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RESEARCH ARTICLE

Dissociating role of Bassoon in glutamatergic and dopaminergic neurons in alcohol-related behaviour and affective state in mice

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Abstract

Background and Purpose: Alcohol abuse and affective disorders are severe comorbid psychiatric diseases characterized by impaired brain synaptic transmission. The role of presynaptic scaffolding proteins coordinating presynaptic plasticity and neurotransmitter release, such as Bassoon (*Bsn*), in the pathogenesis of these disorders remains elusive. Considering the key roles of the dopaminergic and glutamatergic systems in the pathogenesis of affective disorders and alcohol use disorder, we investigated the role of *Bsn* in these neuronal systems in the regulation of disease-related behaviours.

Experimental Approach: We employed two mouse models conditionally lacking *Bsn* in glutamatergic neurons of the forebrain or in dopaminergic neurons. Depression- and anxiety-like and alcohol-related behaviour was evaluated using a battery of behavioural tests in a sex-specific way. Brain monoamine levels were evaluated in several brain regions of mice with *Bsn* deletion in dopaminergic neurons.

Key Results: *Bsn* deletion in forebrain glutamatergic neurons reduced alcohol consumption and preserved affective state in male mice. In females, loss of *Bsn* in these neurons.

enhanced anxiety-like behaviour. A *Bsn* knockout in dopaminergic neurons of males was associated with increased alcohol consumption and anxiety, while depression-like behaviour was attenuated. Females with *Bsn* deletion in dopaminergic neurons showed no alterations in affective state and alcohol drinking behaviour, but increased dopamine levels in amygdala, indicating a potentially compensatory mechanism.

Abbreviations: AUD, alcohol use disorder; Bsn, Bassoon; CC, conditioned compartment; CPP, conditioned place preference; DAT, dopamine transporter; DH, dorsal hippocampus; EPM, elevated plus maze; FST, forced swim; LDB, light–dark box; LORR, loss of righting reflex; NSF, novelty suppressed feeding; OF, open field; PC, pseudo-conditioned compartment; SPT, sucrose preference test; VS, ventral striatum; wt, wild type.

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Conclusion and Implications: Our findings suggest that *Bsn* is a sex-specific regulator of affective state and alcohol consumption behaviour in a neuron type-specific way. *Bsn* exerts dissociating behavioural effects depending on its action in glutamatergic versus dopaminergic neurons.

KEYWORDS
alcohol, anxiety, Bassoon, depression, dopaminergic neurons, glutamatergic neurons, sex differences

1 | INTRODUCTION

Alcohol use disorder (AUD) is a widespread disease condition with poor pharmacotreatment options (Degenhardt et al., 2018). In the vast majority of consumers, alcohol is used not for its euphoric effects but is instrumentalized for achieving certain behavioural goals such as amelioration of anxiety and depression (Müller et al., 2023; Müller & Schumann, 2011). However, chronic over-instrumentalization of alcohol may result in the escalation of intake. Repeated withdrawals may lead to a depression-like mental state. In turn, depressed mood facilitates alcohol consumption resulting in the development of depression-associated addiction (Müller et al., 2023). It should be emphasized that alcohol use and addiction as well as depression are highly sex-dependent (Degenhardt et al., 2018; Müller et al., 2023). A lack of sex-specific understanding of the neuropsychopharmacology of these comorbid disorders limits the development of efficient personalized treatments.

Synaptic transmission is a crucial mechanism mediating functional activity of the central nervous system. Pathogenesis of addictive and affective disorders is highly linked to aberrant excitatory and modulatory neurotransmission (Markou, 1998). Two key systems, the dopaminergic and the glutamatergic system, mediate different aspects of these disorders and shape the pathophysiological landscape. Neurotransmitter release to the synaptic cleft is controlled by a multiprotein complex of the active zones in the presynaptic cytomatrix (Ackermann et al., 2015). One of the largest presynaptic scaffolding proteins omnipresent in the brain is Bassoon (*Bsn*) (Gundelfinger et al., 2015). It controls synaptic release site reloading and synaptic vesicle cycle, thus determining plasticity of neurotransmitter release (Montenegro-Venegas et al., 2022; Okerlund et al., 2017). Bsn also regulates protein homeostasis through modulation of ubiquitination, proteosomal activity and autophagy and links the presynaptic activity to neuronal gene expression reprogramming (Hallermann et al., 2010; Juranek et al., 2013; Montenegro-Venegas et al., 2021; Okerlund et al., 2017; Waites et al., 2013). Loss of *Bsn* in mice increases the number of presynaptically silent synapses and might result in the development of an epileptic phenotype (Altrock et al., 2003; Blondiaux et al., 2023). Recently, disruptive *BSN* variants have been identified in patients with broad spectra of brain disorders presenting epilepsy, neurodevelopmental and neurodegenerative features indicating an indispensable role of *BSN* for normal brain function (Guzman et al., 2025; Wakita et al., 2025). The present study aims to investigate the role of *Bsn* in affective state regulation and alcohol consumption behaviour. To examine the functions of *Bsn* in different types of neurons and its contribution to behavioural control, we used two mouse models conditionally lacking *Bsn* in **glutamatergic** neurons of the forebrain (Annamneedi et al., 2018) and in **dopaminergic** neurons brain-wide. Given the high gender and sex specificity of depression and AUD (Hyde et al., 2008; Wilsnack et al., 2000), we performed the analysis separately for female and male mice.

What is already known?

- Bassoon is a key presynaptic protein in the brain determining plasticity of neurotransmitter release

What does this study add?

- Bassoon is a sex-specific regulator of emotional and alcohol consumption behaviour
- Bassoon exerts dissociating behavioural effects in a neuron-type specific way

What is the clinical significance?

- Bassoon may serve as a male-predominant mechanism in affective disorders and use disorder

2 | METHODS

2.1 | Animals

In this study, transgenic mice of both sexes 8–14 weeks old were used. Bsn^{Emx1} strain *with* functional inactivation of the *Bsn* gene in the glutamatergic neurons of forebrain was generated by crossing of line $Bsn^{tm1.1Arte}$ (RRID: MGI:7484796) with $Emx1^{tm1(cre)Krj}$/J (RRID: MGI:

2684610) and characterized previously (Annamneedi et al., 2018). *Bsn*^{DAT} strain with deletion of *Bsn* in dopaminergic neurons in the entire brain were generated for this study by crossing of line *Bsn*$^{tm1.1Arte}$ (Annamneedi et al., 2018) with Slc6a3$^{tm1.1(cre)Bkmn}$/J (RRID: MGI:3689434) (Bäckman et al., 2006; Costa et al., 2021). All animals used in behavioural experiments and for neurochemical measurements were floxed in both *Bsn*$^{tm1.1Arte}$ alleles. Animals used in the experimental groups (mutants) were heterozygote for the Slc6a3$^{tm1.1(cre)}$ allele in the *Bsn*^{DAT} and for *Emx1*$^{tm1(cre)}$ in the *Bsn*^{Emx1} strain, while controls were wild types for the driver allele. For validation of cell-type specific excision of floxed locus in *Bsn*^{DAT} strain samples from *Bsn*$^{tm1.2Arte}$ (*Bsn*^{KO}, MGI:7484797) animals with constitutive deletion of *Bsn* were used as positive controls. These animals have been characterized previously (Schattling et al., 2019). The experimental animals and controls were littermates. All animals were bred on C57Bl6N background for more than 10 generations. Mice were group housed (3–5 animals per cage; except for the drinking studies) under standard conditions on a 12- to 12-h light–dark cycle with food and water ad libitum. All experiments were carried out in accordance with the National Institutes of Health guidelines for the humane treatment of animals and the European Communities Council Directive (86/609/EEC) and approved by the local governmental commission for animal health (Regierung von Unterfranken).

Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). Testing was performed in mixed cohorts of female and male mice. Data analysis was performed separately in two sexes as multiple studies indicated pronounced sex-specific innate differences in pathological mechanisms determining psychiatric disorders, particularly AUD and affective disorders (Bangasser & Cuarenta, 2021; Grace et al., 2021; Seney et al., 2018; Toivainen et al., 2024). All the tests were performed by an investigator blinded to the genotype.

