) towards greater Aβ deposition in EtOH-treated APP/PS1 mice compared to EtOH-naïve APP/PS1 mice. f) Average number of plaques in the cortex, normalized to area quantified, trended towards increased in EtOH-treated APP/PS1 mice. g) Frequency distribution of amyloid plaque size (in pixels) in the cortex of EtOH-treated and EtOH-naïve APP/PS1 mice shows greater number of smaller plaques in EtOH-treated APP/PS1 mice. h) Percentage of hippocampus occupied by Aβ deposition. Unpaired t-test showed no differences in Aβ deposition between EtOH-treated APP/PS1 mice compared to EtOH-naïve APP/PS1 mice. i) Plaque number increased in the hippocampus of APP/PS1 mice after EtOH treatment. j) Frequency distribution of amyloid plaque size (in pixels) in the hippocampus of EtOH-naïve and EtOH-treated APP/PS1 mice shows greater number of smaller plaques in EtOH-treated APP/PS1 mice.

*$p<0.05$, **$p<0.01$, ****$p<0.0001$

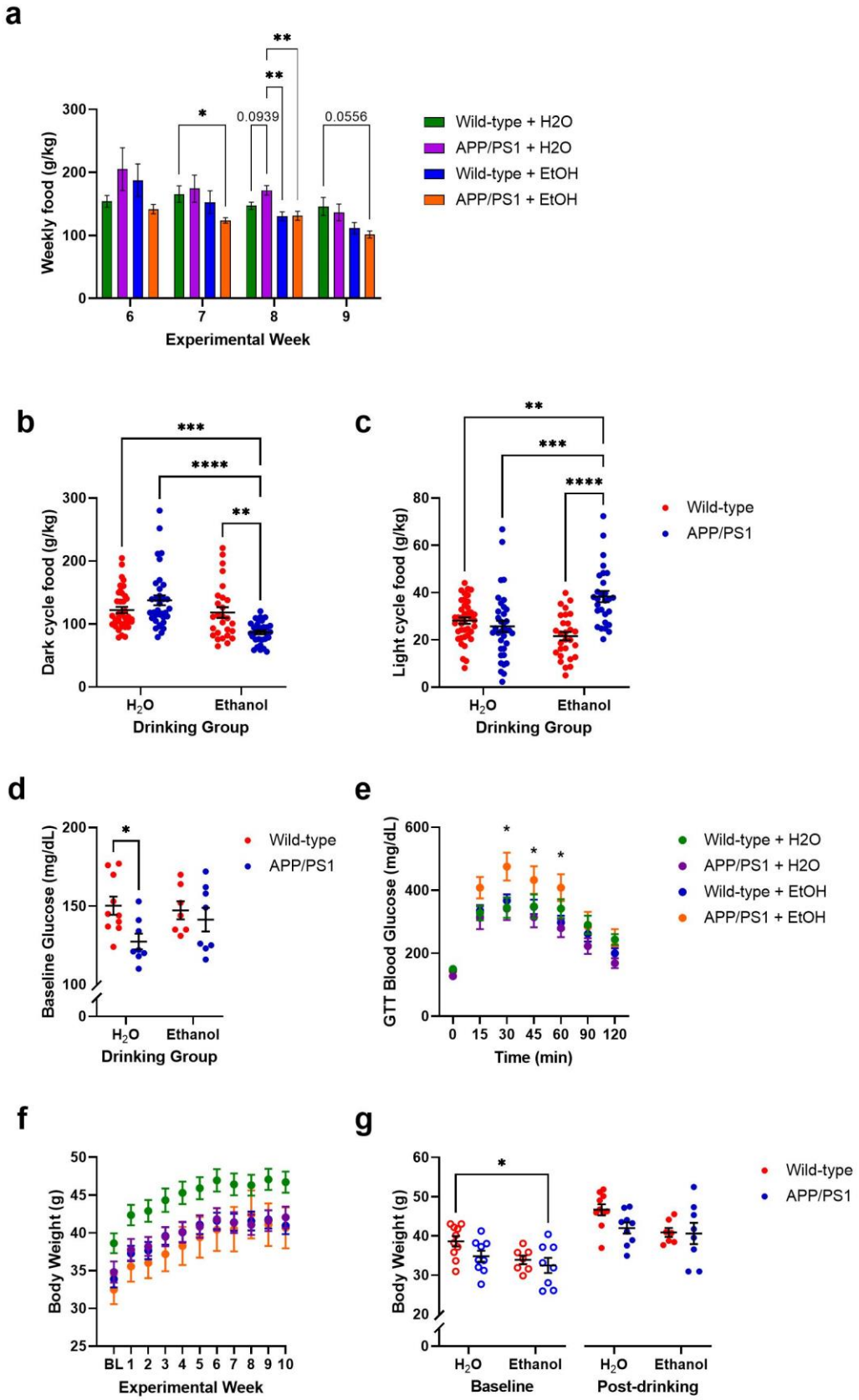


Figure 3: Ethanol exposure dysregulates metabolism in APP/PS1 mice.

