Sex-specific pleiotropic changes in emotional behavior and alcohol consumption in human α-synuclein A53T transgenic mice during early adulthood

Liubov S. Kalinichenko, Zacharias Kohl, Christiane Mühle, Zurina Hassan, Agnes Hahn, Eva-Maria Schmitt, Kilian Macht, Lyubomir Stoyanov, Schayan Moghaddami, Roberto Bilbao, Volker Eulenburg, Jürgen Winkler, Johannes Kornhuber, Christian P. Müller

Angaben zur Veröffentlichung / Publication details:

Kalinichenko, Liubov S., Zacharias Kohl, Christiane Mühle, Zurina Hassan, Agnes Hahn, Eva-Maria Schmitt, Kilian Macht, et al. 2024. "Sex-specific pleiotropic changes in emotional behavior and alcohol consumption in human α-synuclein A53T transgenic mice during early adulthood." Journal of Neurochemistry 168 (3): 269–87. https://doi.org/10.1111/jnc.16051.

Received: 18 July 2023 | Revised: 15 December 2023 | Accepted: 7 January 2024

DOI: 10.1111/jnc.16051

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

ORIGINAL ARTICLE

Sex-specific pleiotropic changes in emotional behavior and alcohol consumption in human α-synuclein A53T transgenic mice during early adulthood

Liubov S. Kalinichenko[1] | Zacharias Kohl[2,3,4] | Christiane Mühle[1] | Zurina Hassan[5] | Agnes Hahn[1] | Eva-Maria Schmitt[1] | Kilian Macht[1] | Lyubomir Stoyanov[1] | Schayan Moghaddami[1] | Roberto Bilbao[1] | Volker Eulenburg[6] | Jürgen Winkler[2,3] | Johannes Kornhuber[1] | Christian P. Müller[1,5,7]

[1]Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany

[2]Division of Molecular Neurology, Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany

[3]Center for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Germany

[4]Department of Neurology, University of Regensburg, Regensburg, Germany

[5]Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia

[6]Department for Anesthesiology and Intensive Care, Faculty of Medicine, University of Leipzig, Leipzig, Germany

[7]Institute of Psychopharmacology, Central Institute of Mental Health, Faculty of Medicine Mannheim, University of Heidelberg, Heidelberg, Germany

Correspondence
Liubov S. Kalinichenko, Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University of Erlangen-Nürnberg, Schwabachanlage 6, Erlangen 91054, Germany.
Email: liubov.kalinichenko@uk-erlangen.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: KO 947/20-1 and MU 2789/18-1; Johannes und Frieda

Abstract

Point mutations in the α-synuclein coding gene may lead to the development of Parkinson's disease (PD). PD is often accompanied by other psychiatric conditions, such as anxiety, depression, and drug use disorders, which typically emerge in adulthood. Some of these point mutations, such as *SNCA* and *A30T*, have been linked to behavioral effects that are not commonly associated with PD, especially regarding alcohol consumption patterns. In this study, we investigated whether the familial PD point mutation *A53T* is associated with changes in alcohol consumption behavior and emotional states at ages not yet characterized by α-synuclein accumulation. The affective and alcohol-drinking phenotypes remained unaltered in female PDGF-hA53T-synuclein-transgenic (A53T) mice during both early and late adulthood. Brain region-specific activation of ceramide-producing enzymes, acid sphingomyelinase (ASM), and neutral sphingomyelinase (NSM), known for their neuroprotective properties, was observed during early adulthood but not in late adulthood. In males, the *A53T* mutation was linked to a reduction in alcohol consumption in both early and late adulthood. However, male A53T mice displayed increased anxiety- and depression-like behaviors during both early and late adulthood. Enhanced ASM activity in the dorsal mesencephalon and ventral hippocampus may potentially contribute to these adverse behavioral effects of the mutation in males during late adulthood. In summary, the *A53T* gene mutation was associated with diverse changes in emotional states and alcohol consumption behavior long before the onset of PD, and these effects varied by sex. These alterations in behavior may be linked to changes in brain ceramide metabolism.

Abbreviations: A53T, PDGF-hA53T-synuclein-transgenic mice; AC, acid ceramidase; ANOVA, analysis of variance; ASM, acid sphingomyelinase; BAC, blood alcohol concentration; DH, dorsal hippocampus; DM, dorsal mesencephalon; DS, dorsal striatum; EPM, elevated plus maze; FST, forced swim test; LDB, light–dark box; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NC, neutral ceramidase; NSF, novelty suppressed feeding; NSM, neutral sphingomyelinase; OF, open field; PD, Parkinson's disorder; PFC, prefrontal cortex (PFC); SMS, sphingomyelin synthase; SPT, sucrose preference test; VH, ventral hippocampus; VM, ventral mesencephalon; VS, ventral striatum; wt, wild type.

Marohn-Stiftung; Interdisciplinary Center for Clinical Research, University of Erlangen, Grant/Award Number: J93

KEYWORDS
A53T, alcohol, ceramide, depression, neutral sphingomyelinase, Parkinson's disorder

1 | INTRODUCTION

Parkinson's disease (PD) is a severe chronic neurodegenerative condition that affects approximately 5% of individuals aged 85 years and older (Farrer, 2006). It is a complex disorder influenced by both genetic and environmental factors. Several genetic factors have been identified as contributing to the risk of PD, including mutations in *PARK*, *A53T*, *A30T*, *SNCA*, and *GBA1* (Kouli et al., 2018). These mutations initiate the development of PD, primarily through α-synuclein-induced degeneration of dopaminergic neurons in the substantia nigra pars compacta (Nuber et al., 2013, 2018), resulting in characteristic motor symptoms such as dyskinesia, rigidity, and tremors (Wang et al., 2018).

PD is frequently comorbid with other psychiatric conditions, notably anxiety and depression (Winkler et al., 2011), and a prodromal stage of PD accompanied by these non-motor symptoms can extend for several decades. In particular, depression, which affects 17% of PD patients, may precede motor symptoms by up to 20 years (Qamhawi et al., 2015; Shiba et al., 2000). However, some studies have suggested potential protective effects of PD against certain mental illnesses, such as alcohol use disorder (Bharucha et al., 1986; Fall et al., 1999; Jiméanez-Jiméanez et al., 1992). Conversely, moderate alcohol consumption has been associated with a reduced risk of PD development (Gentile et al., 2020; Palacios et al., 2012). In contrast, recent research has indicated that point mutations like SNCA and A30T, which are involved in synucleopathies and PD, may contribute to the pathogenesis of alcohol use disorder (Liang et al., 2003; Rotermund et al., 2017). Therefore, it is conceivable that mutations in genes responsible for early PD onset, such as the A53T gene, have pleiotropic effects, influencing the development of certain comorbid psychiatric conditions while potentially protecting against others long before the onset of PD symptoms.

Ceramides are part of the sphingolipid family, a group of lipids with high abundance in the brain that play significant roles in various physiological functions, including apoptosis, autophagy, and cell signaling (Holthuis et al., 2001). Alongside their precursors and the enzymes responsible for their metabolism, ceramides have been implicated in the pathogenesis of several psychiatric disorders, including PD, anxiety, depression, and alcohol use disorder (Gulbins et al., 2013; Kalinichenko et al., 2022, 2023; Kalinichenko, Hammad, et al., 2019; Kalinichenko, Mühle, et al., 2019, 2021; Müller et al., 2017; Zoicas et al., 2020). However, it remains uncertain whether members of the ceramide family may potentially link these psychiatric disorders or serve as early markers for PD.

In this study, we explored whether the *A53T* point mutation in the α-synuclein gene could be linked to diverse alterations in the emotional state and alcohol consumption behavior of mice during both early and late adulthood. These stages correspond to the prodromal phases of PD, which are not accompanied by motor symptoms. Additionally, we examined the predictive potential of ceramide metabolism enzymes in these psychiatric conditions comorbid with PD. Given the higher prevalence of PD incidence in male patients (Moisan et al., 2016), we conducted a comparative study involving both male and female A53T mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Male and female transgenic mice, which overexpressed human α-syn carrying the *A53T* mutation under the regulatory control of the PDGFβ promoter (referred to as A53T mice; received from the University of California San Diego; described in Hashimoto et al. (2006)), along with wild-type (wt) littermates, totalling 306 mice, were utilized in the experiments. The mice were maintained in a standard light–dark cycle (housed between two and five per cage or individually, depending on experimental requirements), with lights off from 07:00 pm to 07:00 am. All animals had ad libitum access to food and water.

The emotional phenotype, drinking behavior (assessed using constant and intermittent access models), blood alcohol concentration analysis, and ceramide metabolism enzyme activities were evaluated in separate batches of animals. Testing was conducted either during early adulthood, commencing at week 8 of age (weighing 22.3 ± 0.5 g), or during late adulthood, commencing at week 24 of age (weighing 32.5 ± 0.7 g), as illustrated in Figure 1. Previous literature had indicated the onset of motor impairments in these mice at 32 weeks of age (Giasson et al., 2002); thus, our assessments were performed during the prodromal stage of PD.

All experiments adhered to the guidelines set forth by the European Communities Council Directive (86/609/EEC) and the National Institutes of Health for the ethical treatment of animals. Ethical approval was obtained from the German Animal Protection Law Authority, Regierung von Unterfranken (55.2-2532-2-317). No animals were excluded from the experiments during testing. The experimenter conducting the tests remained blind to the study groups or genotypes of the animals. Following each experiment, the animals were humanely euthanized by cervical dislocation.

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

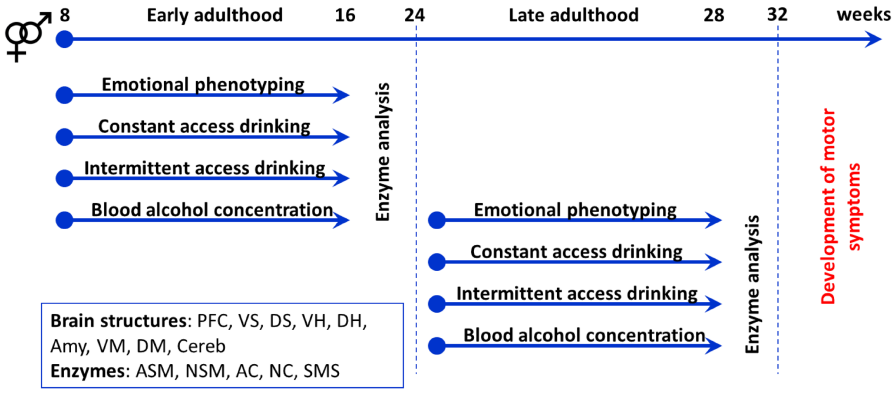


FIGURE 1 Experimental schedule. AC, acid ceramidase; Amy, amygdala; ASM, acid sphingomyelinase; Cereb, cerebellum; DH, dorsal hippocampus; DM, dorsal mesencephalon; NC, neutral ceramidase; NSM, neutral sphingomyelinase; PFC, prefrontal cortex; SMS, sphingomyelin synthase; VH, ventral hippocampus; VM, ventral mesencephalon; VS, ventral striatum.

2.2 | Anxiety- and depression-like phenotype

Naïve mice of both sexes, divided into early adulthood (A53T: $n = 14$ males/8 females; wt: $n = 8$ males/16 females) and late adulthood (A53T: $n = 10$ males/8 females; wt: $n = 11$ males/9 females) groups, underwent a battery of behavioral tests in the following sequence: open field (OF), light–dark box (LDB), elevated plus maze (EPM), novelty-suppressed feeding (NSF), forced swim (FST), and sucrose preference tests (SPT). All tests were conducted on separate days between 09:00 and 16:00 h. The mice were assessed in a pseudorandom order and were acclimated to the behavioral suite for at least 1 h before each test. To prevent any influence of olfactory cues on behaviors, each test apparatus was thoroughly cleaned with 70% ethanol (prepared from Cat. No. 9065.1, Roth) between subjects.

After completing a test, the mice were returned to their home cages and allowed to recover for a minimum of 2 days before undergoing further testing. Behaviors in all tests were recorded for subsequent scoring, following the protocols outlined in prior studies (Kalinichenko et al., 2023; Kalinichenko, Mühle, et al., 2021; Müller et al., 2017).

2.2.1 | OF

Each mouse was introduced into a square white acrylic arena measuring 50 × 50 cm, initially positioned facing one of the outer walls. They were given the freedom to explore the arena for 20 min, with the space uniformly illuminated by white light at an intensity of 100×. Video recordings of the mice's behavior were captured and subsequently analyzed using Biobserve Viewer III, a software provided by Biobserve GmbH, Germany.

To assess their behavior, a virtual square area located at an equal distance from the periphery and measuring 36 × 36 cm was defined as the "central zone." This designation allowed us to record the number of entries into the central zone and the amount of time (in seconds) spent within it. We also recorded the distance covered by the mice in both the outer and central zones (measured in centimeters), as well as the number of entries and the duration of time spent specifically in the central zone.

2.2.2 | EPM

The EPM was crafted from black opaque acrylic material with white lining on the floor. The maze consisted of arms measuring 30 × 5 cm each and a central platform measuring 5 × 5 cm. One pair of opposing arms was entirely enclosed by a 15-cm-high wall made of opaque acrylic, while the other pair featured open arms with narrow ledges of 0.5 cm on either side. The open arms were brightly illuminated at 100×. The maze was elevated to a height of 50 cm above the ground and supported by a transparent acrylic stand.

Each mouse was positioned on the central platform, facing a closed arm, and allowed to explore the maze freely for 5 min. The Biobserve Viewer III tracking software provided by Biobserve GmbH, Germany, was employed to record locomotor activity during the test, including the distance covered in both the open and closed arms, the number of entries into the closed and open arms, and the time spent in each type of the arm as well as in the center.