2.2 | Validation of Bassoon (*Bsn*) gene deletion in *Bsn*^{DAT} mice

To evaluate Bassoon (*Bsn*) gene recombination in dopaminergic neurons, *Bsn*^{DAT} animals and their wild type (wt) littermates were killed by decapitation, the substantia nigra region was dissected form the brains and genomic DNA was extracted using 5% Chelex® 100 resin (Bio-Rad, Hercules, USA, #1422822) according the protocol of the provider. The deletion of the exon 2 of *Bsn* gene (flanked by lox sites) was detected by PCR using Q5® High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, USA, #M0491) *Bsn* genotyping primers F: GTTGCCTAATGTATGCAGAGTCC, R1: TGCTAGGGTTAAAGCCATGTG, and R2: GGCTGCCCAGGGGCTCTTTG. PCR was done under the following cycling conditions: 98°C for 30s; 40 cycles of (98°C for 10s, 66°C for 20s and 72°C for 30s) followed 72°C for 2 min. PCR products were resolved on a 2% agarose gel, visualized using ethidium bromide (Carl Roth, Karlsruhe, Germany, #2218.1) and iBright FL1500 imaging system (Invitrogen, California, USA).

2.3 | Immunohistochemistry, microscopy and image processing

Immunohistochemical procedures and confocal microscopy were performed as described previously (Annamneedi et al., 2018). The 30-µm coronal sections of ventral striatum from both the wt and *Bsn*^{DAT} mice were used to analyse the expression of Bassoon in dopaminergic terminals. Primary antibodies: mouse anti-Bassoon; (Enzo Life Sciences Inc. New York, USA; RRID: 1:1000) and rat anti-DAT (Abcam, Cambridge, UK; RRID: 1:500). Secondary antibodies: goat anti-mouse Cy3 (Jackson Immuno Research Labs, Pennsylvania, USA; RRID: 1:500) and goat anti-rat Alexa 488 (Invitrogen, California, USA; RRID: 1:200). Confocal images were acquired as Z-stacks with a 0.8 µm Z-step size (~10-µm Z-stack volume) using a Leica SP5 confocal microscope equipped with a 63X objective (HCX PL APO CS 63.0x1.40 OIL) and LCS software (Leica Microsystems, Wetzlar, Germany). Maximum projection images were obtained from the Z-stack and deconvoluted images were generated using Diffraction PSF 3D and Interactive Deconvolve 3D plugins (https://www.optinav.info/Iterative-Deconvolve-3D.htm) in ImageJ (https://imagej.net/ij/).

2.4 | Alcohol-related behaviour in mice

To isolate the role of *Bsn* in alcohol-related behaviour, alcohol drinking, taste preference and alcohol deprivation effects were tested in naïve *Bsn*^{Emx1}, *Bsn*^{DAT} and respective wt mice of both sexes using a two-bottle free-choice drinking paradigm. The following group size was used: *Bsn*^{Emx1}–wt—12 males/12 females, *Bsn*^{Emx1}—9 males/9 females; *Bsn*^{DAT}–wt—7 males/8 females; *Bsn*^{DAT}—8 males/8 females. The animals were single housed for 2 weeks before the start of the experiment. Each cage was equipped with two constantly available bottles containing tap water. After an acclimatization period to establish a water-drinking baseline, one of the bottles was filled with tap water and another bottle with alcohol (Carl Roth). Animals received alcohol at increasing concentrations of 2, 4, 8, 12 and 16 % vol. Mice were exposed to each concentration of alcohol for 4 days (Figure 1a). The bottles were weighed and their positions were changed every 24 h. Thereafter, alcohol concentration was maintained at 16 % vol for 12 days. In order to measure the alcohol deprivation effect, alcohol was removed for 3 weeks (both bottles containing tap water) before it was re-introduced for 4 days (Figure 1a). This procedure was repeated two more times. Bottles were changed and weighed daily. The consumed amount of alcohol relative to body weight and the preference vs. water were measured (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

Alcohol experienced *Bsn*^{Emx1}, *Bsn*^{DAT} and respective wt mice (29 days of exposure to free-choice drinking, as described above) were used for the taste preference test (Figure 1a). Sucrose (0.5% and 5%; Merc Chemicals, Darmstadt, Germany) and quinine (2 and 20 mg·dl^{-1}; Merc Chemicals) preference was measured in a two-bottle free-choice test versus water, 3 days after the last alcohol exposure. Each dose was offered for 2 days with the position of the bottles being changed and weighed daily with 1 day wash out

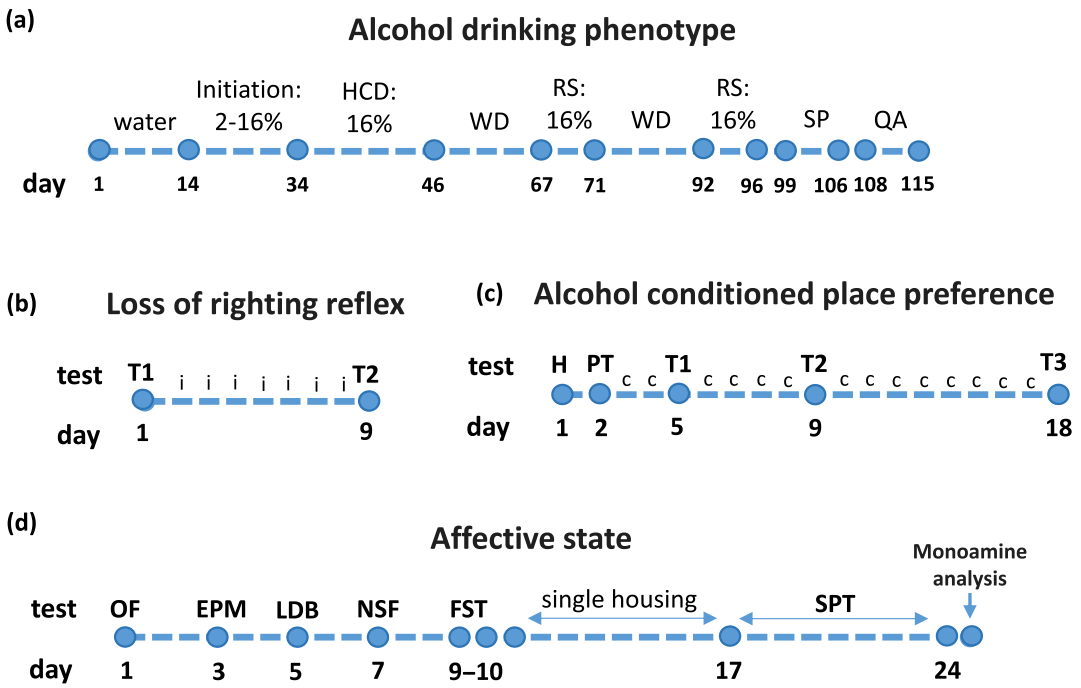

FIGURE 1 Schematic presentation of the experimental schedule. (a) Alcohol drinking phenotype in naïve mice was investigated in a two-bottle free-choice paradigm and included the following phases: initiation high-concentration drinking (HCD), withdrawal (WD), reinstatement (RS), sucrose preference (SP) and quinine aversion (QA). (b) To investigate the loss of righting reflex (LORR), naïve mice were injected with alcohol (i) for 7 days, and LORR was tested at Days 1 and 9 of the treatment (T1–T2). (c) Alcohol conditioned place preference test was performed in naïve mice and consisted of a habituation (H), baseline test (pre-test, PT), 14 conditioning trials (c) and three preference tests (T1–T3). (d) Analysis of affective state of naïve mice included several behavioural tests: open field (OF), elevated plus maze (EPM), light–dark box (LDB), novelty supressed feeding (NSF) and forced swim test (FST) performed with 2-day interval in between. After the FST the animals were single housed in cages with two water bottles constantly available, and 1 week later the sucrose preference test (SPT) took place. .

between sucrose and quinine testing (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

Loss of righting reflex (LORR) was tested in a separate cohort of alcohol naïve 8- to 14-week-old Bsn^{DAT} and wt mice (wt—14 males/8 females; Bsn^{DAT}—14 males/9 females). Animals were administered with an alcohol injection of 3.5 g/kg (i.p., 20 ml·kg^{-1}) for seven consecutive days at the same time. Testing of LORR was performed at Days 1 and 9 of the treatment (Figure 1b). After an alcohol injection, the animals were immediately placed in an empty cage. LORR was observed when the animals became ataxic and stopped moving for at least 30 s. Each animal was then placed on its back. Recovery from LORR was defined as the animal being able to right itself three times within a minute. Time taken for the animals to lose its righting reflex, and time to recovery from the alcohol effect was recorded (Kalinichenko et al., 2021; Stürzenberger et al., 2025).