a) Decreased weekly food consumption in APP/PS1 with or without ethanol treatment during experimental weeks 6-9.

b) Ethanol-treated APP/PS1 mice showed decreased food consumption during the dark period. Two-way ANOVA revealed significant differences between drinking group ($p<0.0001$) and genotype x drinking group ($p=0.0003$). c) Ethanol-treated APP/PS1 mice showed increased food consumption during light cycle. 2-way ANOVA revealed significant interactions of genotype ($p=0.0005$) and genotype x drinking group ($p<0.0001$); d) ethanol-naïve APP/PS1 mice showed lower fasted blood glucose concentrations prior to glucose tolerance test. 2-way ANOVA revealed significant genotype effect ($p = 0.0273$). e) Ethanol-treated APP/PS1 mice displayed glucose intolerance during glucose tolerance test. Two-way ANOVA revealed significance over time ($p<0.0109$), and time x group ($p<0.0001$). Tukey's multiple comparisons revealed that EtOH-treated APP/PS1 mice had significantly higher blood glucose concentrations at 30-, 45-, and 60-minutes post-glucose injection. f) Average weekly body weights throughout the course of the experiment. g) Baseline and post-treatment body weights. Baseline differences between groups at baseline were no longer present by the end of the study.

*$p<0.05$, **$p<0.01$, ****$p<0.0001$;