2.2.3 | LDB

In the LDB test, a white acrylic box measuring 50 × 50 cm was utilized. The box was divided by a white acrylic partition, with one-third of the total area covered from the top and designated as the "dark chamber" and the remaining two-thirds open and brightly illuminated at 100×, serving as the "light chamber." A small entry door (5 × 7 cm) within the partition allowed mice to move freely between these chambers.

Each mouse was introduced into the dark chamber, facing the end wall parallel to the partition. Over 5 min, the activity was recorded using Biobserve Viewer III, a software provided by Biobserve GmbH, Germany. Parameters measured included locomotor activity, indicated by the distance moved in centimeters, and the time spent (in seconds) in both the dark and light chambers. Additionally, the latency period for the first entry into the light chamber was recorded.

2.2.4 | NSF

Prior to the NSF test, animals were subjected to a 24-h food deprivation period. Following the food deprivation, each mouse was

positioned in the corner of a square white acrylic arena measuring 50×50×50 cm, with its orientation toward an outer wall. The arena was uniformly illuminated with white light at an intensity of 100×.

In the center of the arena, a food pellet was placed. Video recordings of the mouse's behavior were captured and subsequently analyzed using Biobserve Viewer III, a software provided by Biobserve GmbH, Germany. The recorded parameters included the time (in seconds) it took for the mouse to commence eating after the 24-h fasting period and the distance it covered before initiating eating.

2.2.5 | FST

Each mouse underwent the forced swim test by being placed into a transparent glass cylinder measuring 17 cm in diameter and 18 cm in height, filled with water to a depth of 12 cm at a temperature of 25°C. This immersion lasted for 15 min, after which the mouse was returned to its home cage.

After a 24-h interval, the mice were once more introduced into the same water-filled cylinder for 5 min. During this session on the second day, the latency period for the initial occurrence of floating and the total duration of floating were recorded manually.

2.2.6 | SPT

Prior to the SPT, the animals were individually housed and were provided access to two water bottles for 7 days. On the eighth day, one of the water bottles was replaced with a 2% sucrose solution, and the position of the bottles containing water and the sucrose solution was altered daily over the subsequent 4 days.

Both before and after the test, the animals' weights were recorded, and the daily estimates of water and sucrose solution consumption were noted. The sucrose preference was calculated as a percentage of the total fluid intake as described previously (Kalinichenko et al., 2023; Kalinichenko, Mühle, et al., 2021; Müller et al., 2017).

2.3 | Alcohol-drinking phenotype in the free-choice unlimited access paradigm

Naïve mice of both genders (early adulthood: A53T—$n=4$ males/5 females; wt—$n=4$ males/6 females; late adulthood: A53T—$n=16$ males; wt—$n=15$ males) were individually housed with two constantly available drinking bottles for 2 weeks prior to the initiation of alcohol exposure. Each cage was equipped with two bottles, one containing tap water and the other containing alcohol at various concentrations. The positions of the bottles were changed daily. Following an acclimatization phase to establish a drinking baseline, the animals were exposed to alcohol at increasing concentrations of 2, 4, 8, 12, and 16 vol.% for 4 days each. Subsequently, the alcohol concentration was maintained at 16 vol.% for 8 days.

To assess the alcohol deprivation effect, alcohol was withdrawn for 3 weeks, during which both bottles contained tap water, before alcohol was reintroduced at a concentration of 16 vol.% for 4 days. This procedure was repeated once more. The bottles were changed and weighed on a daily basis. The amount of alcohol consumed relative to body weight and the preference for alcohol over water were measured (Kalinichenko et al., 2023; Kalinichenko, Mühle, et al., 2021; Müller et al., 2017).

2.4 | Taste preference test

Alcohol-experienced animals, which had been exposed to two-bottle free-choice unlimited access for a total of 42 free-choice drinking days, were employed for this test. Sucrose preferences (0.5% and 5%; Cat. No. S9378, Sigma) and quinine preferences (2 mg/dL and 20 mg/dL; Cat. No. W297607, Sigma) were assessed in a two-bottle free-choice test against water, conducted 3 days after their last exposure to alcohol. Each dose of sucrose and quinine was provided for 3 days, with the positions of the bottles being switched and their weights recorded daily. A 1-day washout period was observed between the sucrose and quinine testing phases (Kalinichenko et al., 2023; Kalinichenko, Mühle, et al., 2021; Müller et al., 2017).

2.5 | Alcohol-drinking phenotype in the intermittent alcohol consumption paradigm

A distinct alcohol consumption phenotype can be observed with intermittent access schedules, where alcohol is available during three 24-h periods each week. This protocol is proposed as a model for human binge-like alcohol drinking and is intended to reflect the mechanisms underlying alcohol-dependent behaviors in humans (Smutek et al., 2014). Naïve mice of both sexes (early adulthood: A53T—$n=5$ males/5 females; wt—$n=5$ males/5 females) were individually housed with two constantly available drinking bottles for 2 weeks before the commencement of alcohol exposure. Subsequently, a 20 vol.% alcohol solution was introduced for 6 weeks within an intermittent access schedule. Under this schedule, animals received alcohol in one bottle only on Monday, Wednesday, and Friday of each week, with a 24-h access period. Following this period, alcohol was withheld for 3 weeks before being reinstated for 1 week. This procedure was repeated three times.

During the third reinstatement, mice were exposed to forced swimming stress by being placed into a transparent glass cylinder (17 cm in diameter, 18 cm in height) filled with water (12 cm deep, 25°C) for 15 min. This stress-inducing procedure was repeated three times, occurring on Monday, Wednesday, and Friday, directly before the reintroduction of the 20% alcohol bottle into the cage. The bottles were changed and weighed daily, and the amount of alcohol consumed relative to body weight and the preference for alcohol over water were measured (König et al., 2020).

2.6 | Blood alcohol determination

Naïve mice of both sexes (early adulthood: A53T—$n=11$ males/6 females; wt—$n=8$ males/13 females; late adulthood: A53T—$n=10$ males/8 females; wt—$n=11$ males/9 females) were administered an alcohol injection (3.0 g/kg, i.p.). Subsequently, 20-μL blood samples were collected from the submandibular vein at 1, 2, and 3 h after the injection, without the use of anesthesia. These blood samples were immediately mixed with 80 μL of 6.25% (w/v) trichloroacetic acid (Cat. No. T4885, Sigma). After centrifugation, 15 μL of the supernatant was subjected to enzymatic alcohol determination using the alcohol dehydrogenase method, as described elsewhere (Zheng et al., 2016).

2.7 | Sphingolipid regulatory enzyme analysis

The brains of naïve mice of both sexes (early adulthood: A53T—$n=11$ males/6 females; wt—$n=8$ males/13 females; late adulthood: A53T—$n=10$ males/8 females; wt—$n=11$ males/9 females) were isolated, frozen in dry ice, and stored at −80°C. Subsequently, the prefrontal cortex (PFC), dorsal striatum (DS) and ventral striatum (VS), dorsal (DH) and ventral hippocampus (VH), dorsal (DM) and ventral mesencephalon (VM), amygdala, and cerebellum were isolated. The activity of enzymes responsible for ceramide generation from sphingomyelin (acid and neutral sphingomyelinases, ASM and NSM), enzymes involved in ceramide degradation to sphingosine [acid ceramidases (AC) and neutral ceramidases (NC)], and sphingomyelin (sphingomyelin synthase, SMS) in these brain structures was determined. For this purpose, we utilized the fluorescence substrate BODIPY-FL-C12-SM (D-7711, Thermo Fisher Scientific, Waltham, MA, USA) for ASM and NSM, and NBD-C12-ceramide (Cat. No. Cay24330-100, Cayman, obtained from Biomol, Hamburg, Germany) for AC and NC, as previously described (Mühle & Kornhuber, 2017). Similarly, SMS activity was evaluated using a newly developed method. SMS activity was quantified in a mixture of 50 mM Tris–HCl pH 7.4 (Cat. No. 1185-53-1, Sigma), 25 mM KCl (Cat. No. P9541, Sigma), 0.5 mM EDTA pH 8 (Cat. No. 6381-92-6, Sigma) with 1 μM C6-NBD-Ceramide (Cay62527, Cayman, obtained from Biomol, Hamburg, Germany), and 0.1 mM phosphatidylcholine as substrates. A solvent mixture of chloroform/methanol/ammonium hydroxide/water (25% NH_3 in water; 70:30:4:1, v/v/v/v; Cat. No. 650498, 1.06008, 30 501-M, respectively, Sigma) was used for thin-layer chromatography to separate the labeled sphingomyelin product from unreacted ceramide. All activity assays were conducted with four replicates for each sample and were normalized to protein concentrations. Enzymatic activities were calculated as the hydrolysis rate of sphingomyelin or ceramide (pmol or fmol, respectively) per unit of time (h) and per unit of protein (μg) (Huston et al., 2016; Kalinichenko, Abdel-Hafiz, et al., 2021).

2.8 | Statistical analyses

The data were analyzed using two-way analysis of variance (ANOVA), with repeated measures applied where appropriate, as well as two-tailed *t*-tests (the data were not assessed for normality; IBM SPSS Statistics 28.0.0.0). Group sizes were determined in advance based on previous studies (Kalinichenko et al., 2023; Kalinichenko, Mühle, et al., 2021; Müller et al., 2017). Randomization could not be performed as a result of genotype-based grouping. Data analysis was conducted by an investigator who was blind to the experimental conditions, age, gender, and genotype of the animals. Outlier control was carried out using mean ± 2 standard deviations using one main parameter of each test (e.g., center time in the OF, latency of eating in the NSF test, and alcohol consumption in the drinking studies) (Berger & Kiefer, 2021), resulting in the exclusion of six animals from the analysis. Pre-planned comparisons using Bonferroni-corrected LSD-tests (Ramsey, 1993) were calculated for single-group and time-point effects, with a significance threshold set at $p<0.05$.

3 | RESULTS

3.1 | Behavioral and neurochemical changes in A53T transgenic mice in early adulthood

3.1.1 | Intact emotional status in female, but not male mice in young adulthood

Anxiety and depression often co-occur with PD and are frequently diagnosed as early as the prodromal stage of the disorder (Qamhawi et al., 2015; Shiba et al., 2000). In this study, we aimed to investigate whether affective states develop in young adult animals carrying the familial PD human *A53T* mutation, starting as early as 8 weeks of age.

In young female A53T mice, we did not observe a pronounced anxiety-like phenotype in various behavioral tests, including the OF, EPM, and LDB tests. In the OF test, there were no genotype-specific differences in terms of the time spent in the center, the number of visits to the center, visit latency, and center and relative locomotion in female mice (Figure 2a,b; Figure S1; Table 1). Furthermore, the total locomotion did not show any alterations in A53T mice compared to their wild-type (wt) littermates, indicating preserved motor function (Figure S1). Similarly, in both the EPM and LDB tests, none of the parameters we studied exhibited differences between female A53T mice and wildtype mice (Figure 2c,d; Figure S1).

Consistent with these findings, we did not observe any significant depression-like behavior or anhedonia in young female A53T mice. In the forced swim test (FST), there were no differences in the latent period of first floating or the total floating time between A53T and wildtype mice (Figure 2e,f). In the NSF test, although the track length passed before the first eating tended to increase in young female A53T mice ($t=-2.922$, $p=0.080$), the latency of the first eating