Blood alcohol concentration (BAC) was measured in alcohol naïve 8–14-weeks-old Bsn^{DAT} and wt mice (wt—9 males/9 females; Bsn^{DAT}—6 males/6 females). Mice received alcohol injections (3 g·kg^{-1}, 20 ml·kg^{-1}, i.p.) and 20-µl blood samples were obtained from the submandibular vein 1, 2 and 3 h after alcohol injection. The blood samples were directly mixed with 80 µl 6.25% (w/v) trichloroacetic acid (Sigma, St. Louis, MO, USA). After centrifugation 15 µl of the supernatant were subjected to enzymatic alcohol determination using the alcohol dehydrogenase method as described elsewhere (Kalinichenko et al., 2021).

2.5 | Alcohol conditioned place preference

To address the effects of *Bsn* loss for reinforcing properties as well as conditioned rewarding properties of alcohol-associated environments of alcohol, the establishment of conditioned place preference (CPP) for alcohol was tested in naive Bsn^{Emx1} (wt—6 males/5 females; Bsn^{Emx1}—4 males/4 females) and Bsn^{DAT} (wt—7 males/8 females; Bsn^{DAT}—8 males/8 females) mice. The TSE Place Preference test boxes (Bad Homburg, Germany) were made of non-transparent PVC with standard inside dimensions of 40 cm (*L*) × 15 cm (*W*) × 20 cm (*H*). The apparatus was divided into three fully automated compartments; the outer chambers measured 17 cm in length, and the centre chamber 6 cm. The floor of the left chamber was covered with a smooth black rubber mat. The floor of the right chamber was covered with a patterned black rubber mat. The centre chamber was not covered and coloured white. Activity was recorded in each compartment using high-resolution infrared sensors. The system automatically recorded the number of entries made, the sojourn time, and distance moved in each compartment for each trial. An unbiased design was used, i.e. half the mice were conditioned to their preferred compartment, and half to their non-preferred compartment. Animals were injected (i.p.) immediately before each trial with either saline, or alcohol (2 g·kg^{-1}). Mice were instantly transferred to the testing suite and placed into the CPP boxes, signifying the beginning of the trial period. The experiment

involved three phases; baseline testing (Bl), conditioning trials (14 sessions) and preference tests (three sessions, T1–T3). Trials were performed once daily. Baseline-test: The pre-test was designed to establish a baseline level of preference for each individual animal. Mice were injected with saline and introduced into the centre compartment with free access to all three compartments for 20 min. Conditioning trials: Conditioning trials were performed in pairs, odd numbered pairings were conditioned with alcohol, and even numbered pairings were conditioned with saline, this was balanced across groups. All animals received seven pairings with saline and seven pairings with alcohol. Mice were injected with either saline or an ethanol solution and introduced into one of two compartments, conditioned or pseudo-conditioned compartments (CC and PC), for 5 min. Preference tests: In order to monitor the time course of CPP establishment, preference tests were systematically performed after one, three and seven conditioning trials. Before each test, mice were injected with saline and introduced into the centre compartment with free access to all three compartments for 20 min (Figure 1c) (Kalinichenko et al., 2025).

2.6 | Affective state

The affective state of naïve Bsn^{Emx1} (wt—13 males/9 females, Bsn^{Emx1}—12 males/9 females) and Bsn^{DAT} mice (wt—16 males/13 females; Bsn^{DAT}—17 males/16 females) of both sexes was tested in a battery of behavioural tests in the following order: open field (OF), light–dark box (LDB), elevated plus maze (EPM), novelty suppressed feeding (NSF), forced swim (FST) and sucrose preference test (SPT). All tests were performed on separate days between 09:00 and 15:00 h with 2 days' interval between them. Mice were moved to the behavioural test room 1 h before testing and were tested in a pseudorandom order. Each test apparatus was cleaned with 60% alcohol between subjects. The day after the FST, the mice were single housed in cages with two bottles, and 1 week later the SPT was performed (Figure 1d) (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.6.1 | Open field

Each mouse was placed in a square white acrylic arena (50 × 50 cm), facing a wall, for 20 min and allowed to freely explore the arena. The area was lighted with white light (100 lx) (Hall, 1934). Video recordings were taken and analysed using Biobserve Viewer III (Biobserve GmbH, Bonn, Germany). A virtual square of equal distance from the periphery (25 × 25 cm) was defined as the 'central zone', and the outer part of the arena was defined as 'peripheral zone'. Distance moved in the peripheral and central zones, number of entries, latent period of the first entrance and time spent in the central zone were registered (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.6.2 | Elevated plus maze (EPM)

The EPM was constructed from black opaque acrylic with white lining on the floor, each arm measuring 30 × 5 cm and the central platform 5 × 5 cm. Two opposite arms were enclosed by a 15-cm wall of opaque acrylic, while the other two arms were open with a ledge of 0.5 cm on the sides. The maze was elevated 50 cm from the ground and illuminated with 100 lx. Each mouse was placed on the central platform, facing towards a closed arm, and allowed to freely explore the maze for 5 min. Biobserve Viewer III tracking software (Biobserve GmbH, Germany) was used to record the distance moved in the open and closed arms, and the number of entries into the closed and open arms and time spent in them (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.6.3 | Light dark box

For the light–dark test, a box of white acrylic was used (50 × 50 cm). The box was partitioned with a white acrylic wall, so that one-third of the total area was dark ('dark chamber'). The remaining two thirds were brightly lit (100 lx) with white light, which served as the 'light chamber'. A small entry door within the partition (5 × 7 cm), allowed mice to move freely between chambers. Each mouse was placed into the dark chamber facing the end wall parallel to the partition. Activity was recorded for the following 5 min using Biobserve Viewer III (Biobserve GmbH, Germany). Locomotor activity (distance moved in cm) and time (s) in the light chamber, and latent period of first entrance into the light chamber were measured. A single transition was counted when two paws had entered a chamber (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.6.4 | Novelty suppressed feeding

Animals were deprived of food for 24 h before the test. After deprivation, each mouse was put in the corner of a square white acrylic arena (50 × 50 × 50 cm, 100 lx), facing a wall. A piece of standard food was placed in the centre of the arena. Video recordings were taken and analysed using Biobserve Viewer III (Biobserve GmbH, Germany). The time (s) before a mouse began eating after placing in the arena and the distance moved before eating were registered (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.6.5 | Forced swim test

At the Day 1 of the test, each mouse was placed into a glass transparent cylinder (18-cm diameter, 19-cm height) filled with water (13 cm, 25°C) for 15 min. After 24 h, mice were again placed in this cylinder with water for 5 min. The latency of first floating, and total floating time during the Day 2 were recorded (Biobserve Viewer III, Biobserve GmbH, Germany) and analysed manually (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.7 | Post mortem neurochemistry

The brain tissue monoamine levels were evaluated in Bsn^{DAT} and respective wt mice (wt—16 males/13 females; Bsn^{DAT}—17 males/16 females) after affective state testing (Figure 1d). The ventral striatum (VS), dorsal hippocampus (DH) and amygdala (Amy) were isolated from mice after cervical dislocation. Brain samples were homogenized in 500 µl of 0.05 perchloric acid. Then, 1377-pg dihydroxybenzylamine was added as an internal standard, and the samples were analysed by HPLC with electrochemical detection. The column was an ET 125/2, Nucleosil 120-5, C-18 reversed phase column (Macherey and Nagel, Düren, Germany). The mobile phase consisted 75-mM NaH_2PO_4, 4-mM KCl, 20-µM EDTA, 1.5-mM sodium dodecylsulfate, 100-µl·L^{-1} diethylamine, 12% methanol and 12% acetonitril adjusted to pH 6.0 using phosphoric acid (Sigma, USA). The electrochemical detector (Intro, Antec, Leyden, Netherlands) was set at 500 mV versus an ISAAC reference electrode (Antec, Netherlands) at 30°C. Monoamine tissue concentration was expressed as pg·mg^{-1} wet tissue (Kalinichenko et al., 2021; Kalinichenko et al., 2023).