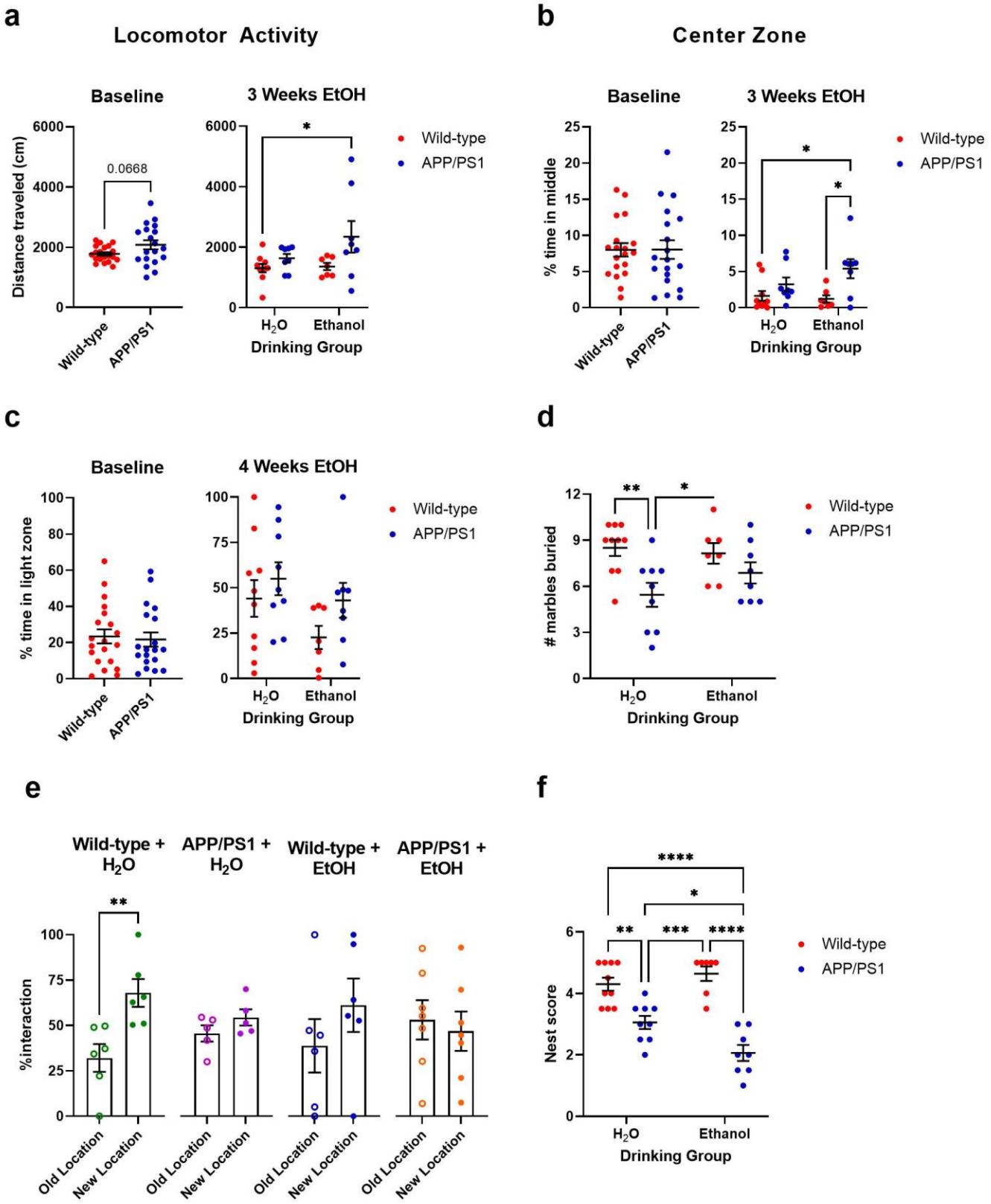


Figure 4: Chronic ethanol consumption alters anxiety-related and dementia-related behaviors in APP/PS1 mice.

a) At baseline, APP/PS1 mice showed a trend towards increased locomotor activity during the OFA (unpaired t-test, $p = 0.0668$). After 3 weeks of ethanol exposure APP/PS1 mice showed more locomotor activity than other groups. b) There were no differences in the % time spent in the center zone at baseline. After 3 weeks of ethanol treatment, APP/PS1 mice spent more time in central zone than wild-type controls. c) Mice exhibited no differences spent in the light zone in the LD box at baseline or following treatment. d) Ethanol-naïve APP/PS1 mice buried more marbles than wild-type controls. e) Ethanol-naïve wild-type spent significantly more time interacting with the relocated object than with the object in the familiar location (unpaired t-test, $p = 0.0078$), while other groups spent similar amounts of time interacting with both objects. f) Two-way ANOVA revealed significant differences in nest building scores between groups after 9 weeks of EtOH treatment. APP/PS1 mice scored lower than wild-type mice, which was even lower in ethanol-treated APP/PS1 mice. Ethanol had no effect on nest scores in wild-type mice