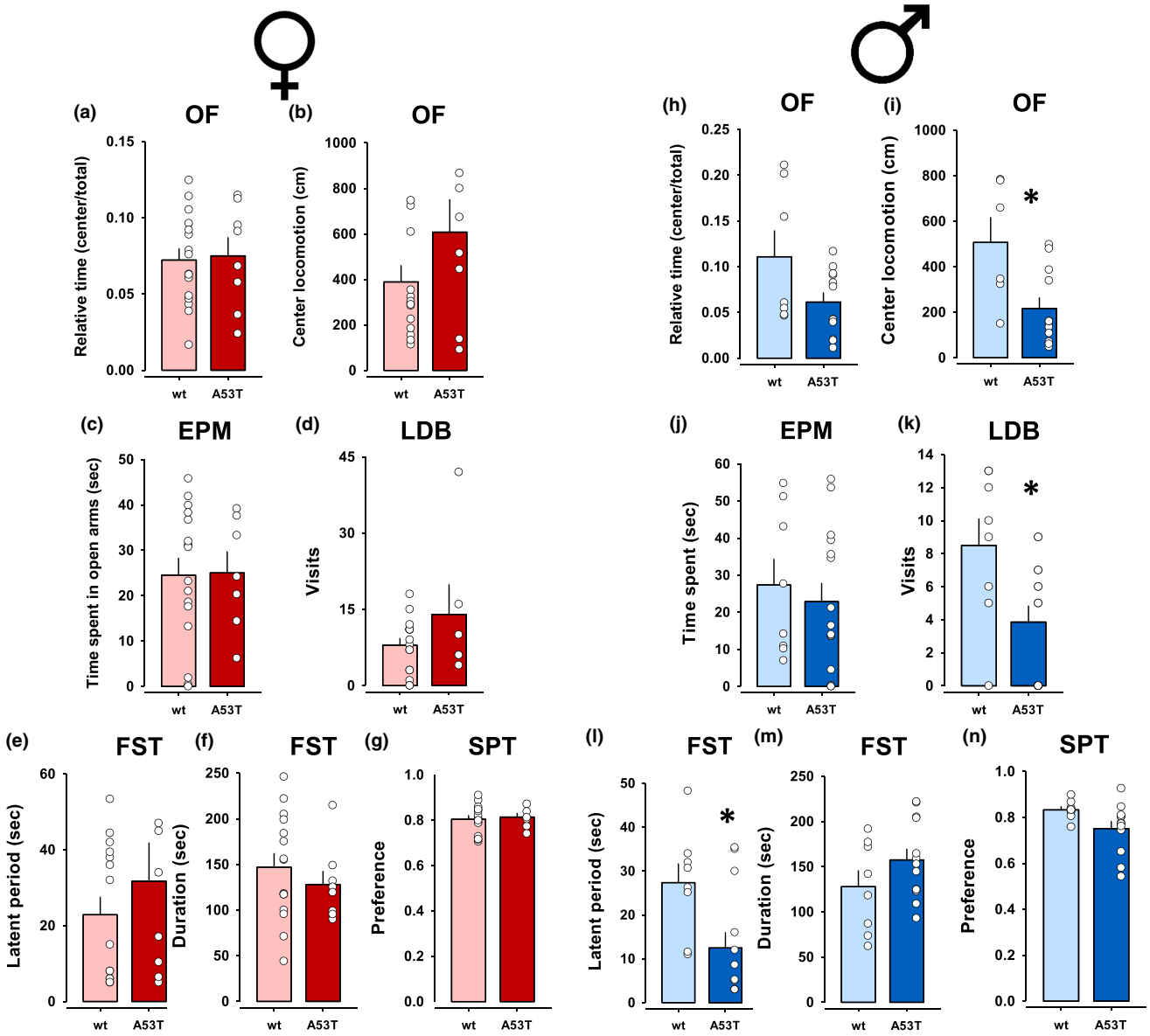


FIGURE 2 Emotional behavior of female, but not male A53T mice in early adulthood. In young female A53T mice, no changes in relative time spent in the center of the open field (OF) and center locomotion (a, b), time spent in the open arms of the elevated plus maze (EPM) (c), number of visits to the light compartment of the light–dark box (LDB) (d), latent period (e) and duration of floating (f) in the forced swim test (FST), and sucrose preference in the sucrose preference test (SPT) (g) were observed ($p > 0.05$). Young male A53T mice were characterized by the diminished relative time ($t = 0.459$, $p = 0.052$; (h) and locomotion in the center ($t = 2.592$, $p = 0.019$; (i) of the OF and number of visits in the light compartment of the LDB ($t = 2.460$, $p = 0.030$; (k). The time spent in the open arms of the EPM tended to decrease in male A53T mice ($p > 0.05$; (j). Reduced latent period of floating in the FST ($t = 2.653$, $p = 0.016$; (l) and slightly diminished sucrose preference in the SPT ($t = 1.977$, $p = 0.064$; (n), but not duration of floating in the FST ($p > 0.05$; m) indicated depression-like behavior in male A53T mice at the early adulthood of PD. Animal number: A53T—$n = 14$ males/8 females; wt—$n = 8$ males/16 females.

was similar in A53T and wt littermates (Figure S1). Additionally, in the SPT, no genotype-specific differences were observed in sucrose preference (Figure 2g). Therefore, we did not observe any alterations in the emotional phenotype of female A53T mice during early adulthood.

In contrast to the findings in female mice, young male A53T mice exhibited an enhanced anxiety-like phenotype. We observed a reduction in both the center and total track length in the OF test in young male A53T mice compared to their wild-type (wt) littermates (Figure 2i; Figure S2; Table 1). There was also a tendency for a decrease in the relative time spent in the center of the OF in A53T mice (Figure 2h). In the LDB test, we recorded a pronounced reduction in the time spent, the number of visits, and track length in the light compartment for young male A53T mice (Figure 2k; Figure S2). However, we did not observe any significant genotype-specific differences in all the parameters studied in the EPM (Figure 2j; Figure S2). It is important to note that the locomotion of male A53T mice in the EPM was not altered (Figure S2).

TABLE 1 Emotional and alcohol consumption phenotype of A53T mice at early and late adulthood compared to wild-type littermates.

		Adulthood	
Sex	**Phenotype**	**Early**	**Late**
Females	Anxiety-like behavior	–	–
	Depression-like behavior	–	–/↓
	Alcohol consumption		
	• Constant access	–	n.t.
	• Intermittent access	↓	
	• Taste preference	–	
	• BAC	–	
Males	Anxiety-like behavior	↑	↑
	Depression-like behavior	↑	↑
	Alcohol consumption		
	• Constant access	↓	↓
	• Intermittent access	↑	n.t.
	• Taste preference	–	–/↓
	• BAC	–	–

Abbreviations: –, no changes; ↑, increase; ↓, decrease; BAC, blood alcohol concentration; n.t., not tested.

In the FST, young male A53T mice exhibited a reduced latency to first floating (Figure 2l) compared to their wt counterparts. However, the duration of floating only showed a tendency to increase in A53T mice (Figure 2m). Similarly, sucrose preference in the SPT tended to decrease in young male A53T mice (Figure 2n). Nevertheless, no significant genotype-specific differences were observed in the latency to first eating and track length before eating in the NSF test (Figure S2). Therefore, unlike female mice, young male A53T mice displayed enhanced depression-like behavior and a mild anhedonic state. In summary, even at an early adulthood stage, anxiety and depression-like behaviors were observed in male A53T mice, while the emotional behavior of female mice remained unchanged.

3.1.2 | Young A53T transgenic males, but not females, are characterized by reduced alcohol consumption

Previous studies have indicated the role of PD point mutations, such as *SNCA* and *A30T*, in alcohol use disorder (Liang et al., 2003; Rotermund et al., 2017). In this experiment, we tested whether A53T mice exhibit altered alcohol consumption during ongoing unlimited access drinking and during relapse to alcohol drinking after a period of abstinence, using a two-bottle free-choice drinking paradigm (König et al., 2020). In young female mice, no genotype-specific differences were observed in alcohol consumption, alcohol preference, and water intake (Figure 3a,b; Figure S3; Table 1). The alcohol deprivation effect (ADE) was slightly diminished in young female A53T mice compared to wt littermates. A pre-planned comparison showed a significantly reduced alcohol consumption in A53T females only on reinstatement day 6 compared to wt mice (Figure 3c). Additionally, genotype-driven differences in sucrose preference and quinine avoidance were not found across all studied doses (Figure S3). Overall, in early adulthood, female A53T mice exhibit an intact alcohol consumption pattern but a slightly attenuated sensitivity to the reinstatement of drinking after withdrawal.

In young adult males, the A53T mutation was associated with significantly lower alcohol consumption compared to wt animals, reaching statistical significance at an alcohol concentration of 16% (Figure 4a). The ADE was not altered in young adult male A53T mice compared to wt littermates (Figure 4c). Conversely, during the first reinstatement period, young male A53T mice exhibited an enhanced alcohol preference, but this effect was not observed during the second reinstatement period (Figure S3). Analysis of taste preference did not reveal any genotype-driven differences in the preference for sucrose or avoidance of quinine at all studied doses (Figure S3). In summary, adult male A53T mice exhibit reduced alcohol consumption but maintain intact sensitivity to the reinstatement of drinking after withdrawal.

3.1.3 | Reduced alcohol consumption in A53T female but not male mice during intermittent alcohol access

A distinct alcohol consumption phenotype may develop under limited access where alcohol is repeatedly available but only for a limited time (24 h, three times a week). This model represents situations in small urban or rural environments where access to alcohol is restricted to specific time windows (Heath, 2000). Additionally, alcohol consumption is often used as a coping strategy to reduce stress, which can lead to excessive alcohol intake (McGrath et al., 2016). Here, we investigated whether the A53T mutation is associated with altered alcohol consumption during intermittent access, relapse to alcohol drinking, and after repeated stress episodes.

In young adult female mice, the *A53T* mutation was accompanied by a significantly lower average daily alcohol consumption (Figure 3d) and preference (Figure S3; Table 1), but not water consumption (Figure 3e). Alcohol withdrawal induced opposite changes in alcohol consumption between young A53T and wt animals after reinstatement. A pre-planned comparison revealed that alcohol consumption in female A53T mice significantly increased during the first reinstatement period but returned to baseline levels during the second reinstatement period (Figure 3f). Conversely, in female wt mice, alcohol consumption dropped during alcohol reinstatement, with these changes reaching significance only during the second reinstatement period (Figure 3f; Table 1). A similar ADE effect was observed in the alcohol preference of A53T mice after reinstatement (Figure S3), while water intake did not significantly change in female A53T and wt mice after reinstatement (Figure S3). Interestingly, exposure to stress in a forced swim paradigm directly before reinstatement nullified the observed differences, and no genotype-induced

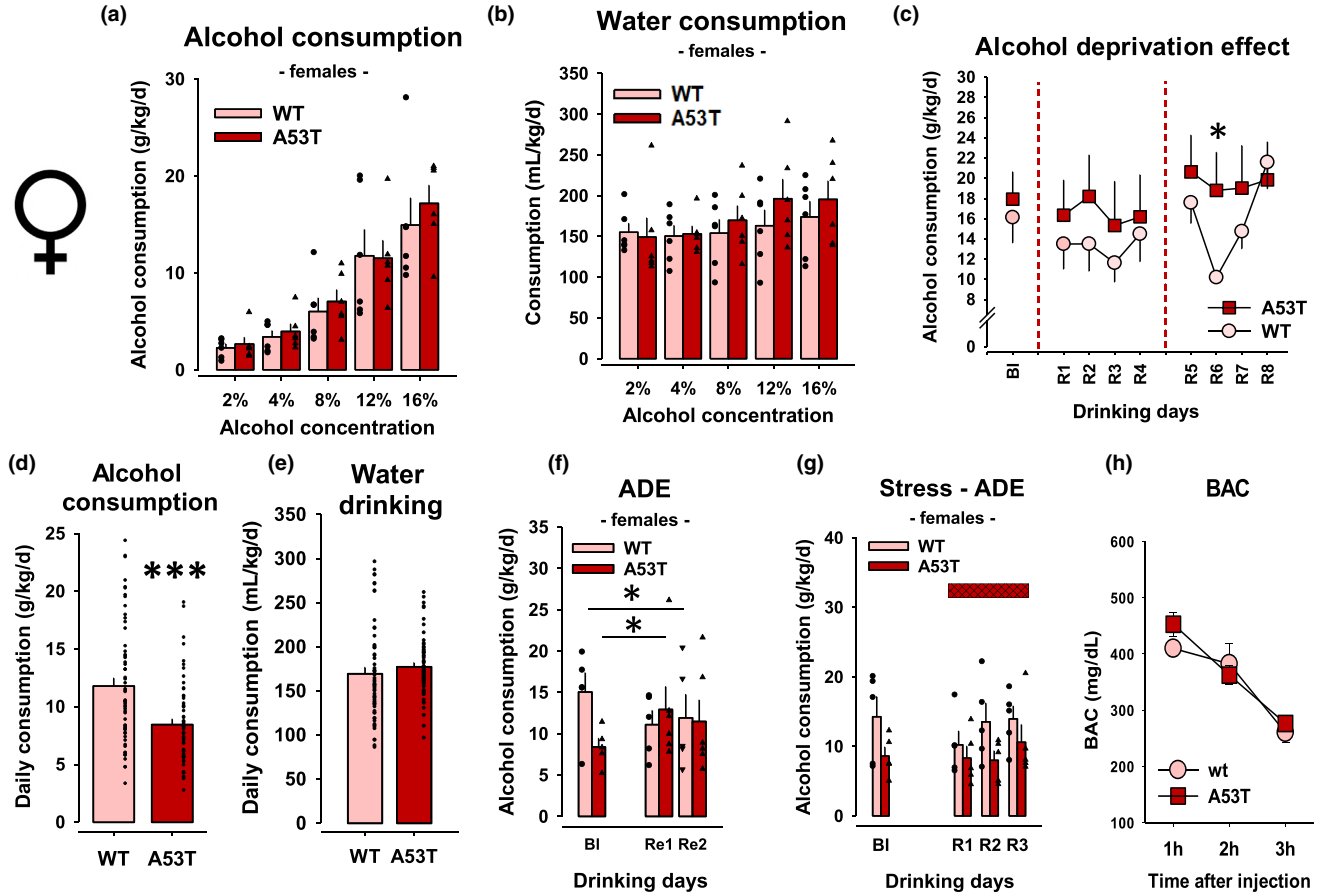


FIGURE 3 Alcohol consumption behavior of A53T females in early adulthood. In the model of constant access to alcohol, intact alcohol (genotype: $F(1, 10)=0.036$, $p=0.854$; concentration: $F(4, 40)=43.712$, $p<0.0001$; genotype × concentration interaction: $F(4, 40)=0.864$, $p=0.4933$), (a) and water consumption (genotype: $F(1, 10)=0.412$, $p=0.535$; concentration: $F(4, 40)=3.445$, $p=0.016$; genotype × concentration interaction: $F(4, 40)=0.855$, $p=0.498$; Figure 2a,b; (b), but slightly attenuated sensitivity for the reinstatement of drinking after withdrawal (genotype: $F(1, 9)=0.359$, $p=0.564$; time: $F(12, 108)=11.085$, $p<0.001$; genotype × time interaction: $F(12, 108)=2.528$, $p=0.006$; R6: $p=0.033$; (c) was observed in female A53T mice. Animal number: A53T—$n=5$; wt—$n=6$. In the model of intermittent access to alcohol, young A53T female mice were characterized by reduced alcohol consumption ($t=-4.082$, $p<0.0001$; (d) but intact water intake ($p>0.05$; (e) Alcohol withdrawal induced opposite changes in alcohol consumption of young A53T and wt animals after reinstatement (genotype: $F(1, 28)=3.05$, $p=0.091$; time: $F(2, 56)=1.0059$, $p=0.3722$, genotype × time: $F(2, 56)=4.2927$, $p=0.018$; first reinstatement period: $p=0.028$ in A53T and $p>0.05$ in wt; second reinstatement period: $p>0.05$ in A53T and $p=0.043$ in wt; (f), while stress exposure overwrote these differences (genotype: $F(1, 8)=2.957$, $p=0.124$; time: $F(3, 24)=1.584$, $p=0.219$; genotype × time interaction: $F(3, 24)=0.798$, $p=0.507$; (g) Animal number: A53T—$n=5$; wt—$n=5$. No genotype-specific differences were observed in the blood alcohol concentration (BAC) of young female mice (genotype: $F(1, 17)=0.019$, $p=0.893$; time: $F(2, 34)=70.999$, $p<0.001$; genotype × time: $F(2, 34)=0.360$, $p=0.700$; (h). Animal number: A53T—$n=6$; wt—$n=13$.

differences in alcohol consumption, alcohol preference, and water consumption were observed under these conditions (Figure 3g; Figure S3). In summary, young adult female A53T mice exhibit reduced alcohol consumption and preference in the model of intermittent drinking. However, alcohol withdrawal induced an increase in alcohol consumption and preference in transgenic mice but not in wt mice. This increase was diminished after exposure to stress.