2.8 | Statistical analyses

The manuscript complies with the recommendations and requirements of the *British Journal of Pharmacology* on experimental design and analysis (Curtis et al., 2025). Data were examined with one- or two-way analysis of variance (ANOVA), for repeated measures where appropriate. Post-hoc tests were run only if F achieved P<0.05 and there was no significant variance inhomogeneity. For single group and time point effects, pre-planned comparisons were calculated using least significant difference (LSD)test or two-tailed *t* test (Ramsey, 1993). Pre-planned comparisons were selected as they are hypothesis-driven and decisions regarding the null hypothesis of one contrast are not influenced by the decisions regarding any other comparisons. Pre-planned comparisons help to isolate specific sources of variance in the dependent variable, determine forms or patterns of relationships, and provide greater statistical power against Type II error and higher temporal resolution (Benton, 1989; Ramsey, 1993). Declared group size corresponded to the number of independent values, and statistical analysis was done using these independent values. Statistical analysis was carried out only where the number of independent experiments, n, was 5 or more. $P< 0.05$ was considered indicative of statistical significance.

2.9 | Nomenclature of targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2025/26 (Alexander, Fabbro et al., 2025; Alexander, Gibb et al., 2025; Alexander, Striessnig et al., 2025).

3 | RESULTS

3.1 | Generation and validation of Bsn^{DAT} animals

The animals with specific deletion of *Bsn* in forebrain glutamatergic neurons were generated and characterized previously (Annamneedi et al., 2018; Annamneedi et al., 2021). To investigate effect of deletion of *Bsn* in dopaminergic neurons, we generated Bsn^{DAT} animals homozygote for floxed *Bsn* allele and expressing Cre recombinase specifically in dopaminergic neurons. These animals were born at expected rates, were normally fertile and overall indistinguishable from their wt littermates. In genomic DNA extracted from the substantia nigra brain region of Bsn^{DAT} animals we could detect efficient deletion of floxed exon 2 of *Bsn*, confirming successful cell-type specific recombination (Figure 2a–c). Next, we employed immunohistochemical staining and confocal imaging with deconvolution to examine expression of Bsn in dopaminergic projections labelled with a specific antibody against the dopamine transporter (**DAT**) in striatum of Bsn^{DAT} and respective controls (Figure 2d). The co-labelling for DAT and Bsn was evident in controls (Figure 1d, white arrowheads) but was largely reduced in Bsn^{DAT} tissue (Figure 2d, yellow arrows show DAT signal without Bsn co-labelling). These findings confirm the specific loss of Bassoon in the dopaminergic terminals in the Bsn^{DAT} animals.

3.2 | Bassoon (*Bsn*) deletion in glutamatergic neurons has anxiogenic effect in females

Anxiety and depression are among the most severe psychiatric disorders; thus, we investigated the role of *Bsn* in the mechanisms of these affective diseases. Female Bsn^{Emx1} mice were characterized by enhanced neophobia and anxiety. In the OF test, centre time and tracklength, and number of centre visits were significantly lower in Bsn^{Emx1} females compared to wt (Figures 3a–c and S1). Similar, time and tracklength in the light compartment of the LDB test were reduced in Bsn^{Emx1} females (Figure 3g,h). No significant genotype-driven differences were observed in the EPM test (Figure 1d,f). Therefore, *Bsn* knockout in glutamatergic forebrain neurons enhances anxiety in females. However, depression-like behaviour was not affected in Bsn^{Emx1} females as shown in the NSF, FST and SPT tests (Figures 3j–l and S1).

In male Bsn^{Emx1} mice, no changes in the anxiety- and depression-like behaviour were observed in the used tests (Figures 3m–x and S1). Altogether, higher anxiety was found in female Bsn^{Emx1} mice, while affective phenotype of males was preserved.

3.3 | Bassoon (*Bsn*) deletion in glutamatergic neurons diminishes alcohol consumption in males

A key behaviour of AUD is the consumption of easily available alcohol. We analysed a role of *Bsn* loss in the glutamatergic neurons in voluntary alcohol consumption. No *Bsn*-related differences in alcohol drinking phenotype were observed in Bsn^{Emx1} female mice as no