*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

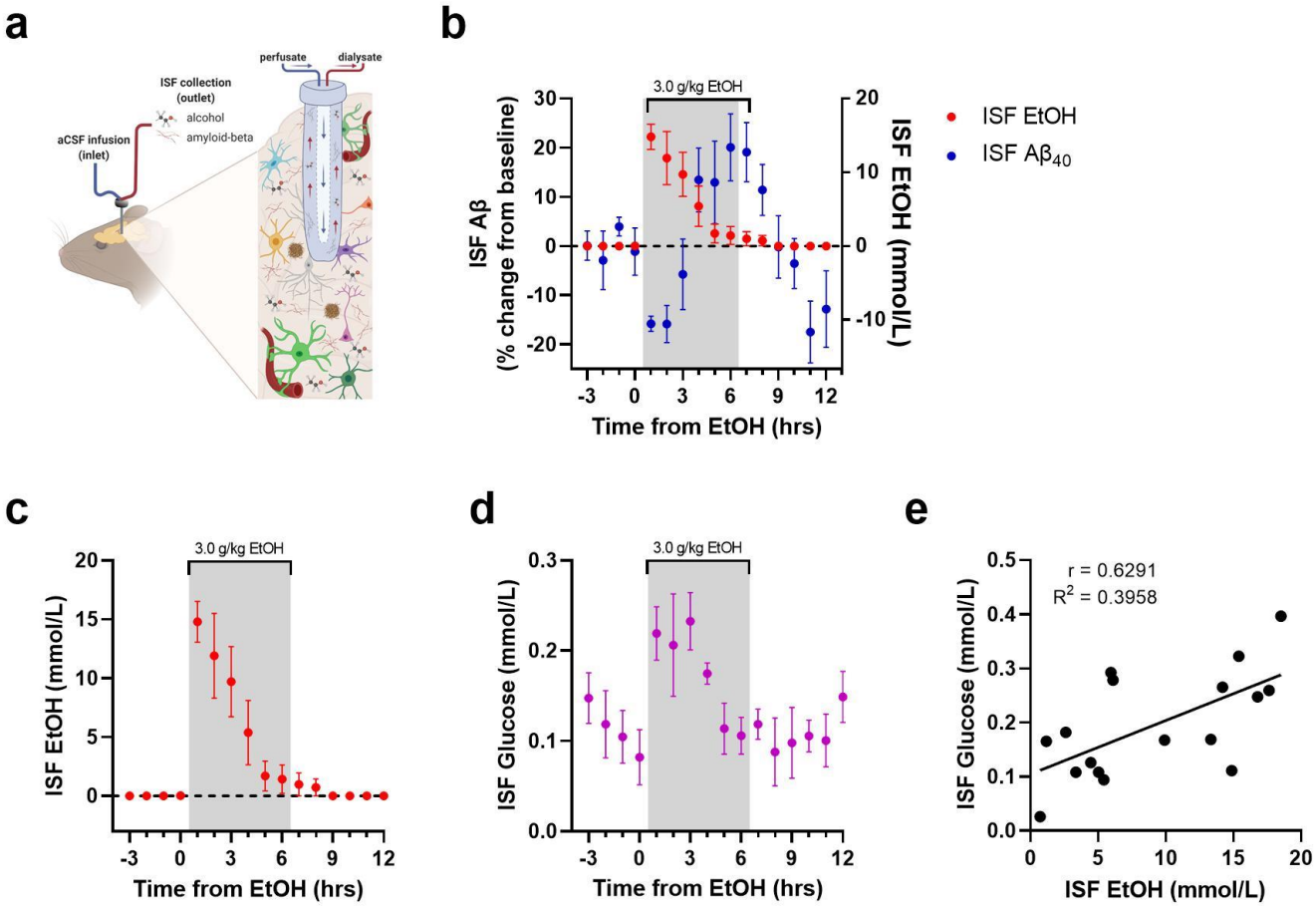


Figure 5: Ethanol acutely modulates ISF Aβ and ISF glucose in APP/PS1 mice.

a) Schematic of 38 kDa in vivo microdialysis to sample brain hippocampal interstitial fluid (ISF). b) Ethanol exposure changes ISF Aβ levels (left y-axis; blue) and ISF EtOH (right y-axis; red) in 3 month-old APP/PS1 mice. c) Changes in ISF ethanol in 3 month-old APP/PS1 mice. c) Changes in ISF glucose in 3 month-old APP/PS1 mice. d) ISF ethanol and ISF glucose are significantly correlated while ethanol is present in the brain.