Young adult male A53T mice exhibited enhanced average daily alcohol consumption and preference in the model of intermittent drinking (Figure 4d; Figure S3; Table 1). Average daily water intake under these conditions was reduced (Figure 4e). Alcohol deprivation led to a reduction in alcohol consumption at both reinstatement episodes in young male A53T mice but not in wt mice (Figure 4f). Similarly, alcohol preference after withdrawal was also reduced in A53T males, reaching significance only during the first reinstatement period (Figure S3). However, a significant enhancement of this parameter during the first reinstatement episode was observed in wt animals, but not during the second reinstatement episode. Water consumption decreased in both wt and A53T mice after alcohol deprivation (Figure S3).

The effects of stress during reinstatement on alcohol consumption and preference were similar to the pattern of changes in non-stressed animals. Male A53T mice exhibited a significant reduction in alcohol consumption and preference, but not water intake, during all reinstatement days (Figure 4g; Figure S3). However, this effect was not as pronounced in wt males. Therefore, young adult male A53T mice are characterized by enhanced alcohol consumption

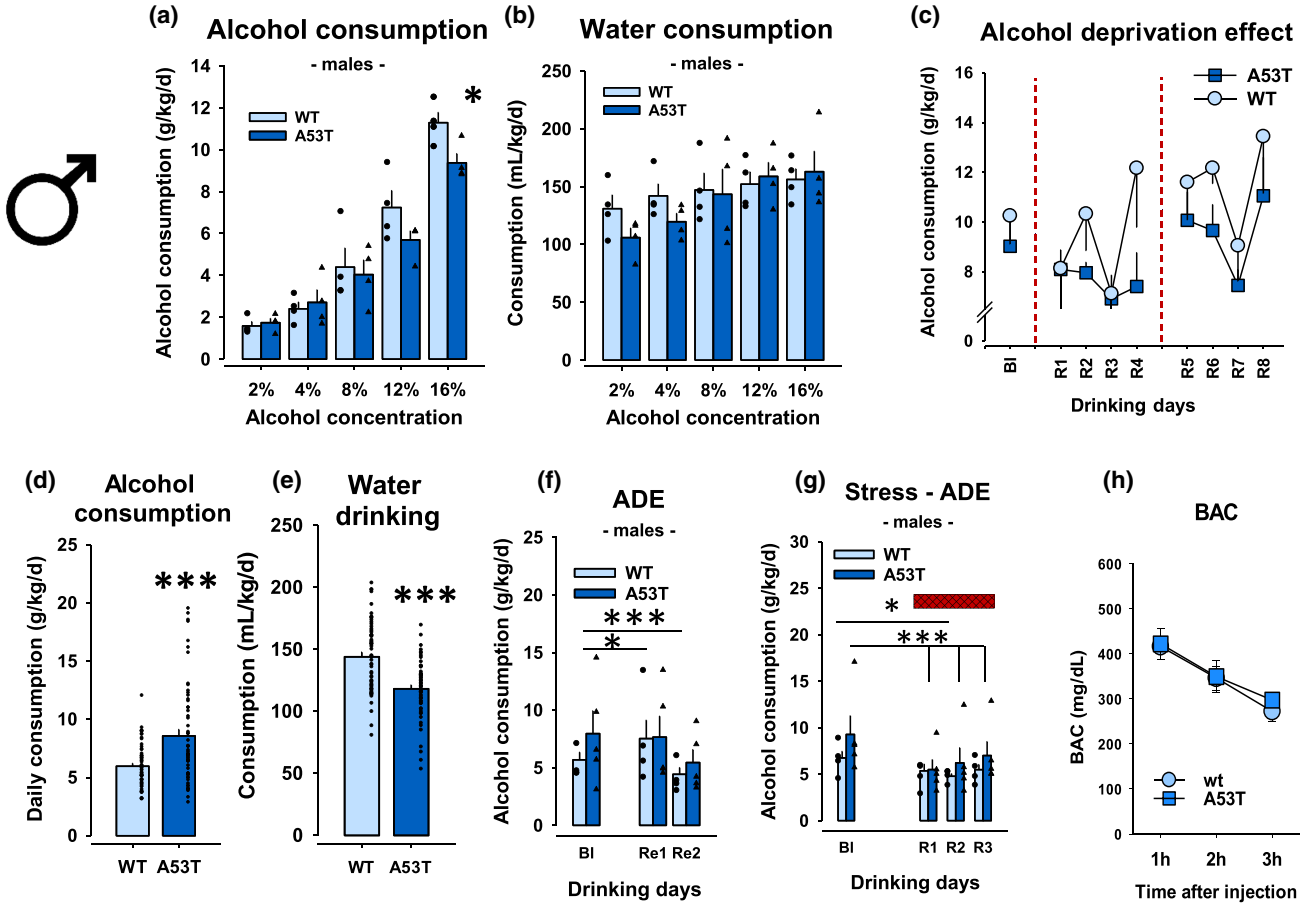


FIGURE 4 Alcohol consumption behavior of A53T males in early adulthood. In the model of constant access to alcohol, reduced alcohol (genotype: $F(1, 6) = 2.058$, $p = 0.2014$; concentration: $F(4, 24) = 211.272$, $p < 0.0001$; genotype × concentration interaction: $F(4, 24) = 4.1866$, $p = 0.010$; 16%: $p = 0.037$; (a) was observed in young adult male A53T mice. Water consumption (genotype: $F(1, 6) = 0.2311$, $p = 0.6477$; concentration: $F(4, 24) = 10.607$, $p < 0.0001$; genotype × concentration interaction: $F(4, 24) = 2.123$, $p = 0.1091$; (b) and sensitivity for the reinstatement of drinking after withdrawal (genotype: $F(1, 28) = 1.151$, $p = 0.292$; time: $F(2, 56) = 7.882$, $p = 0.001$; genotype × time interaction: $F(2, 56) = 3.415$, $p = 0.039$; (c) were not altered under these conditions. Animal number: A53T—$n = 4$; wt—$n = 4$. In the model of intermittent access to alcohol, enhanced alcohol consumption ($t = -4.308$, $p < 0.0001$ (d) but reduced water intake ($t = -5.480$, $p < 0.0001$; (e) was observed in young male A53T mice. The alcohol deprivation-induced reduction in alcohol consumption in A53T mice, both exposed (genotype: $F(1, 28) = 1.151$, $p = 0.292$; time: $F(2, 56) = 7.882$, $p = 0.001$; genotype × time: $F(2, 56) = 3.415$, $p = 0.040$; A53T: Re1: $p = 0.023$; Re2: $p = 0.001$; wt: $p > 0.05$; (f) and not exposed to stress (genotype: $F(1, 8) = 0.809$, $p = 0.395$; time: $F(3, 24) = 10.616$, $p = 0.0001$; genotype × time: ($F(3, 24) = 1.760$, $p = 0.182$; A53T: Re1: $p < 0.0001$; Re2: $p = 0.0004$; Re3: $p = 0.005$; wt: Re2: $p = 0.013$; (g). Animal number: A53T—$n = 5$; wt—$n = 5$. No genotype-specific differences were observed in the blood alcohol concentration (BAC) of young adult male mice (genotype: $F(1, 17) = 0.021$, $p = 0.887$; time: $F(2, 34) = 49.008$, $p < 0.001$; genotype × time interaction: $F(2, 34) = 1.516$, $p = 0.234$; (h). Animal number: A53T—$n = 11$; wt—$n = 8$.

and preference in the model of intermittent access. The alcohol deprivation-induced reduction in alcohol consumption was more pronounced in young adult A53T mice than in wt mice, both when exposed and not exposed to stress.

3.1.4 | Intact blood alcohol concentration in young adult A53T mice

In both young female and male A53T mice, we observed no significant genotype-specific differences in the blood alcohol concentration (BAC) (Figures 3h, 4h; Table 1). Therefore, the observed sex-specific changes in alcohol consumption are not related to differences in alcohol bioavailability.

3.1.5 | Brain region-specific changes in sphingolipid metabolic enzymes in early adulthood

Ceramides, their precursors, and the enzymes involved in their metabolism significantly contribute to the development of neurodegenerative diseases, depressive episodes, and drug use disorders (Gulbins et al., 2013; Kalinichenko et al., 2022, 2023; Kalinichenko, Hammad, et al., 2019; Kalinichenko, Mühle, et al., 2019, 2021;

Müller et al., 2017; Zoicas et al., 2020). In this study, we investigated whether changes in ceramide metabolism appear in A53T mice during early adulthood. We observed an overall brain region-specific increase in the activities of the studied enzymes. Specifically, the activities of ASM, NC, and SMS in the VM, ASM, and NSM in the DH, NSM in the DM and VH, as well as AC activity in the PFC of female A53T mice were higher than in wt littermates (Figure 5). However, no changes were observed in the cerebellum and amygdala (Figure 5j,k; Figure S4).

Conversely, the activity of NSM in the VM and AC in the PFC and the cerebellum was reduced in young male A53T mice (Figure 6; Figure S5). However, the activities of the other studied enzymes remained unaffected (Figure 6; Figure S5). These findings highlight the early effects of the A53T mutation on sphingolipid synthesis and metabolism, which vary based on brain region and sex.

3.2 | Behavioral and neurochemical changes in A53T transgenic mice in late adulthood

3.2.1 | Anxiety- and depression-like phenotype in male, but not female A53T mice in late adulthood

Similar to young animals, no alterations in anxiety-like behavior were observed in female A53T mice in late adulthood (from 24 weeks old). There were no genotype-driven differences in the time spent in the center of the OF, relative time, center track length, visits in the center, or visit latency in female mice. Notably, total locomotion in the OF was similar in older adult female A53T and wt mice (Figure S7), indicating the absence of PD motor symptoms.

In the EPM, a slight, albeit not statistically significant, reduction in the time spent and track length in the open arms, as well as relative locomotion and relative time, was observed in aged female A53T mice compared to wt mice (Figure 7c; Figure S6). Other parameters studied in the EPM test did not show genotype-specific differences (Figure S6). Likewise, no differences were found in the time spent in the light compartment of the LDB test, track length, visits, and visit latency between A53T and wt littermates (Figure 7d; Figure S6). These data indicate that anxiety-like behavior remained unchanged in aged female A53T mice.

Analysis of depression-like behavior in the FST, NSF, and SPT in older adult female A53T mice also did not reveal any significant genotype-driven differences (Figure 7e–g; Figure S6). Altogether, these data indicate that anxiety- and depression-like behavior remains intact in older female A53T mice.

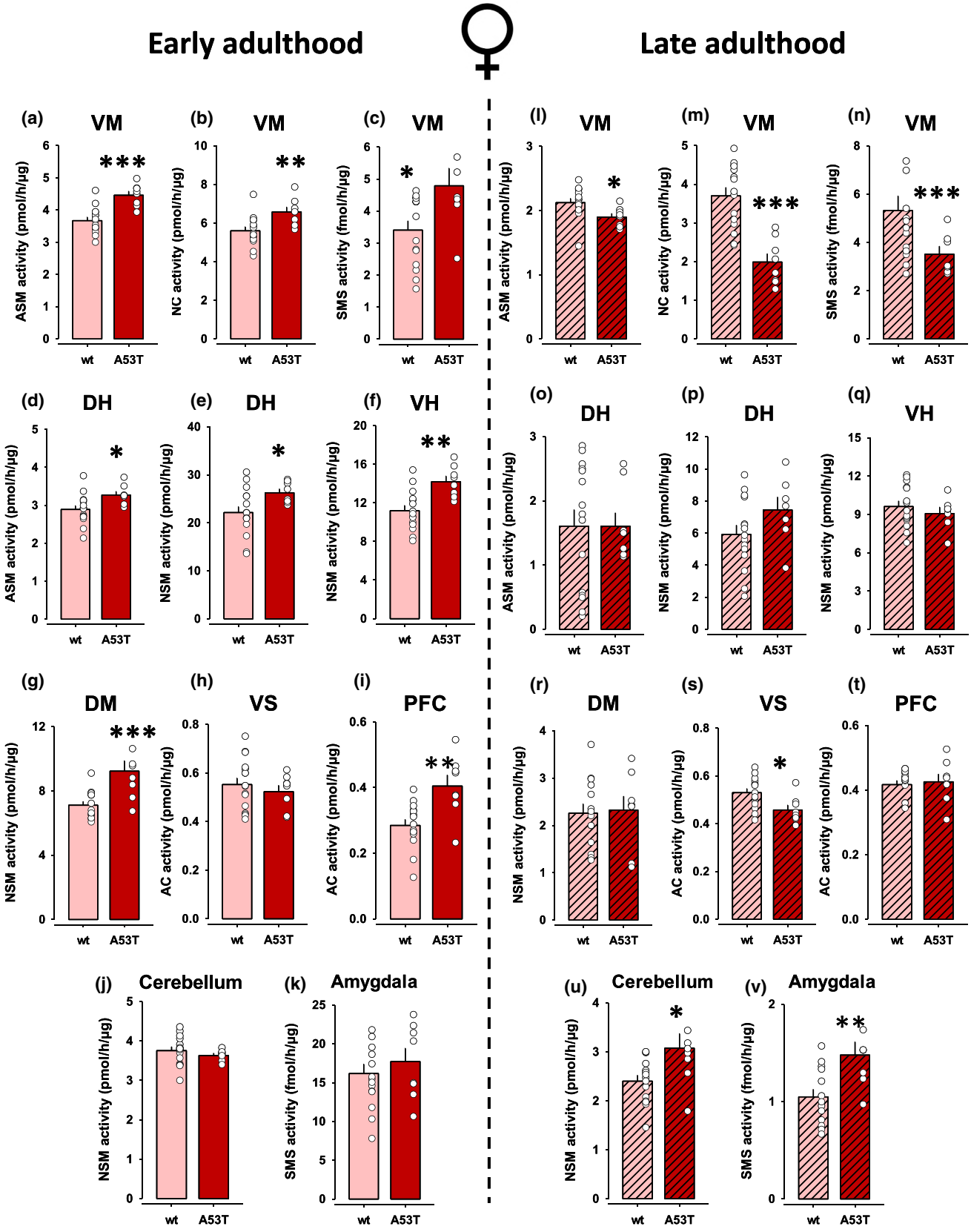
In older adult male A53T mice, pronounced anxiety-like behavior was observed in the EPM test. A53T males spent less time in and made fewer visits to the open arms compared to their wt littermates (Figure 7j; Figure S7; Table 1). Additionally, track length in the open arms and relative track length in the EPM were significantly reduced in A53T males, while relative time spent in these arms showed a slight decrease (Figure S7). However, no significant genotype-driven differences were observed in all the parameters studied in the OF and LDB tests (Figure 7h,i,k; Figure S7).