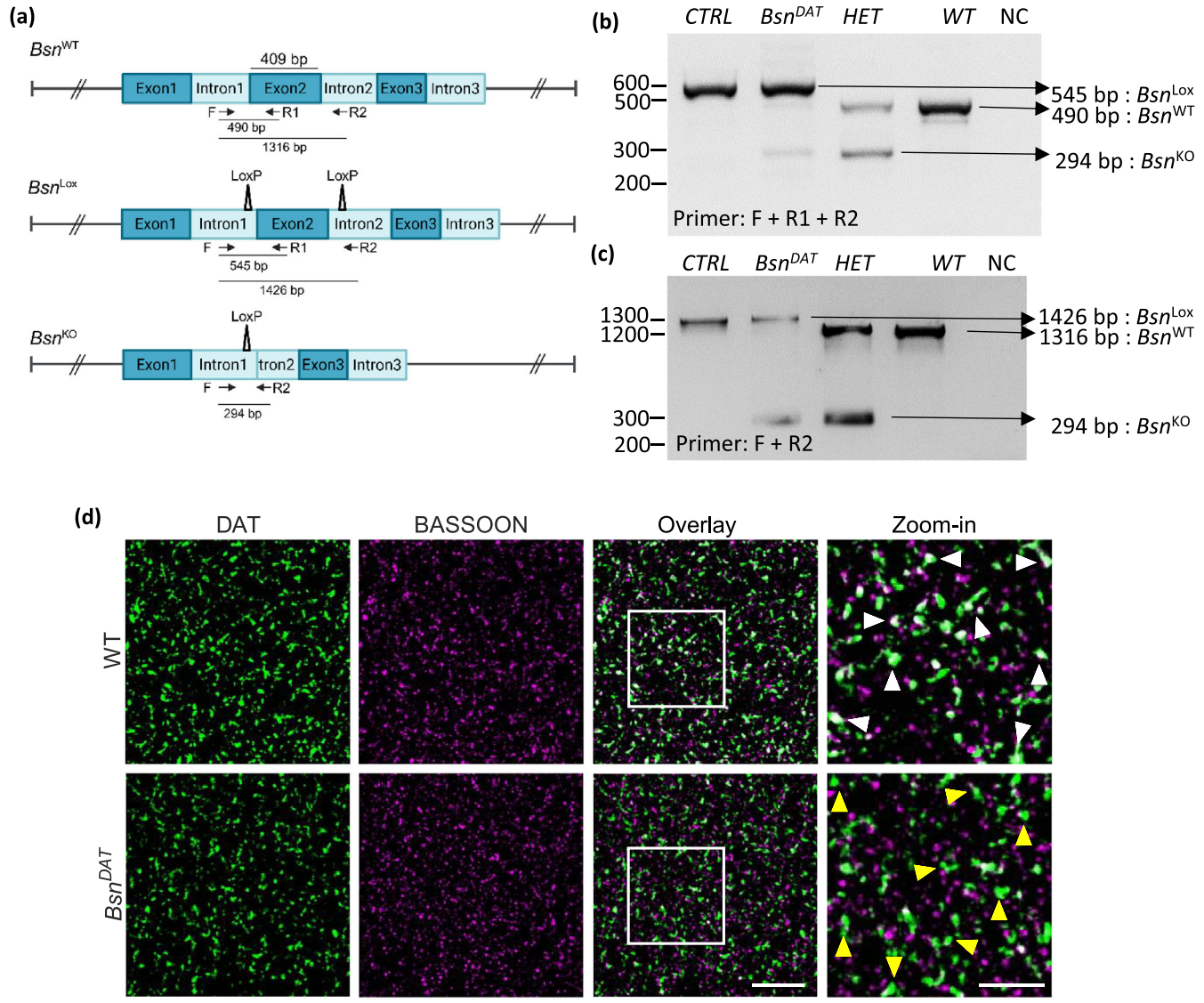

FIGURE 2 Validation of Bsn^{DAT} strain with Bassoon (*Bsn)* deletion in dopaminergic neurons. (a) Schematic representation of the *Bsn* genomic locus. In the $Bsn^{tm1.1Arte}$ stran, exon 2 is flanked by LoxP sites. WT, $Bsn^{tm1.1Arte}$ floxed (Bsn^{Lox}) and recombined (Bsn^{KO}) alleles are depicted. The position of forward (F) and reverse (R1 and R2) primers that were used for validation of the strain and sizes of expected amplicon are shown. (b,c) PCR was performed on genomic DNA isolated from substantia nigra of Bsn^{DAT} animals and their control (CTRL) littermates. DNA from tail biopsies from heterozygote constitutive $Bsn^{tm1.2Arte}$ animals (HET) were used as positive control. The amplicon specific for Bsn^{KO} allele was detected only in samples from Bsn^{DAT} and HET animals but not from controls or WT. In CTRL only non-recombined floxed allele (Bsn^{Lox}) and WT allele were detected. Specificity of PCR (no DNA contamination) was confirmed in reaction, where no DNA was added (negative control, NC). (d) Ventral striatal sections from WT and Bsn^{DAT}cKO mice are stained using anti-bassoon and anti-DAT antibodies. Co-localisation of Bassoon with the dopamine transporter (DAT) is observed in WT (white arrow heads in zoom-in overlay) and reduced co-localization in Bsn^{DAT}cKO mice (yellow arrow heads pointing the non-colocalized DA terminals). Scale bar is 10 and 5 µm (zoom-in).

significant changes in alcohol consumption and preference as well as water and total fluid intake were found (Figures 4a,b and S2). ADE or sucrose preference or quinine aversion were not altered in these females (Figure S2).

Bsn^{Emx1} males consumed less alcohol compared to wt littermates. This effect reached a significance level at 16% of alcohol (Figure 4e). Preference of 12% and 16% alcohol was also diminished in Bsn^{Emx1} males, while water and total fluid intake were preserved (Figures 4f and S2). ADE and taste preference were not affected by the *Bsn* loss in the forebrain glutamatergic neurons in males (Figure S2). Altogether, a moderate reduction in alcohol drinking was observed in male, but not female Bsn^{Emx1} mice.

3.4 | Bassoon (*Bsn*) deletion in glutamatergic neurons slightly attenuates reinforcing properties of alcohol in males

A major driver of AUD is drug-seeking behaviour, which is based on the establishment and retrieval of the conditioned reinforcing effects

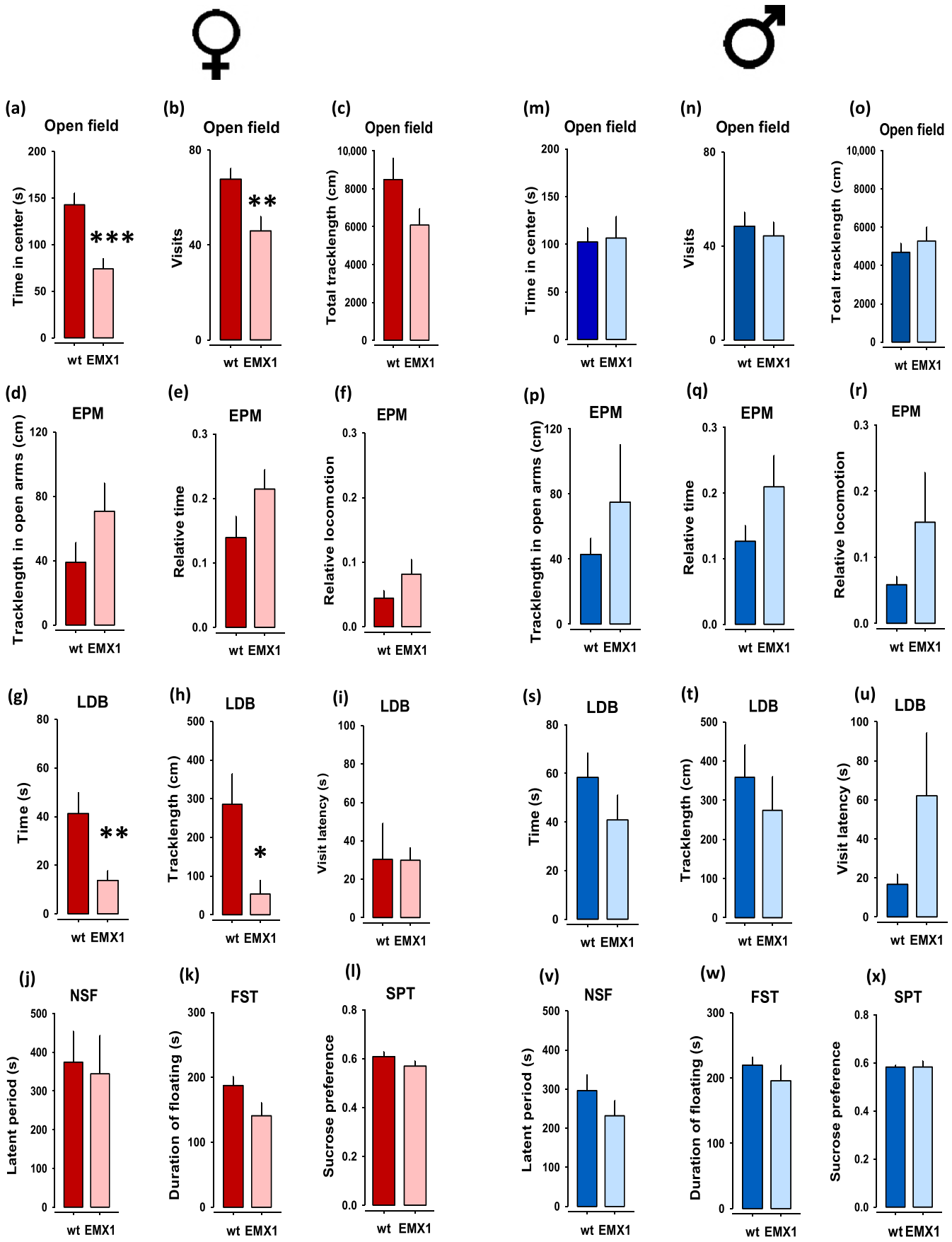

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FIGURE 3 Sex-specific effects of Bassoon (*Bsn)* knockout in the forebrain glutamatergic neurons on emotional behaviour. In females, specific loss of *Bsn* in the glutamatergic neurons was associated with a reduction in the centre time ($t = -4.082$, $P < 0.001$) and number of entries in the centre of the open field (OF) ($t = -2.914$, $P < 0.001$; a,b), while total locomotion in the OF was preserved ($P > 0.05$; c). In the elevated plus maze (EPM), no significant genotype-specific changes was observed ($P > 0.05$; d–f). In the light–dark box (LDB), reduced time ($t = -2.890$, $P = 0.010$; g) and tracklength in the light compartment ($t = -2.701$, $P = 0.016$; h), but intact visit latency ($P > 0.05$; i) were found in Bsn^{Emx1} females. No changes in depression-like behaviour of Bsn^{Emx1} females was found in the novelty suppressed feeding (NSF) (j), forced swim test (FST) (k) and sucrose preference test (SPT) (l) tests ($P > 0.05$). In Bsn^{Emx1} males, no signs of altered anxiety-like behaviour as shown in the OF ($P > 0.05$ for all parameters; m–o), EPM ($P > 0.05$ for all parameters; p–r) and LDB ($P > 0.05$ for all parameters; s–u) tests were found. Depression-like behaviour was also preserved in Bsn^{Emx1} males as shown in the NSF, FST and SPT ($P > 0.05$ for all parameters; v–x). Animal number: wt—13 males/9 females; Bsn^{Emx1}—12 males/9 females. Data were analysed by two-tailed *t* test. EMX1, Bsn^{EMX1} mice.