Supplementary Table 1: Expanded list of statistical results		
Figure	**Stats**	**Result**
Figure 1b	Unpaired t-test	p = 0.0047
Figure 1c	2-way ANOVA	Time: F (2.433, 31.09) = 8.865, p = 0.0005 Group: F (1, 13) = 1.994, p = 0.1814 Time x Group: F (9, 115) = 1.147, p = 0.3357
Figure 1d	Unpaired t-test	p = 0.1936
Figure 1e	2-way ANOVA	Time: F (4.183, 54.38) = 12.28, p < 0.0001 Group: F (1, 13) = 0.8678, p = 0.3685 Time x Group: F (9, 117) = 2.066, p = 0.0381
Figure 1f	Unpaired t-test	p = 0.2434
Figure 1g	2-way ANOVA	Time: F (2.923, 38.01) = 15.27, p < 0.0001 Group: F (1, 13) = 0.02509, p = 8766 Time x Group: F (9, 117) = 2.220, p = 0.0253
Figure 1h	Unpaired t-test	p < 0.0001
Figure 1i	2-way ANOVA	Time: F (1.255, 20.49) = 35.85, p < 0.0001 Group: F (1, 17) = 19.27, p = 0.0004 Time x Group: F (9, 147) = 3.076, p = 0.0021
Figure 2b	2-way ANOVA	Genotype: F (1, 30) = 45.80, p < 0.0001 Drinking Group: F (1, 30) = 8.520, p = 0.0066 Genotype x Drinking Group: F (1, 30) = 5.773, p = 0.0227
Figure 2c	Unpaired t-test	p = 0.2087
Figure 2d	Unpaired t-test	p = 0.2314
Figure 2e	Unpaired t-test	p = 0.0762
Figure 2f	Unpaired t-test	p = 0.0992
Figure 2g	2-way ANOVA	Plaque Size: F (990, 14865) = 56.30, p < 0.0001 Plaque #: F (1, 14865) = 334.7, p < 0.0001 Plaque Size x Plaque #: F (990, 14865) = 6.807, p < 0.0001
Figure 2h	Unpaired t-test	p = 0.2319
Figure 2i	Unpaired t-test	p = 0.0134
Figure 2j	2-way ANOVA	Plaque Size: F (990, 14865) = 40.87, p < 0.0001 Plaque #: F (1, 14865) = 227.1, p < 0.0001 Plaque Size x Plaque #: F (990, 14865) = 7.183, p < 0.0001
Figure 3a	2-way ANOVA	Time: F (1.807, 54.21) = 18.23, p < 0.0001 Group: F (3, 30) = 2.302, p = 0.0972 Time x Group: F (9, 90) = 2.428, p = 0.0161
Figure 3b	2-way ANOVA	Genotype: F (1, 129) = 1.596, p = 0.2088 Drinking Group: F (1, 129) = 18.80, p < 0.0001 Genotype x Drinking Group: F (1, 129) = 13.76, p = 0.0003
Figure 3c	2-way ANOVA	Genotype: F (1, 128) = 12.85, p = 0.0005 Drinking Group: F (1, 128) = 2.260, p = 0.1352 Genotype x Drinking Group: F (1, 128) = 23.31, p < 0.0001
Figure 3d	2-way ANOVA	Genotype: F (1, 29) = 5.402, p = 0.0273 Drinking Group: F (1, 29) = 0.8039, p = 0.3773 Genotype x Drinking Group: F (1, 29) = 1.872, p = 0.1818
Figure 3e	2-way ANVOA	Time: F (3.355, 97.31) = 81.46, p = 0.0109 Group: F (3, 29) = 2.138, p = 0.1170 Time x Group: F (18, 174) = 2.021, p< 0.0001
Figure 3f	2-way ANOVA	Time: F (3.000, 89.99) = 130.7, p < 0.0001 Group: F (3, 30) = 3.050, p = 0.0437 Time x Group: F (30, 300) = 1.411, p = 0.0802

Supplementary Table 1: Expanded list of statistical results

Supplementary Table 1: Expanded list of statistical results (Continuted)		
Figure	**Stats**	**Result**
Figure 3g (baseline)	2-way ANOVA	Genotype: F (1, 30) = 3.042, p = 0.0914 Drinking Group: F (1, 30) = 5.564, p = 0.0250 Genotype x Drinking Group: F (1, 30) = 0.6423, p = 0.4292
Figure 3g (post-ethanol)	2-way ANOVA	Genotype: F (1, 30) = 1.937, p = 0.1743 Drinking Group: F (1, 30) = 4.077, p = 0.0525 Genotype x Drinking Group: F (1, 30) = 1.521, p = 0.2270
Figure 4a	Unpaired t-test (Baseline)	p = 0.0668
	2-way ANOVA (3 weeks EtOH)	Genotype: F (1, 29) = 5.412, p = 0.0272 Drinking Group: F (1, 29) = 1.851, p = 0.1841 Genotype x Drinking Group: F (1, 29) = 1.357, p = 0.2535
Figure 4b	Unpaired t-test (Baseline)	p = 0.9770
	2-way ANOVA (3 weeks EtOH)	Genotype: F (1, 29) = 9.901, p = 0.0038 Drinking Group: F (1, 29) = 0.9061, p = 0.3490 Genotype x Drinking Group: F (1, 29) = 1.957, p = 0.1724
Figure 4c	Unpaired t-test (Baseline)	p = 0.7565
	2-way ANOVA (3 weeks EtOH)	Genotype: F (1, 30) = 2.800, p = 0.1047 Drinking Group: F (1, 30) = 3.173, p = 0.0850 Genotype x Drinking Group: F (1, 30) = 0.2579, p = 0.6153
Figure 4d	2-way ANOVA	Genotype: F (1, 30) = 10.23, p = 0.0033 Drinking Group: F (1, 30) = 0.6305, p = 0.4334 Genotype x Drinking Group: F (1, 30) = 1.749, p = 0.1960
Figure 4e	Unpaired t-tests	Wild-type + H_2O: p = 0.0078 APP/PS1 + H_2O: p = 0.2036 Wild-type + EtOH: p = 0.3082 APP/PS1 + EtOH: p = 0.6890
Figure 4f	2-way ANOVA	Genotype: F (1, 30) = 68.39, p < 0.0001 Drinking Group: F (1, 30) = 1.976, p = 0.1700 Genotype x Drinking Group: F (1, 30) = 8.343, p = 0.0071
Figure 5e	Pearson r	r = 0.6291 R^2 = 0.3958 p = 0.0068