In the FST, older adult male A53T mice exhibited a significantly longer duration of floating, although the reduction in the latency of floating did not reach statistical significance (Figure 7l,m). In the NSF and SPT, the studied parameters did not differ between aged A53T males and wt littermates (Figure 7n; Figure S6). Altogether, similar to young adult A53T males, older adult male A53T animals displayed enhanced levels of anxiety and depression in late adulthood.

3.2.2 | Diminished alcohol consumption in male A53T mice in late adulthood

Aged male A53T mice were exposed to alcohol exposure using the two-bottle free-choice access model. We observed a significantly lower consumption of alcohol, which reached statistical significance at the 16% concentration, in older adult male A53T mice compared to their wt littermates (Figure 8a; Table 1). However, alcohol preference and water consumption did not differ between male A53T and wt mice (Figure 8b; Figure S8). We did not observe genotype-related differences in the ADE either (Figure S8). The taste preference test did not reveal any differences in the avoidance of quinine at the doses of 2 and 20 mg/dL in older adult male A53T and wt mice (Figure 8c). However, the preference for 5% sucrose, but not 0.5% sucrose, was significantly lower in A53T animals (Figure 8c). Altogether, older adult male A53T mice are characterized by reduced consumption of high-concentration alcohol but maintain intact sensitivity for the reinstatement of alcohol drinking after withdrawal.

FIGURE 5 Activity of ceramide metabolizing enzymes in the brain areas of female A53T mice at the early (a–k) and late (l–v) adulthood. AC, acid ceramidase; Amy, amygdala; ASM, acid sphingomyelinase; Cereb, cerebellum; DH, dorsal hippocampus; DM, dorsal mesencephalon; NC, neutral ceramidase; NSM, neutral sphingomyelinase; PFC, prefrontal cortex; SMS, sphingomyelin synthase; VH, ventral hippocampus; VM, ventral mesencephalon; VS, ventral striatum. In young female A53T mice, an increase in the activities of ASM, NC, and SMS in the VM ($t = 4.660$, $p < 0.001$; $t = -2.973$, $p = 0.007$; $t = 2.517$, $p = 0.021$; a–c), ASM and NSM in the DH ($t = 2.543$, $p = 0.019$; $t = 4.120$, $p = 0.033$; d–e), NSM in the DM and VH ($t = 2.120$, $p < 0.001$; $t = 3.581$, $p = 0.002$; f–g), and AC in the PFC ($t = 3.508$, $p = 0.002$; i) were observed. In aged female A53T mice, a decrease in the activity of ASM ($t = -2.377$, $p = 0.028$), NC ($t = -5.149$, $p < 0.0001$), and SMS ($t = -2.013$, $p = 0.058$) in the VM (l–n), AC in the VS ($t = -2.691$, $p = 0.014$; s), and NSM in the cerebellum ($t = 2.521$, $p = 0.019$, u) was observed. In the Amy, the activity of SMS was significantly higher in A53T mice compared to wt animals ($t = 3.008$, $p = 0.007$; v). Animal number: early adulthood—A53T: $n = 6$; wt: $n = 13$; late adulthood—A53T: $n = 8$; wt: $n = 9$.



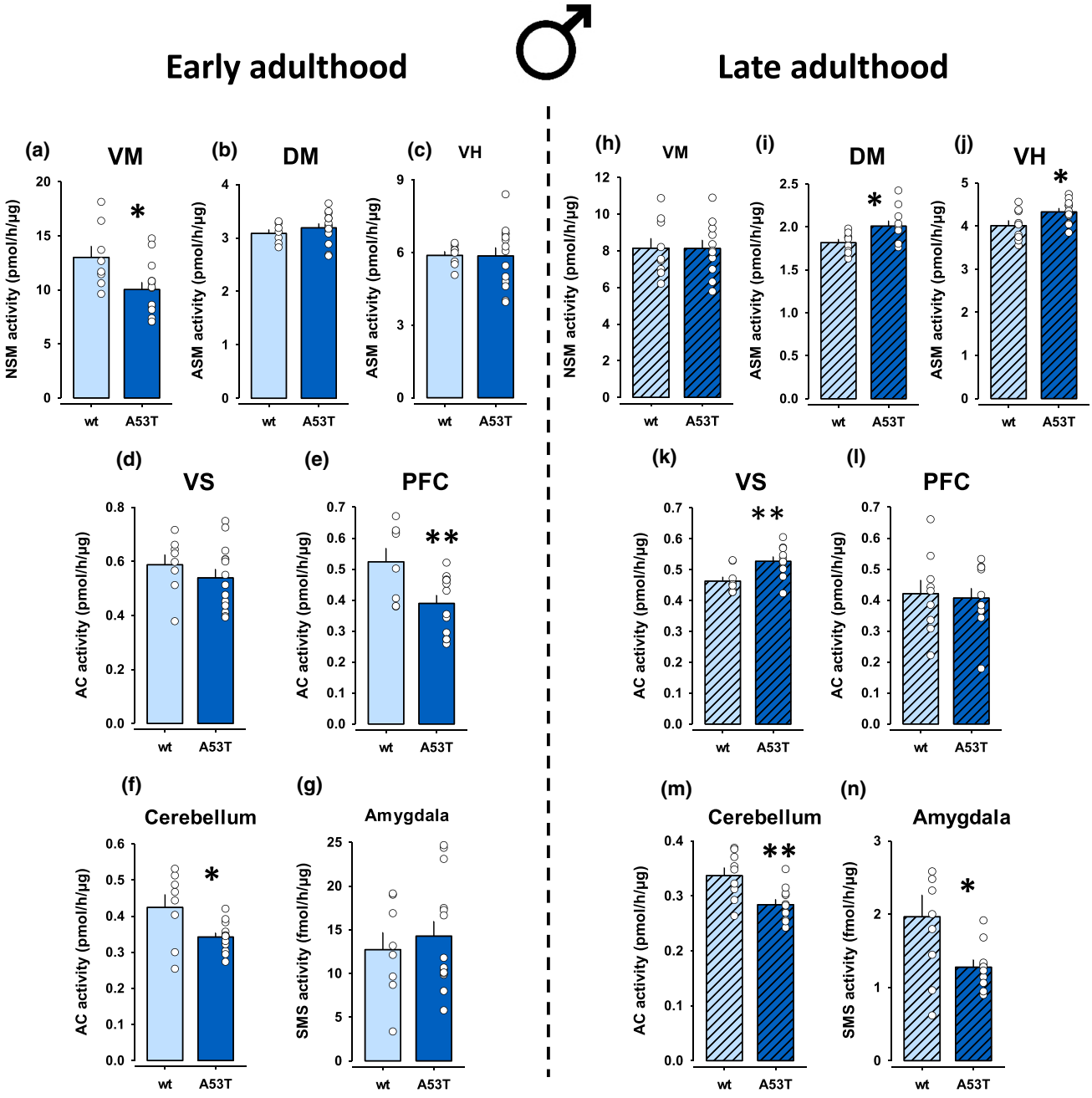


FIGURE 6 Activity of ceramide metabolizing enzymes in the brain areas of male A53T mice at the early (a–g) and late (h–n) adulthood. ASM, acid sphingomyelinase; NSM, neutral sphingomyelinase; AC, acid ceramidase; NC, neutral ceramidase; SMS, sphingomyelin synthase; VM, ventral mesencephalon; DM, dorsal mesencephalon; DH, dorsal hippocampus; VH, ventral hippocampus; VS, ventral striatum; PFC, prefrontal cortex; Cereb, cerebellum; Amy, amygdala. In young male A53T mice, a reduction in the activity of NSM in the VM ($t = -2.555$, $p = 0.019$ (a) and AC in the PFC and cerebellum ($t = -2.848$, $p = 0.010$; $t = -2.657$, $p = 0.016$; (e–f) was found. In older adult male A53T mice, an increase in the activity of ASM in the DM and VH ($t = 2.556$, $p = 0.019$ and $t = 2.270$, $p = 0.036$; i, j) and AC in the VS ($t = 3.137$, $p = 0.006$; k) was registered. The activities of AC in the cerebellum ($t = -3.197$, $p = 0.005$; m) and SMS in the amygdala ($t = -2.304$, $p = 0.034$; n) were reduced in male A53T mice. Animal number: early adulthood—A53T: $n = 11$; wt: $n = 8$; late adulthood—A53T: $n = 10$; wt: $n = 11$.

3.2.3 | Intact blood alcohol concentration in A53T mice of both genders

Similar to young mice, no differences in BAC were found in older adult A53T mice of both genders (Figure 8; Figure S8c; Table 1). Therefore, the observed changes in alcohol consumption in male A53T mice were not related to altered alcohol bioavailability.

3.2.4 | Brain region-specific changes in sphingolipid metabolism enzymes in late adulthood

In contrast to young animals, the activity of the studied enzymes involved in ceramide metabolism was mostly reduced in older female A53T mice. Specifically, the activities of ASM, NC, and SMS in the VM of older female A53T mice were diminished (Figure 5l–n). The

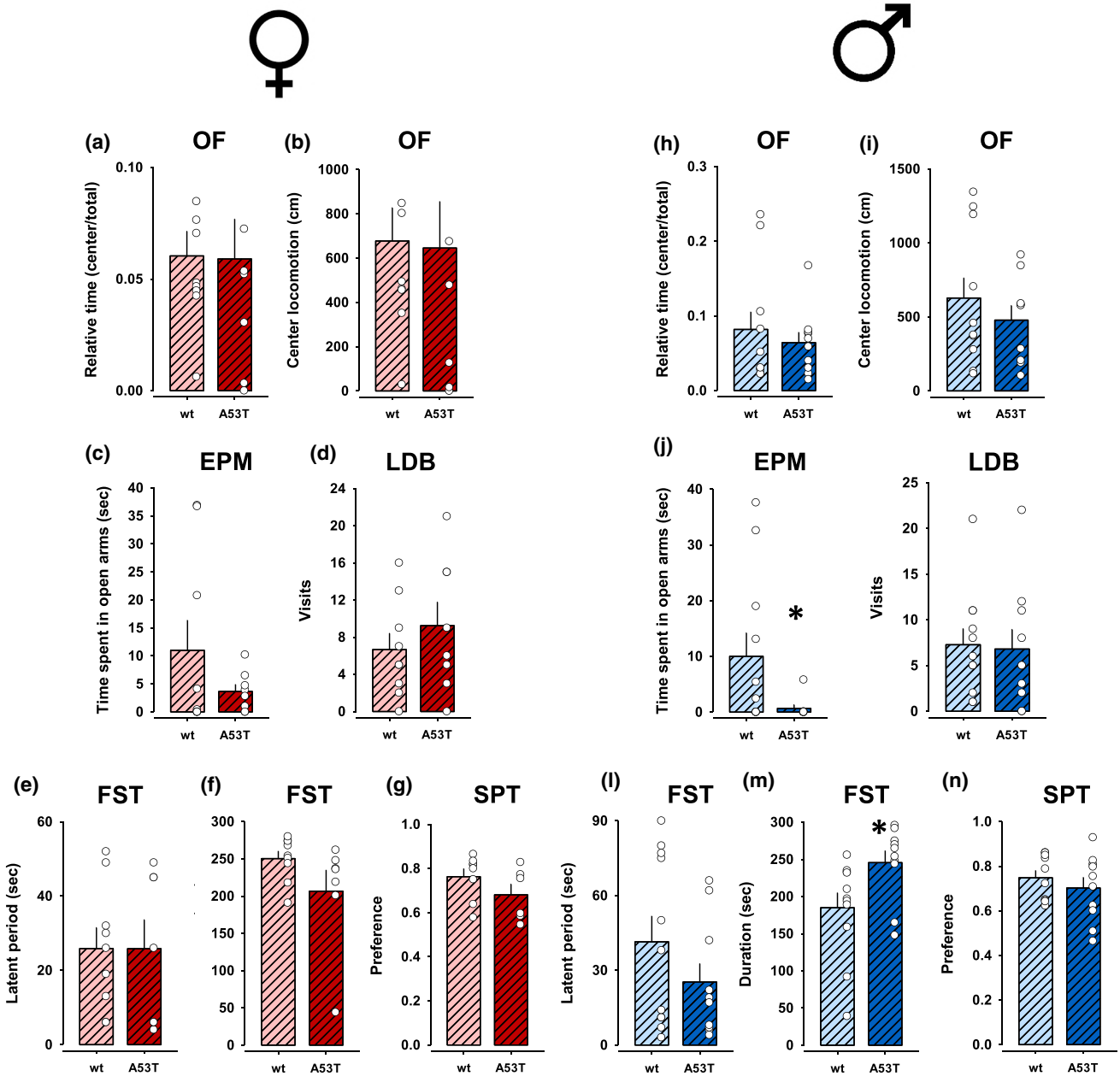


FIGURE 7 Altered emotional behavior in A53T males, but not females in late adulthood. In aged female A53T mice, no changes in relative time spent in the center of the open field (OF) and center locomotion (a, b), time spent in the open arms of the elevated plus maze (EPM) (c), number of visits to the light compartment of the light–dark box (LDB) (d), latent period (e) and duration of floating (f) in the forced swim test (FST), and sucrose preference in the sucrose preference test (g) were observed ($p > 0.05$). Animal number: A53T—$n = 8$; wt—$n = 9$. In aged male A53T mice, the relative time (h) and locomotion in the center (i) of the OF, as well as the number of visits in the light compartment of the LDB (k), tended to decrease ($p > 0.05$). The time spent in the open arms of the EPM was significantly reduced in male A53T mice ($t = -2.200$, $p = 0.050$; j). Increased duration of floating ($t = 2.382$, $p = 0.028$; m) and slightly reduced latency of floating ($t = -1.280$, $p = 0.109$; l) in the FST, but not or sucrose preference in the SPT ($p > 0.05$; n), indicated depression-like behavior in male A53T mice in late adulthood. Animal number: A53T—$n = 10$; wt—$n = 11$.