of alcohol. In a preliminary study on a small cohort of females, *Bsn deletion* in the glutamatergic neurons of forebrain did not affect reinforcing properties of alcohol in the CPP test as shown by preserved time, entries and distance passed in the CC (Figures 4c and S3). Alcohol-induced locomotion at conditioning days was slightly reduced in Bsn^{EMX1} females, and reached significance level at the conditioning Day 6 (Figure 4d).

In males, *Bsn* deletion in the glutamatergic neurons of forebrain had moderate effects on the reinforcing properties of alcohol. Although we found no effects of the mutation on time or entries to the CC, distance in the CC passed at the Test Day 3 was reduced in Bsn^{EMX1} males compared to wt littermates (Figures 4g and S4). Similar to females, alcohol-induced locomotion at conditioning days was reduced in Bsn^{EMX1} males and reached significance level at the conditioning Days 1 and 4 (Figure 4h). Thus, deletion of *Bsn* in forebrain glutamatergic neurons slightly diminishes reinforcing properties of alcohol in males, but not females.

3.5 | Bassoon (*Bsn*) deletion in dopaminergic neurons affects affective state of males

Next, we analysed alcohol-related behaviour and affective state in Bsn^{DAT} animals, in which *Bsn* was deleted in dopaminergic neurons. In female Bsn^{DAT} mice, no changes in the anxiety- and depression-like behaviour were observed in any of the tests employed (Figures 5a–l and S5).

Dopaminergic neuron-specific *Bsn* deletion affected anxiety- and depression-like behaviour in male Bsn^{DAT} mice. In the EPM, tracklength in the open arms, relative time and locomotion were significantly reduced in Bsn^{DAT} males, while time in the open arms only tended to decrease (Figures 5p–r and S5). No genotype-related differences were observed in the OF (Figures 5m–o and S5). Similarly, the main parameters of the LDB did not differ between genotypes, except for visit latency, which was higher in Bsn^{DAT} males (Figures 5s–u and S5). In the NSF test, latency to first eating was reduced in Bsn^{DAT} mice compared to wt littermates (Figure 5v). In the FST and SPT, no pronounced genotype-specific differences were found (Figures 5w,x and S5). In sum, *Bsn* deletion in dopaminergic neurons enhances anxiety and diminished depression-like behaviour in male, but not female mice.

3.6 | Bassoon (*Bsn*) knockout in dopaminergic neurons enhances alcohol drinking in males

No *Bsn*-related differences in alcohol drinking phenotype were observed in Bsn^{DAT} female mice. Neither alcohol consumption and preference during drinking free-choice drinking, nor ADE differed between Bsn^{DAT} and wt females (Figures 6a,b and S6). Sucrose preference was enhanced in Bsn^{DAT} mice at the concentration of 0.5%, but not 5%. Quinine aversion was preserved in Bsn^{DAT} females (Figure S6).

Alcohol consumption phenotype in Bsn^{DAT} males slightly differed from that in wt littermates. Although alcohol consumption remained unchanged, Bsn^{DAT} males had higher alcohol preference than wt littermates, especially at low alcohol concentrations (2% and 8%; Figure 6e,f). ADE did not show genotype-specific differences (Figure S6). Preference for 5% sucrose was reduced in Bsn^{DAT} males, while preference for 0.5% sucrose and quinine avoidance were preserved (Figure S6). Thus, *Bsn* deletion in dopaminergic neurons slightly enhanced alcohol preference in males, but not in females.

3.7 | Bassoon (*Bsn*) deletion in dopaminergic neurons does not influence alcohol metabolism but attenuates sedative effects of alcohol in females

The analysis of blood alcohol concentration (BAC) did not reveal significant genotype effects at any of the studied time points in females and males (Figure S7). Thus, blood alcohol availability was not altered in Bsn^{DAT} mice of both sexes.

We analysed effects of *Bsn* loss for sedative effects of alcohol. In females, the latency of LORR was enhanced in Bsn^{DAT} *mice* compared to wt littermates at Test Day 1, but not at Test Day 8. The duration of LORR was diminished at Day 8 compared to Day 1, but this reduction reached significance only in Bsn^{DAT} *f*emales (Figure S7). In males, the latency of LORR did not differ between the genotypes at the Day 1, while it reduced at Day 8 only in Bsn^{DAT} males. LORR duration did not differ between *the genotypes* at Days 1 and 8 (Figure S7). These data indicate that lack of *Bsn* in dopaminergic neurons reduces sensitivity to sedating effects of alcohol and prevents tolerance development in females after repeated treatment. In males, sedative effects of alcohol were preserved, but *Bsn* deletion in dopaminergic neurons also prevented development of tolerance.