Supplementary Table 1: Expanded list of statistical results (continued)

Supplementary Table 2

Cortex: Plaque Size x Frequency			
Plaque Size	p value	Summary	
10 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
11 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
12 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
13 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
14 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
15 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
16 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
17 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
18 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
19 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
20 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
21 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
22 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
23 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
24 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
25 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
26 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
27 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
28 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
29 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
30 pxls	p=0.0005	***	APP/PS1 + H2O < APP/PS1 + EtOH
31 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
32 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
33 pxls	p=0.0003	***	APP/PS1 + H2O < APP/PS1 + EtOH
34 pxls	p=0.0153	*	APP/PS1 + H2O < APP/PS1 + EtOH
35 pxls	p=0.0003	***	APP/PS1 + H2O < APP/PS1 + EtOH
36 pxls	p=0.0011	**	APP/PS1 + H2O < APP/PS1 + EtOH
37 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
38 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
39 pxls	p=0.0093	**	APP/PS1 + H2O < APP/PS1 + EtOH
40 pxls	p=0.2534	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
41 pxls	p=0.9456	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
42 pxls	p=0.2306	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
43 pxls	p=0.9492	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
44 pxls	p=0.2447	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
45 pxls	p=0.0038	**	APP/PS1 + H2O < APP/PS1 + EtOH

Hippocampus: Plaque Size x Frequency			
Plaque Size	p value	Summary	
10 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
11 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
12 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
13 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
14 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
15 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
16 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
17 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
18 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
19 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
20 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
21 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
22 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
23 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
24 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
25 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
26 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
27 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
28 pxls	p>0.9999	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
29 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
30 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
31 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
32 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
33 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
34 pxls	p=0.0061	**	APP/PS1 + H2O < APP/PS1 + EtOH
35 pxls	p<0.0001	****	APP/PS1 + H2O < APP/PS1 + EtOH
36 pxls	p>0.9999	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
37 pxls	p=0.0635	ns	APP/PS1 + H2O < APP/PS1 + EtOH
38 pxls	p=0.224	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
39 pxls	p=0.9982	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
40 pxls	p>0.9999	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
41 pxls	p=0.1578	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
42 pxls	p=0.6351	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
43 pxls	p>0.9999	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
44 pxls	p>0.9999	ns	APP/PS1 + H2O ≈ APP/PS1 + EtOH
45 pxls	p=0.0003	***	APP/PS1 + H2O < APP/PS1 + EtOH

Supplementary table 2: Results from Šídák's multiple comparisons test for plaque size x mean frequency: figures 2g (left) and 2j (right).

Supplementary Table 3

	ISF EtOH (mmol/L)		ISF Aβ40 (% BL)		ISF Glucose (mmol/L)	
Time (hrs)	Mean	±SEM	Mean	±SEM	Mean	±SEM
-3	0.00	0.00	0.10	2.99	0.15	0.03
-2	0.00	0.00	-2.90	5.94	0.12	0.04
-1	0.00	0.00	3.92	1.92	0.10	0.03
0	0.03	0.03	-1.12	4.79	0.08	0.03
1	14.79	1.73	-15.82	1.58	0.22	0.03
2	11.91	3.60	-15.83	3.79	0.21	0.06
3	9.71	2.99	-5.74	7.20	0.23	0.03
4	5.38	2.73	13.43	6.50	0.17	0.01
5	1.71	1.28	12.92	8.41	0.11	0.03
6	1.43	1.21	20.07	6.83	0.11	0.02
7	0.98	0.98	19.09	6.01	0.12	0.02
8	0.72	0.72	11.41	5.19	0.09	0.04
9	0.00	0.00	-0.19	6.34	0.10	0.04
10	0.00	0.00	-3.55	5.10	0.11	0.02
11	0.00	0.00	-17.49	6.28	0.10	0.03
12	0.00	0.00	-12.82	7.78	0.15	0.03

Supplementary table 3: Mean and SEM results from microdialysis experiments (Figure 5b-e)