A53T mutation was associated with reduced AC activity in the VS and NSM activity in the cerebellum of these animals (Figure 5s,t). However, the activity of SMS in the amygdala was significantly higher in A53T mice compared to wild-type (wt) animals (Figure 5v; Figure S10; Table 2). No significant sex-specific differences in the activities of the studied enzymes were observed in the DH, ventral hippocampus (VH), dorsal mesencephalon (DM), and prefrontal cortex (PFC) of aged mice (Figure S10; Table 2).

In older adult male A53T mice, we observed enhanced activity of ASM in the DM and VH, as well as increased AC activity in the VS (Figure 6i–k; Table 2). However, the activity of AC was reduced in the cerebellum of A53T mice (Figure 6u). In the amygdala (Amy),

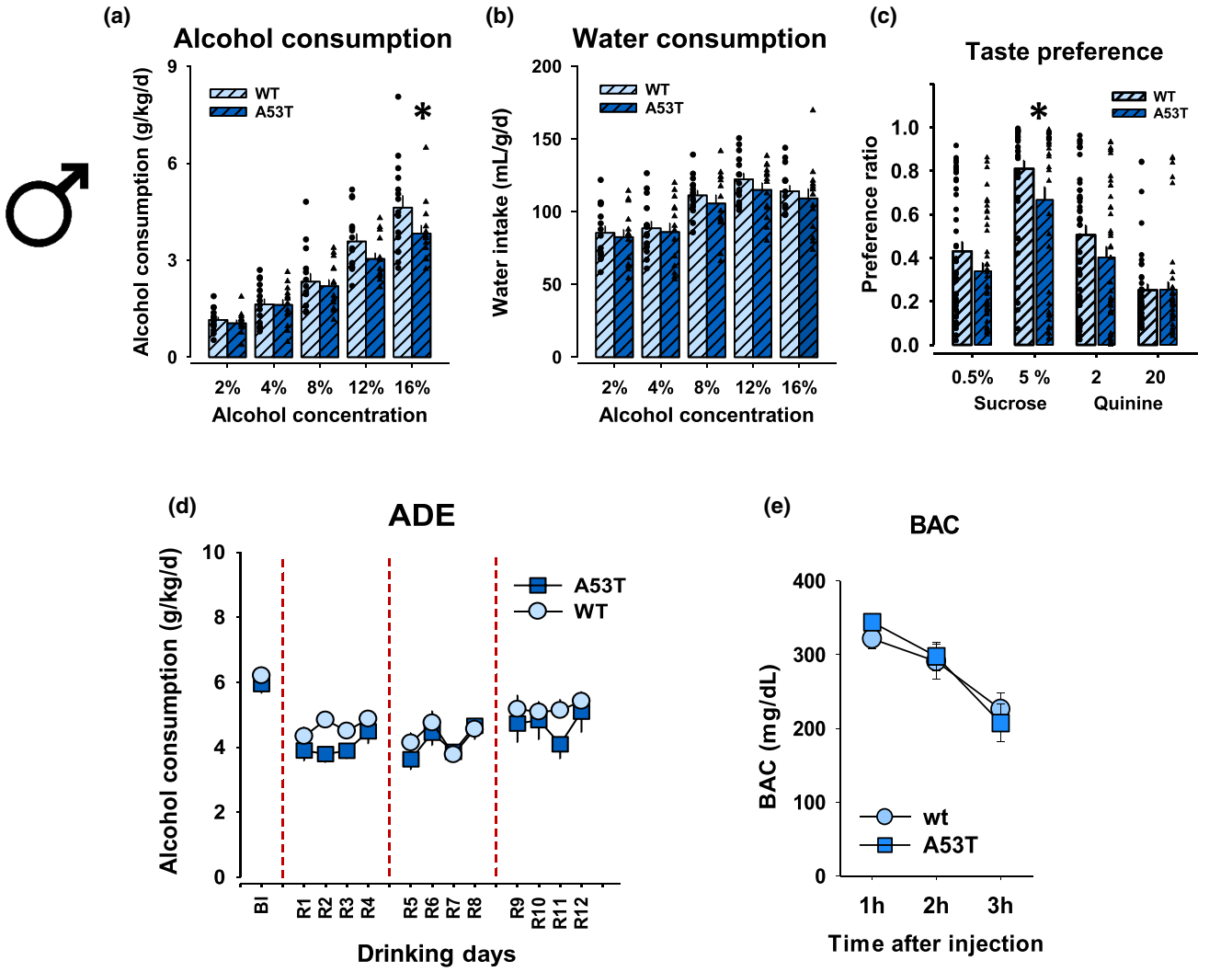


FIGURE 8 Alcohol consumption behavior at the constant access model in A53T males in late adulthood. Aged male A53T mice are characterized by a reduced consumption of alcohol of high concentration (genotype: $F(1, 27) = 1.7090$, $p = 0.2012$; concentration: $F(4, 108) = 146.1697$, $p < 0.0001$; genotype × concentration: $F(4, 108) = 2.6053$, $p = 0.0398$; 16%: $p = 0.011$; a), but intact water consumption (genotype: $F(1, 27) = 0.597$, $p = 0.446$; concentration: $F(4, 108) = 0.218$, $p = 0.928$; genotype × concentration: $F(4, 108) = 55.016$, $p < 0.0001$; b) and sensitivity for the reinstatement of alcohol drinking after withdrawal ($p > 0.05$; d). Preference of 5% sucrose ($t = 2.1076$, $p = 0.038$), but not aversion to quinine of both studied concentrations, was diminished ($t = 1.5832$, $p = 0.1171$ and $t = -0.0605$, $p = 0.9519$) in aged male A53T mice (c). Animal number: A53T—$n = 16$; wt—$n = 15$. No genotype-specific differences were observed in the blood alcohol concentration (BAC) of aged male A53T mice (genotype: $F(1, 19) = 0.151$, $p = 0.702$; time: $F(2, 38) = 25.246$, $p < 0.001$; genotype × time interaction: $F(2, 30) = 0.199$, $p = 0.820$; e). Animal number: A53T—$n = 10$; wt—$n = 11$.

the activity of SMS was slightly lower in male A53T mice compared to their wild-type (wt) littermates (Figure 6v). No significant sex-specific differences in the activities of the studied enzymes were observed in the DH, VH, DM, cerebellum, amygdala, and prefrontal cortex (PFC) of aged male mice (Figure S10).

4 | DISCUSSION

Familial cases of PD are caused by single-gene mutations, such as single-nucleotide polymorphisms in the *A53T* gene. One of the crucial effects of these mutations is the overexpression of α-synuclein, leading to the development of synucleinopathies, particularly PD (Klein & Westenberger, 2012; Nuber et al., 2013, 2018). However, early behavioral changes during the prodromal stages of PD, which occur before α-synuclein overproduction, might still be influenced by these mutations. In general, gene mutations can have pleiotropic effects, impacting both the adaptation and survival of individuals. In our study, we observed no significant changes in alcohol consumption and emotional traits in female A53T mice during both early and late adulthood prior to the onset of the disease. Brain-specific activation of ASM and NSM in these mice during young adulthood may have neuroprotective effects, potentially slowing down the disease progression. In A53T males, there was a decrease in alcohol

TABLE 2 Changes in the enzymes of the sphingomyelinase pathway of ceramide metabolism in A53T mice compared to wild-type littermates at early and late adulthood.

	Female A53T mice		Male A53T mice	
Brain structure	**Young adulthood**	**Late adulthood**	**Young adulthood**	**Late adulthood**
Ventral mesencephalon	↑ASM ↑NC ↑SMS	↓ASM ↓NC ↓SMS	↓NSM	–
Dorsal mesencephalon	↑NSM	–	–	↑ASM
Ventral hippocampus	↑NSM	–	–	↑ASM
Dorsal hippocampus	↑ASM ↑NSM	–	–	–
Ventral striatum	–	↓AC	–	↑AC
Prefrontal cortex	↑AC	–	↓AC	–
Cerebellum	–	↑NSM	↓AC	↓AC
Amygdala	–	↑SMS	–	↓SMS

Abbreviations: –, no changes; ↓, decrease; AC, acid ceramidase; ASM, acid sphingomyelinase; NC, neutral ceramidase; NSM, neutral sphingomyelinase; SMS, sphingomyelin synthase. ↑, increase.

consumption in a free-choice constant access alcohol-drinking model during both early and late adulthood. However, male A53T mice exhibited notable impairments in anxiety- and depression-like behavior during young and late adulthood. These changes in males were accompanied by completely different alterations in the ceramide metabolism compared to female mice. Therefore, we observed that the *A53T* gene mutation is associated with sex-specific changes in emotional and alcohol consumption behavior long before the onset of PD. These behavioral effects may be mediated by region-specific changes in brain ceramide metabolism (Zoicas et al., 2016), which could either protect against or promote the development of adverse neurochemical changes.

The *A53T* mutation was not associated with the changes in alcohol consumption behavior in female mice during both young and late adulthood. However, we did observe specific alterations in brain ceramide metabolism. The increased activity of NSM in the DM, DH, and VH regions of young adult female A53T mice might act as a protective mechanism against the early development of neuronal pathology typically associated with PD. NSM has been identified as a critical regulator of autophagy, which plays a vital role in safeguarding neurons from cellular damage, particularly dopaminergic toxicity (Back et al., 2018). NSM overexpression has been demonstrated to rescue autophagy-associated neurodegeneration in Drosophila blue cheese mutants (Hebbar et al., 2015). Conversely, a reduction in NSM activity in an MPTP-induced mouse model of PD led to increased neuroinflammation and oxidative stress (Cataldi et al., 2017). Similar neuroprotective effects have been reported for ASM, whose activity was heightened in the VM and DH regions of young adult female A53T mice. Diminished ASM activity has been linked to an elevated risk of PD (Gan-Or et al., 2015) and earlier onset of the disease (Alcalay et al., 2019). A study involving HeLa and BE(2)-M17 dopaminergic cells showed increased α-synuclein levels after *SMPD1* knockout or knockdown (Alcalay et al., 2019). Consequently, the substantially heightened activities of ASM and NSM in multiple brain regions of young adult female A53T mice might be proposed as a potential mechanism of the early stage of PD and may offer protective effects against early adverse neurochemical alterations in the brain.

However, it is important to note that the observed changes in sphingomyelinase activities may not fully account for the advantageous behavioral phenotype observed in female mice. Our previous data indicated that an increase in ASM activity resulted in an augmented depression- and anxiety-like phenotype and significantly increased alcohol consumption (Müller et al., 2017), while a reduction in NSM activity induced the opposite phenotype in female mice (Kalinichenko, Mühle, et al., 2021). Moreover, we cannot rule out the potential contribution of other enzymes involved in ceramide metabolism, such as AC, NC, and SMS, as well as enzymes in the *de novo* pathway, as their contribution to emotional and alcohol use behavior has not been extensively studied yet.

Conversely, the preserved emotional phenotype in late adulthood among female A53T mice was associated with different, and at times even opposing, changes in the activity of the examined enzymes. Specifically, the heightened ceramide metabolism observed in the VM of female A53T mice during early adulthood shifted to inhibition during late adulthood. These findings align with those of a recent study demonstrating that inhibiting the *de novo* pathway of ceramide synthesis significantly enhances α-synuclein toxicity in yeast cells expressing A53T (Lee et al., 2011). This interaction between ceramide synthesis and α-synuclein functioning could potentially serve as one of the pathogenetic mechanisms triggering the transition from late adulthood to the onset of PD. However, these data were not consistent with those of previous preclinical and cellular studies that have shown increased ceramide production and associated α-synuclein proteinopathy in PD (Kurzawa-Akanbi et al., 2021; Mingione et al., 2021). It is important to note that the vast majority of previous data were generated in cell cultures or animal models without gender segregation. Therefore, we

observed a fundamentally new ceramide-mediated mechanism specific to females, which might be related to PD pathogenesis.