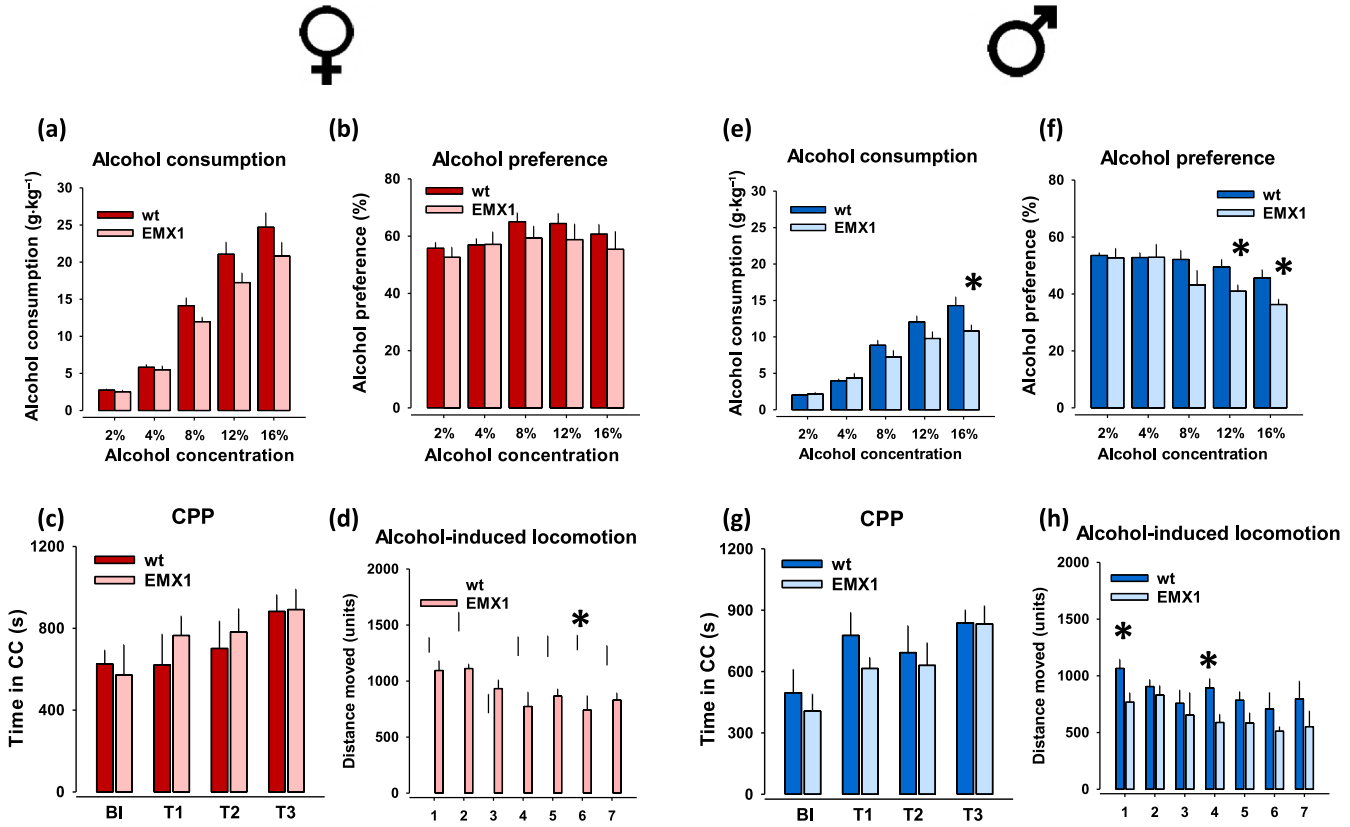

FIGURE 4 Sex-specific effects of Bassoon (*Bsn)* knockout in the forebrain glutamatergic neurons on alcohol consumption behaviour. No *Bsn*-related differences in alcohol drinking phenotype were observed in Bsn^{Emx1} female mice. Analysis of variance (ANOVA) for repeated measurements did not observed effects of genotype on alcohol consumption ($F[1,16] = 3.059$, $P = 0.099$) and preference ($F[1,16] = 1.373$, $P = 0.258$). Pre-planned comparison also did not reveal genotype-driven differences in these parameters ($P > 0.05$; Figure 2a,-b). In females, *Bsn* knockout in glutamatergic neurons did not affect reinforcing properties of alcohol in the conditioned place preference (CPP) test. Alcohol CPP was successfully established as factor test day had significant effects on time in the conditioned compartment ($F[3,21] = 7.345$, $P = 0.002$). However, the factor genotype did not significantly affect any of the studied parameters ($P > 0.05$). Pre-planned comparison showed reduced distance passed by Bsn^{EMX1} females at the Test Day 3 in the conditioned compartment (CC) ($P = 0.037$; Figure 2c). Alcohol-induced locomotion at conditioning days was reduced in Bsn^{EMX1} females and reached significance level at the conditioning Day 6 ($P = 0.026$; Figure 2d). Bsn^{Emx1} males consumed less alcohol comparing to wt littermates. ANOVA did not reveal a genotype effect on alcohol consumption ($F[1,18] = 3.461$, $P = 0.079$), but a strong effect of alcohol concentration on this parameter ($F[4,72] = 109.565$, $P < 0.001$). Genotype* concentration interaction was significant ($F[4,72] = 3.817$, $P = 0.007$). Pre-planned comparison showed a reduction in alcohol consumption by Bsn^{Emx1} males, which reached significance at 16% of alcohol ($P = 0.034$; Figure 2e). Effects of the factor concentration on alcohol preference was also significant ($F[4,72] = 8.120$, $P < 0.001$), while a slight effect of the genotype ($F[1,18] = 3.950$, $P = 0.062$) and no interaction between these factors ($F[4,72] = 1.683$, $P = 0.163$) were found. Pre-planned comparison revealed significantly reduced preference of 12% and 16% of alcohol in Bsn^{Emx1} males ($P = 0.028$ and $P = 0.020$; Figure 2f). In males, *Bsn* knockout in the forebrain had moderate effects on the reinforcing properties of alcohol. Significant effects of the factor test day on time in the conditioned compartment ($F[3,24] = 9.142$, $P < 0.001$) confirmed successful establishment of the CPP. However, no significant effects of the factor genotype on any of the time in the CC was observed ($P > 0.05$; Figure 2g). Similar to females, alcohol-induced locomotion at conditioning days was reduced in Bsn^{EMX1} males and reached significance level at the conditioning Days 1 and 4 ($P = 0.039$ and $P = 0.031$; Figure 2h). Animal number in the drinking study: wt—12 males/12 females; Bsn^{Emx1}—9 males/9 females; animal number in the CPP test: wt—6 males/5 females; Bsn^{Emx1}—4 males/4 females. Data were analysed by ANOVA for repeated measurements followed by pre-planned comparison using least significance difference (LSD) test. For experiments where n=4, statistical analysis was not carried out and results should be regarded as preliminary. BI, baseline; EMX1, Bsn^{Emx1} mice.

3.8 | Bassoon (*Bsn*) deletion in dopaminergic neurons does not affect reinforcing properties of alcohol

In females, *Bsn* deletion in dopaminergic neurons did not affect reinforcing properties of alcohol as shown in any of the studied parameters of the CPP test (Figures 6c,d and S8).

Similar to females, no *Bsn* deletion-induced changes in the reinforcing properties of alcohol were observed in males (Figures 6e,f and S9). Among the studied parameters, only alcohol-induced locomotion in the CC at the conditioning day 6 was higher in Bsn^{DAT} males than in wt mice (Figure 6h). Altogether, *Bsn* deletion in dopaminergic neurons does not affect reinforcing properties of alcohol in both sexes.