In contrast to female mice, the *A53T* mutation in males was associated with impaired emotional status during both early and late adulthood. These findings align with those of a human twin study that demonstrated a link between trait neuroticism and the risk of PD development (Sieurin et al., 2016). On the contrary, the A53T mutation was accompanied by reduced alcohol consumption in the constant access model in both young and aged adult male *A53T* mice, which contradicts previous preclinical data from transgenic mice expressing human mutant [A30P]αSYN, indicating enhanced reinforcing effects of alcohol under operant self-administration conditions during PD development (Rotermund et al., 2017). Another critical gene implicated in PD development, SNCA, has been identified as a candidate gene for alcoholism (Levey et al., 2014). Single-nucleotide polymorphisms in this gene have been associated with increased alcohol-craving behavior (Agrawal et al., 2013) and responses to alcohol taste cues (Wilcox et al., 2013). However, several epidemiological and clinical studies have suggested an inverse relationship between alcohol use disorder and PD (Bharucha et al., 1986; Fall et al., 1999; Jiméanez-Jiméanez et al., 1992). Specifically, allele length variability of the dinucleotide repeat sequence within the α-synuclein gene promoter (*SNCA REP1*) has been linked to PD but does not correlate with alcohol consumption (Brighina et al., 2009). Similarly, reduced expression of α-synuclein in the dorsolateral prefrontal cortex of long-term alcoholics as a result of a shorter *REP1* allele of the *SNCA* gene indicates an inverse correlation between α-synuclein expression and alcohol consumption (Janeczek et al., 2014). Therefore, α-synuclein overexpression during proteinopathies does not necessarily lead to increased alcohol consumption.

Interestingly, unlike female mice, changes in the activities of the studied enzymes involved in ceramide synthesis in male mice may contribute to the observed emotional phenotype. While no neuroprotective increase in the activities of ASM and NSM was observed in young adult male mice, the *A53T* mutation-induced reduction in NSM activity in the VM might contribute to the development of the affective state already in early adulthood as previously shown in NSM knockout mice (Kalinichenko et al., 2023). However, this change does not explain the reduced alcohol consumption in these animals (Kalinichenko et al., 2023). Notably, the ceramide-based mechanism of anxiety and depression-like behavior in A53T mice appears to change with age. In late adulthood, the affective state, as well as reduced alcohol consumption, might be mediated by an increase in ASM activity in the DM and VH (Müller et al., 2017). This could be influenced by the negative effects of ASM overexpression on neurogenesis in mice (Gulbins et al., 2013, 2015).

Additionally, human *A53T* and *A30T* mutations in transgenic mice, even at the prodromal stages of PD (4–6 months), have been associated with impaired hippocampal neurogenesis, potentially leading to changes in emotional patterns (Kohl et al., 2012; Marxreiter et al., 2013). Previous studies have also observed profound dysfunction in the serotonergic system in different PD models (Deusser et al., 2015; Kohl et al., 2016; Wihan et al., 2019). Considering the interaction between enhanced ASM activity and reduced serotonin levels in several murine brain structures, as shown by Müller et al. (2017), ASM activation in male A53T mice in late adulthood might contribute to serotonin deficiency and associated anxiety- and depression-like behavior (Müller et al., 2017). On the other hand, ASM activation in several brain structures of male A53T mice in late adulthood might induce α-synuclein overproduction (Alcalay et al., 2019). These neurochemical changes might collectively contribute to the adverse emotional state and altered alcohol consumption phenotype. However, it should be noted that the observed changes in other enzymes involved in ceramide metabolism might also contribute to the pathogenesis of early stages of PD.

In our study, we observed gender-specific differences in PD pathogenesis that align with the sex-specificity observed in clinical data. Human studies have shown a significantly higher frequency of PD in men (Baldereschi et al., 2000; Solla et al., 2012), but women exhibit a higher mortality rate and faster progression of the disorder (Solla et al., 2012). Additionally, a positive association between anxiety and depression and PD was observed in male patients, but not in female patients (Jacob et al., 2010), which is consistent with our findings. Animal studies conducted on aged A53T mice also indicate reduced dopamine neuron degeneration and TH immunoreactivity in the striatum and substantia nigra in female mice compared to males (Costa et al., 2020; Sirabella et al., 2018). Male A53T mice, on the other hand, exhibit an acceleration of PD development characterized by enhanced neuroinflammation, microglia activation, degeneration of dopaminergic neurons, and reduced dopamine levels after stress (Wu et al., 2016). In summary, these data suggest that females demonstrate higher resistance to the development and manifestations of PD, which may be influenced by various mechanisms, including innate differences in ceramide metabolism (Kalinichenko, Abdel-Hafiz, et al., 2021; Kalinichenko et al., 2022, 2023; Kalinichenko, Mühle, et al., 2021).

In conclusion, the *A53T* mutation is linked to a range of changes in emotional responses and alcohol consumption behavior, which manifest long before the onset of PD symptoms. Notably, the protective effects of this mutation, specific to each sex, were observed already during early adulthood, likely contributing to its persistence within the population. The alterations in emotional responses and alcohol consumption behavior in *A53T* mice appear to be associated with brain ceramide metabolism. Nevertheless, a more comprehensive exploration of the various pathways involving ceramide transitions is necessary to establish early predictive criteria for the development of PD.

AUTHOR CONTRIBUTIONS

Liubov S. Kalinichenko: Data curation; formal analysis; funding acquisition; investigation; project administration; supervision; writing – original draft; writing – review and editing. **Zacharias Kohl:** Conceptualization; resources. **Christiane Mühle:** Formal analysis; investigation. **Zurina Hassan:** Investigation. **Agnes Hahn:** Investigation. **Eva-Maria Schmitt:** Investigation. **Kilian Macht:** Investigation. **Lyubomir Stoyanov:** Investigation.

Schayan Moghaddami: Investigation. **Roberto Bilbao Canalejas:** Investigation; methodology. **Volker Eulenburg:** Investigation. **Jürgen Winkler:** Conceptualization; resources. **Johannes Kornhuber:** Conceptualization; funding acquisition; resources. **Christian P. Müller:** Conceptualization; funding acquisition; resources; supervision.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) grants MU 2789/18-1 and KO 947/20-1, the Interdisciplinary Center for Clinical Research (project J93), and the Johannes und Frieda Marohn-Stiftung. Open Access funding enabled and organized by Projekt DEAL.

All experiments were conducted in compliance with the ARRIVE guidelines.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Liubov S. Kalinichenko https://orcid.org/0000-0003-0729-2299
Christian P. Müller https://orcid.org/0000-0002-5325-9900

REFERENCES

Agrawal, A., Wetherill, L., Bucholz, K. K., Kramer, J., Kuperman, S., Lynskey, M. T., Nurnberger, J. I., Schuckit, M., Tischfield, J. A., Edenberg, H. J., Foroud, T., & Bierut, L. J. (2013). Genetic influences on craving for alcohol. *Addictive Behaviors*, *38*, 1501–1508.

Alcalay, R. N., Mallett, V., Vanderperre, B., Tavassoly, O., Dauvilliers, Y., Wu, R. Y. J., Ruskey, J. A., Leblond, C. S., Ambalavanan, A., Laurent, S. B., Spiegelman, D., Dionne-Laporte, A., Liong, C., Levy, O. A., Fahn, S., Waters, C., Kuo, S. H., Chung, W. K., Ford, B., … Gan-Or, Z. (2019). SMPD1 mutations, activity, and α-synuclein accumulation in Parkinson's disease. *Movement Disorders*, *34*, 526–535.

Back, M. J., Ha, H. C., Fu, Z., Choi, J. M., Piao, Y., Won, J. H., Jang, J. M., Shin, I. C., & Kim, D. K. (2018). Activation of neutral sphingomyelinase 2 by starvation induces cell-protective autophagy via an increase in Golgi-localized ceramide. *Cell Death & Disease*, *9*, 670.

Baldereschi, M., Carlo, A., Di, R. W. A., Vanni, P., Maggi, S., Perissinotto, E., Grigoletto, F., Amaducci, L., & Inzitari, D. (2000). Parkinson's disease and parkinsonism in a longitudinal study: Two-fold higher incidence in men. *Neurology*, *55*, 1358–1363.

Berger, A., & Kiefer, M. (2021). Comparison of different response time outlier exclusion methods: A simulation study. *Frontiers in Psychology*, *12*, 675558.

Bharucha, N. E., Stokes, L., Schoenberg, B. S., Ward, C., Ince, S., Nutt, J. G., Eldridge, R., Calne, D. B., Mantel, N., & Duvoisin, R. (1986). A case-control study of twin pairs discordant for Parkinson's disease: A search for environmental risk factors. *Neurology*, *36*, 284–288.

Brighina, L., Schneider, N. K., Lesnick, T. G., de Andrade, M., Cunningham, J. M., Mrazek, D., Rocca, W. A., & Maraganore, D. M. (2009). α-Synuclein, alcohol use disorders, and Parkinson disease: A case–control study. *Parkinsonism & Related Disorders*, *15*, 430–434.

Cataldi, S., Arcuri, C., Hunot, S., Légeron, F.-P., Mecca, C., Garcia-Gil, M., Lazzarini, A., Codini, M., Beccari, T., Tasegian, A., Fioretti, B., Traina, G., Ambesi-Impiombato, F. S., Curcio, F., & Albi, E. (2017). Neutral sphingomyelinase behaviour in hippocampus neuroinflammation of MPTP-induced mouse model of Parkinson's disease and in embryonic hippocampal cells. *Mediators of Inflammation*, *2017*, 1–8.

Costa, G., Sisalli, M. J., Simola, N., Notte, S., Della, C. M. A., Serra, M., Pinna, A., Feliciello, A., Annunziato, L., Scorziello, A., & Morelli, M. (2020). Gender differences in neurodegeneration, neuroinflammation and Na^+-Ca^{2+} exchangers in the female A53T transgenic mouse model of Parkinson's disease. *Frontiers in Aging Neuroscience*, *12*, 118.

Deusser, J., Schmidt, S., Ettle, B., Plötz, S., Huber, S., Müller, C. P., Masliah, E., Winkler, J., & Kohl, Z. (2015). Serotonergic dysfunction in the A53T alpha-synuclein mouse model of Parkinson's disease. *Journal of Neurochemistry*, *135*, 589–597.

Fall, P.-A., Fredrikson, M., Axelson, O., & Granrus, A.-K. (1999). Nutritional and occupational factors influencing the risk of Parkinson's disease: A case-control study in southeastern Sweden. *Movement Disorders*, *14*, 28–37.

Farrer, M. J. (2006). Genetics of Parkinson disease: Paradigm shifts and future prospects. *Nature Reviews. Genetics*, *7*, 306–318.

Gan-Or, Z., Orr-Urtreger, A., Alcalay, R. N., Bressman, S., Giladi, N., & Rouleau, G. A. (2015). The emerging role of SMPD1 mutations in Parkinson's disease: Implications for future studies. *Parkinsonism & Related Disorders*, *21*, 1294–1295.

Gentile, F., Doneddu, P. E., Riva, N., Nobile-Orazio, E., & Quattrini, A. (2020). Diet, microbiota and brain health: Unraveling the network intersecting metabolism and neurodegeneration. *International Journal of Molecular Sciences*, *21*, 7471.

Giasson, B. I., Duda, J. E., Quinn, S. M., Zhang, B., Trojanowski, J. Q., & Lee, V. M.-Y. (2002). Neuronal α-synucleinopathy with severe movement disorder in mice expressing A53T human α-Synuclein. *Neuron*, *34*, 521–533.

Gulbins, E., Palmada, M., Reichel, M., Lüth, A., Böhmer, C., Amato, D., Müller, C. P., Tischbirek, C. H., Groemer, T. W., Tabatabai, G., Becker, K. A., Tripal, P., Staedtler, S., Ackermann, T. F., van Brederode, J., Alzheimer, C., Weller, M., Lang, U. E., Kleuser, B., … Kornhuber, J. (2013). Acid sphingomyelinase–ceramide system mediates effects of antidepressant drugs. *Nature Medicine*, *19*, 934–938.

Gulbins, E., Walter, S., Becker, K. A., Halmer, R., Liu, Y., Reichel, M., Edwards, M. J., Müller, C. P., Fassbender, K., & Kornhuber, J. (2015). A central role for the acid sphingomyelinase/ceramide system in neurogenesis and major depression. *Journal of Neurochemistry*, *134*, 183–192.

Hashimoto, M., Rockenstein, E., & Maskiah, E. (2006). Transgenic models of α-Synuclein pathology. *Annals of the New York Academy of Sciences*, *991*, 171–188.

Heath, D. (2000). *Drinking occasions: Comparative perspectives on alcohol and culture*. Brunner/Mazel.

Hebbar, S., Sahoo, I., Matysik, A., Argudo, G. I., Osborne, K. A., Papan, C., Torta, F., Narayamaswami, P., Hui Fun, X., Wenk, M. R., Shevchenko, A., Schwudke, D., & Kraut, R. (2015). Ceramides and stress signalling intersect with autophagic defects in neurodegenerative *Drosophila* blue cheese (*bchs*) mutants. *Scientific Reports*, *5*, 15926.

Holthuis, J. C. M., Pomorski, T., Raggers, R. J., Sprong, H., & Van Meer, G. (2001). The organizing potential of sphingolipids in intracellular membrane transport. *Physiological Reviews*, *81*, 1689–1723.

Huston, J. P., Kornhuber, J., Mühle, C., Japtok, L., Komorowski, M., Mattern, C., Reichel, M., Gulbins, E., Kleuser, B., Topic, B., de Souza Silva, M. A., & Müller, C. P. (2016). A sphingolipid mechanism for behavioral extinction. *Journal of Neurochemistry*, *137*, 589–603.

Jacob, E. L., Gatto, N. M., Thompson, A., Bordelon, Y., & Ritz, B. (2010). Occurrence of depression and anxiety prior to Parkinson's disease. *Parkinsonism & Related Disorders*, *16*, 576–581.

Janeczek, P., MacKay, R. K., Lea, R. A., Dodd, P. R., & Lewohl, J. M. (2014). Reduced expression of α-synuclein in alcoholic brain: Influence of SNCA -Rep1 genotype. *Addiction Biology*, *19*, 509–515.

Jiméanez-Jiméanez, F. J., Mateo, D., & Giméanez-Roldan, S. (1992). Premorbid smoking, alcohol consumption, and coffee drinking habits in Parkinson's disease: A case-control study. *Movement Disorders*, *7*, 339–344.

Kalinichenko, L. S., Abdel-Hafiz, L., Wang, A.-L., Mühle, C., Rösel, N., Schumacher, F., Kleuser, B., Smaga, I., Frankowska, M., Filip, M., Schaller, G., Richter-Schmidinger, T., Lenz, B., Gulbins, E., Kornhuber, J., Oliveira, A. W. C., Barros, M., Huston, J. P., & Müller, C. P. (2021). Neutral sphingomyelinase is an affective valence-dependent regulator of learning and memory. *Cerebral Cortex*, *31*, 1316–1333.

Kalinichenko, L. S., Gulbins, E., Kornhuber, J., & Müller, C. P. (2022). Sphingolipid control of cognitive functions in health and disease. *Progress in Lipid Research*, *86*, 101162.

Kalinichenko, L. S., Hammad, L., Reichel, M., Kohl, Z., Gulbins, E., Kornhuber, J., & Müller, C. P. (2019). Acid sphingomyelinase controls dopamine activity and responses to appetitive stimuli in mice. *Brain Research Bulletin*, *146*, 310–319.

Kalinichenko, L. S., Mühle, C., Eulenburg, V., Praetner, M., Reichel, M., Gulbins, E., Kornhuber, J., & Müller, C. P. (2019). Enhanced alcohol preference and anxiolytic alcohol effects in Niemann-pick disease model in mice. *Frontiers in Neurology*, *10*, 731.

Kalinichenko, L. S., Mühle, C., Jia, T., Anderheiden, F., Datz, M., Eberle, A.-L., Eulenburg, V., Granzow, J., Hofer, M., Hohenschild, J., Huber, S. E., Kämpf, S., Kogias, G., Lacatusu, L., Lugmair, C., Taku, S. M., Meixner, D., Sembritzki, N. K., Praetner, M., … Müller, C. P. (2023). Adult alcohol drinking and emotional tone are mediated by neutral sphingomyelinase during development in males. *Cerebral Cortex*, *33*, 844–864.

Kalinichenko, L. S., Mühle, C., Jia, T., Anderheiden, F., Datz, M., Eberle, A.-L., Eulenburg, V., Granzow, J., Hofer, M., Hohenschild, J., Huber, S. E., Kämpf, S., Kogias, G., Lacatusu, L., Lugmair, C., Taku, S. M., Meixner, D., Tesch, N., Praetner, M., … Müller, C. P. (2021). Neutral sphingomyelinase mediates the co-morbidity trias of alcohol abuse, major depression and bone defects. *Molecular Psychiatry*, *26*, 7403–7416.

Klein, C., & Westenberger, A. (2012). Genetics of Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *2*, a008888.

Kohl, Z., Abdallah, N., Ben, V. J., Tischer, L., Deusser, J., Amato, D., Anderson, S., Mueller, C. P., Riess, O., Masliah, E., Nuber, S., & Winkler, J. (2016). Severely impaired hippocampal neurogenesis associates with an early serotonergic deficit in a BAC α-synuclein transgenic rat model of Parkinson's disease. *Neurobiology of Disease*, *85*, 206–217.

Kohl, Z., Winner, B., Ubhi, K., Rockenstein, E., Mante, M., Münch, M., Barlow, C., Carter, T., Masliah, E., & Winkler, J. (2012). Fluoxetine rescues impaired hippocampal neurogenesis in a transgenic A53T synuclein mouse model. *The European Journal of Neuroscience*, *35*, 10–19.

König, L., Kalinichenko, L. S., Huber, S. E., Voll, A. M., Bauder, M., Kornhuber, J., Hausch, F., & Müller, C. P. (2020). The selective FKBP51 inhibitor SAFit2 reduces alcohol consumption and reinstatement of conditioned alcohol effects in mice. *Addiction Biology*, *25*, e12758.

Kouli, A., Torsney, K. M., & Kuan, W.-L. (2018). Parkinson's disease: Etiology, neuropathology, and pathogenesis. In *Parkinson's disease pathology clinical Asp* (pp. 3–26). Codon Publications.

Kurzawa-Akanbi, M., Tammireddy, S., Fabrik, I., Gliaudelytė, L., Doherty, M. K., Heap, R., Matečko-Burmann, I., Burmann, B. M., Trost, M., Lucocq, J. M., Gherman, A. V., Fairfoul, G., Singh, P., Burté, F., Green, A., McKeith, I. G., Härtlova, A., Whitfield, P. D., & Morris, C. M. (2021). Altered ceramide metabolism is a feature in the extracellular vesicle-mediated spread of alpha-synuclein in Lewy body disorders. *Acta Neuropathologica*, *142*, 961–984.

Lee, Y. J., Wang, S., Slone, S. R., Yacoubian, T. A., & Witt, S. N. (2011). Defects in very long chain fatty acid synthesis enhance alpha-Synuclein toxicity in a yeast model of Parkinson's disease. *PLoS One*, *6*, e15946.

Levey, D. F., Le-Niculescu, H., Frank, J., Ayalew, M., Jain, N., Kirlin, B., Learman, R., Winiger, E., Rodd, Z., Sheckar, A., Schork, N., Kiefer, F., Wodarz, N., Mueller-Muhsok, B., Dahmen, N., GESGA Consortium, Noethen, M., Sherav, R., Farrer, L., & Niculescu, A. B. (2014). Genetic risk prediction and neurobiological understanding of alcoholism. *Translational Psychiatry*, *4*, e391.

Liang, T., Spence, J., Liu, L., Strother, W. N., Chang, H. W., Ellison, J. A., Lumeng, L., Li, T.-K., Foroud, T., & Carr, L. G. (2003). α-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. *Proceedings of the National Academy of Sciences*, *100*, 4690–4695.

Marxreiter, F., Ettle, B., May, V. E. L., Esmer, H., Patrick, C., Kragh, C. L., Klucken, J., Winner, B., Riess, O., Winkler, J., Masliah, E., & Nuber, S. (2013). Glial A30P alpha-synuclein pathology segregates neurogenesis from anxiety-related behavior in conditional transgenic mice. *Neurobiology of Disease*, *59*, 38–51.

McGrath, E., Jones, A., & Field, M. (2016). Acute stress increases ad-libitum alcohol consumption in heavy drinkers, but not through impaired inhibitory control. *Psychopharmacology*, *233*, 1227–1234.

Mingione, A., Pivari, F., Plotegher, N., Dei, C. M., Zulueta, A., Bocci, T., Trinchera, M., Albi, E., Maglione, V., Caretti, A., Bubacco. L., Paroni, R., Bottai, D., Ghidoni, R., & Signorelli, P. (2021). Inhibition of ceramide synthesis reduces α-synuclein proteinopathy in a cellular model of Parkinson's disease. *International Journal of Molecular Sciences*, *22*, 6469.

Moisan, F., Kab, S., Mohamed, F., Canonico, M., Guern, M., Le, Q. C., Carcaillon, L., Nikolau, J., Duport, N., Singh-Manoux, A., Boussac-Zarbeska, M., & Elbaz, A. (2016). Parkinson disease male-to-female ratios increase with age: French nationwide study and meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *87*, 952–957.

Mühle, C., & Kornhuber, J. (2017). Assay to measure sphingomyelinase and ceramidase activities efficiently and safely. *Journal of Chromatography. A*, *1481*, 137–144.

Müller, C. P., Kalinichenko, L. S., Tiesel, J., Witt, M., Stöckl, T., Sprenger, E., Fuchser, J., Beckmann, J., Praetner, M., Huber, S. E., Amato, D., Mühle, C., Büttner, C., Ekici, A. B., Smaga, I., Pomierny-Chamiolo, L., Pomierny, B., Filip, M., Eulenburg, V., … Kornhuber, J. (2017). Paradoxical antidepressant effects of alcohol are related to acid sphingomyelinase and its control of sphingolipid homeostasis. *Acta Neuropathologica*, *133*, 463–483.

Nuber, S., Harmuth, F., Kohl, Z., Adame, A., Trejo, M., Schönig, K., Zimmermann, F., Bauer, C., Casadei, N., Giel, C., Calaminus, C., Pichler, B. J., Jensen, P. H., Müller, C. P., Amato, D., Kornhuber, J., Teismann, P., Yamakado, H., Takahashi, R., … Riess, O. (2013). A progressive dopaminergic phenotype associated with neurotoxic conversion of α-synuclein in BAC-transgenic rats. *Brain*, *136*, 412–432.

Nuber, S., Rajsombath, M., Minakaki, G., Winkler, J., Müller, C. P., Ericsson, M., Caldarone, B., Dettmer, U., & Selkoe, D. J. (2018). Abrogating native α-synuclein tetramers in mice causes a L-DOPA-responsive motor syndrome closely resembling Parkinson's disease. *Neuron*, *100*, 75–90.e5.

Palacios, N., Gao, X., O'Reilly, E., Schwarzschild, M., McCullough, M. L., Mayo, T., Gapstur, S. M., & Ascherio, A. A. (2012). Alcohol and risk of Parkinson's disease in a large, prospective cohort of men and women. *Movement Disorders*, *27*, 980–987.

Qamhawi, Z., Towey, D., Shah, B., Pagano, G., Seibyl, J., Marek, K., Borghammer, P., Brooks, D. J., & Pavese, N. (2015). Clinical

correlates of raphe serotonergic dysfunction in early Parkinson's disease. *Brain*, *138*, 2964–2973.

Ramsey, P. (1993). Multiple comparisons of independent means. In L. Edwards (Ed.), *Applied Analysis of variance Behavioural sciences* (pp. 25–61). Dekker, M.

Rotermund, C., Reolon, G. K., Leixner, S., Boden, C., Bilbao, A., & Kahle, P. J. (2017). Enhanced motivation to alcohol in transgenic mice expressing human α-synuclein. *Journal of Neurochemistry*, *143*, 294–305.

Shiba, M., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., Schaid, D. J., & Rocca, W. A. (2000). Anxiety disorders and depressive disorders preceding Parkinson's disease: A case-control study. *Movement Disorders*, *15*, 669–677.

Sieurin, J., Gustavsson, P., Weibull, C. E., Feldman, A. L., Petzinger, G. M., Gatz, M., Pedersen, N. L., & Wirdefeldt, K. (2016). Personality traits and the risk for Parkinson disease: A prospective study. *European Journal of Epidemiology*, *31*, 169–175.

Sirabella, R., Sisalli, M. J., Costa, G., Omura, K., Ianniello, G., Pinna, A., Morelli, M., Renzo, G. M., Di, A. L., & Scorziello, A. (2018). NCX1 and NCX3 as potential factors contributing to neurodegeneration and neuroinflammation in the A53T transgenic mouse model of Parkinson's disease. *Cell Death & Disease*, *9*, 725.

Smutek, M., Turbasa, M., Sikora, M., Piechota, M., Zajdel, J., Przewlocki, R., & Parkitna, J. R. (2014). A model of alcohol drinking under an intermittent access schedule using group-housed mice. *PLoS One*, *9*, e96787.

Solla, P., Cannas, A., Ibba, F. C., Loi, F., Corona, M., Orofino, G., Marrosu, M. G., & Marrosu, F. (2012). Gender differences in motor and non-motor symptoms among Sardinian patients with Parkinson's disease. *Journal of the Neurological Sciences*, *323*, 33–39.

Wang, W., Song, N., Jia, F., Tang, T., Bao, W., Zuo, C., Xie, J., & Jiang, H. (2018). Genomic DNA levels of mutant alpha-synuclein correlate with non-motor symptoms in an A53T Parkinson's disease mouse model. *Neurochemistry International*, *114*, 71–79.

Wihan, J., Grosch, J., Kalinichenko, L. S., Müller, C. P., Winkler, J., & Kohl, Z. (2019). Layer-specific axonal degeneration of serotonergic fibers in the prefrontal cortex of aged A53T α-synuclein–expressing mice. *Neurobiology of Aging*, *80*, 29–37.

Wilcox, C. E., Claus, E. D., Blaine, S. K., Morgan, M., & Hutchison, K. E. (2013). Genetic variation in the alpha Synuclein gene (SNCA) is associated with BOLD response to alcohol cues. *Journal of Studies on Alcohol and Drugs*, *74*, 233–244.

Winkler, J., Ehret, R., Büttner, T., Dillmann, U., Fogel, W., Sabolek, M., Winkelmann, J., & Kassubek, J. (2011). Parkinson's disease risk score: Moving to a premotor diagnosis. *Journal of Neurology*, *258*, 311–315.

Wu, Q., Yang, X., Zhang, Y., Zhang, L., & Feng, L. (2016). Chronic mild stress accelerates the progression of Parkinson's disease in A53T α-synuclein transgenic mice. *Experimental Neurology*, *285*, 61–71.

Zheng, F., Puppel, A., Huber, S. E., Link, A. S., Eulenburg, V., van Brederode, J. F., Müller, C. P., & Alzheimer, C. (2016). Activin controls ethanol potentiation of inhibitory synaptic transmission through GABAA receptors and concomitant behavioral sedation. *Neuropsychopharmacology*, *41*, 2024–2033.

Zoicas, I., Huber, S. E., Kalinichenko, L. S., Gulbins, E., Müller, C. P., & Kornhuber, J. (2020). Ceramides affect alcohol consumption and depressive-like and anxiety-like behavior in a brain region and ceramide species-specific way in male mice. *Addiction Biology*, *25*, e12847.

Zoicas, I., Reichel, M., Gulbins, E., & Kornhuber, J. (2016). Role of acid sphingomyelinase in the regulation of social behavior and memory. *PLoS One*, *11*, e0162498.

SUPPORTING INFORMATION

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

How to cite this article: Kalinichenko, L. S., Kohl, Z., Mühle, C., Hassan, Z., Hahn, A., Schmitt, E.-M., Macht, K., Stoyanov, L., Moghaddami, S., Bilbao, R., Eulenburg, V., Winkler, J., Kornhuber, J., & Müller, C. P. (2024). Sex-specific pleiotropic changes in emotional behavior and alcohol consumption in human α-synuclein A53T transgenic mice during early adulthood. *Journal of Neurochemistry*, *168*, 269–287. https://doi.org/10.1111/jnc.16051