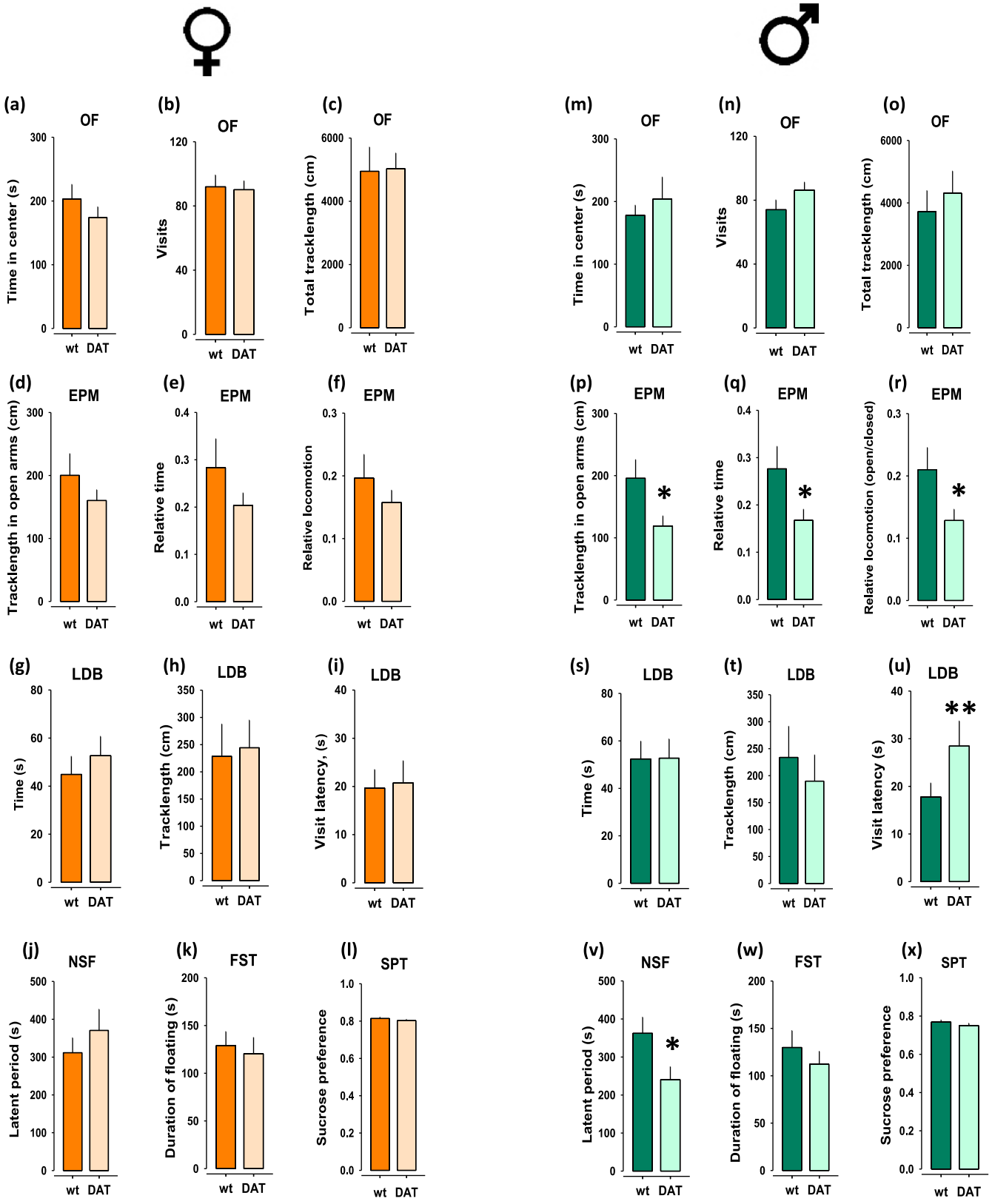

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FIGURE 5 Sex-specific effects of Bassoon (*Bsn)* knockout in dopaminergic neurons on emotional behaviour. In females, loss of *Bsn* specifically in dopaminergic neurons did not affect anxiety-like behaviour as shown in the open field (OF) (a–c), elevated plus maze (EPM) (d–f) and light–dark box (LDB) (g–i) tests ($P > 0.05$ for all parameters). Depression-like behaviour was also preserved in Bsn^{DAT} females as shown in the novelty suppressed feeding (NSF), forced swim test (FST) and sucrose preference test (SPT) ($P > 0.05$ for all parameters; j–l). In Bsn^{DAT} males, no changes in the OF were observed ($P > 0.05$ for all parameters; m–o). In the EPM, tracklength in the open arms ($t = -2.112$, $P = 0.039$; p), relative time ($t = -1.901$, $P = 0.006$; q) and relative locomotion ($t = -2.024$, $P = 0.048$; r) were reduced in Bsn^{DAT} males indicating higher anxiety. In the LDB, enhanced visit latency ($t = 1.712$, $P = 0.009$; u), but preserved time and tracklength in the light compartment ($P > 0.05$; s,t) were found in male Bsn^{DAT} mice. Latent period to first eating in the NSF was lower in Bsn^{DAT} males comparing to wt littermates ($t = -2.228$, $P = 0.034$; v). Depression-like behaviour in Bsn^{DAT} males was intact as shown by duration of floating the FST ($P > 0.05$; w) and sucrose preference in the SPT ($P > 0.05$; x). Animal number: wt—16 males/13 females; Bsn^{DAT}—17 males/16 females. Data were analysed by two-tailed *t* test. DAT, Bsn^{DAT} mice.

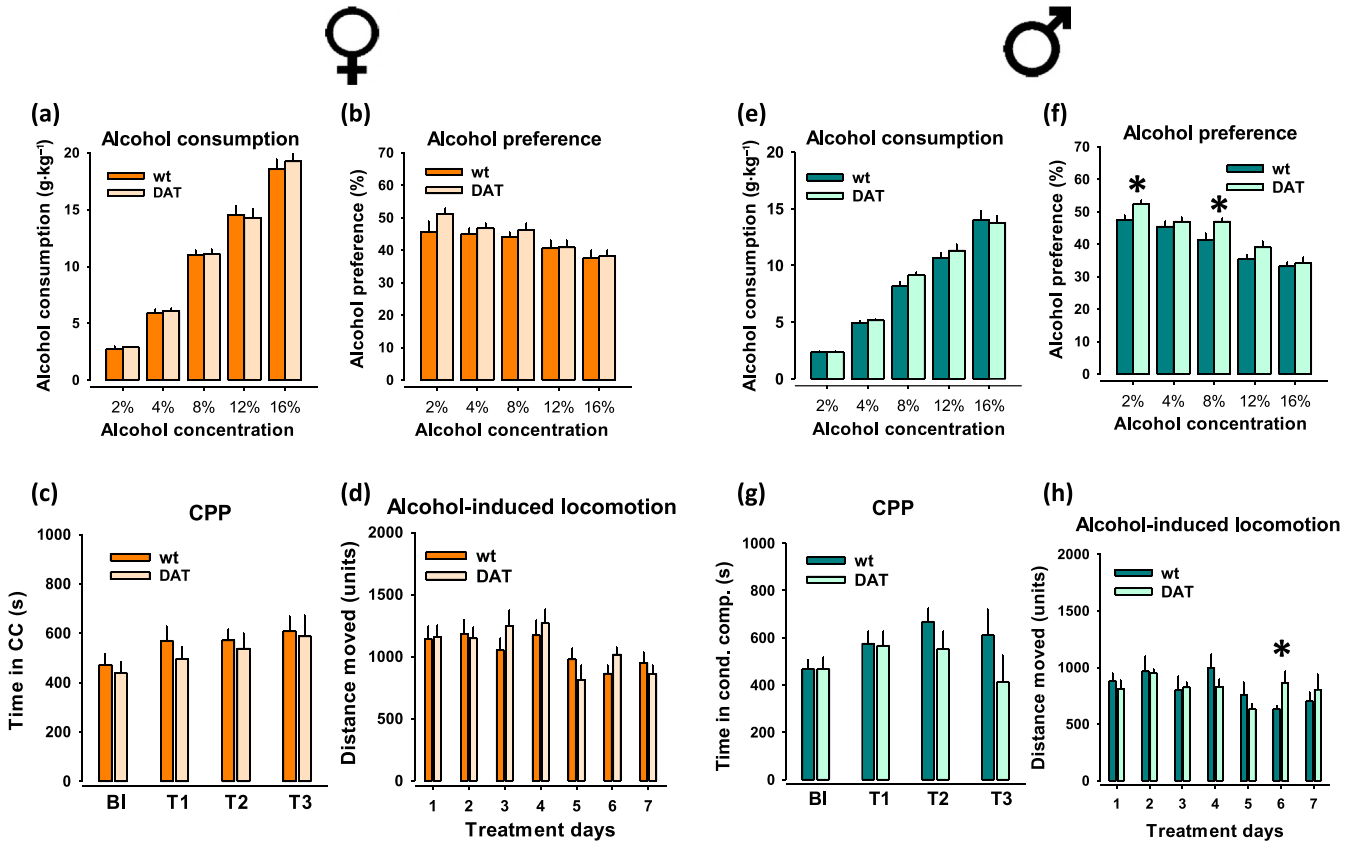

FIGURE 6 Sex-specific effects of *Bsn* knockout specifically in dopaminergic neurons on alcohol consumption behaviour. In females, loss of *Bsn* specifically in dopaminergic neurons did not induce changes in alcohol consumption (genotype effect: $F[1,15] = 0.081$, $P = 0.780$; Figure 4a) or preference (genotype effect: $F[1,15] = 0.643$, $P = 0.435$; Figure 4b) in a two-bottle free-choice paradigm. Alcohol conditioned place preference (CPP) was successfully established as factor test day has significant effects on conditioned compartment (CC) ($F[3,42] = 3.3447$, $P = 0.025$; Figure 4c). However, the factor genotype did not have significant effects on time in the CC ($P > 0.05$; Figure 4c). Analysis of variance (ANOVA) did not reveal significant effects of parameters genotype ($F[1,28] = 0.237$, $P = 0.634$) on the locomotion in the CC, while effects of the factor day were significant ($F[3,84] = 14.858$, $P = 0.001$; Figure 4d). Alcohol consumption phenotype in Bsn^{DAT} males slightly differed from that in wt littermates. ANOVA did not reveal effects of genotype on alcohol consumption ($F[1,17] = 0.569$, $P = 0.461$; Figure 4e). However, ANOVA showed the effects of the genotype and concentration on alcohol preference ($F[1,17] = 4.622$, $P = 0.046$; $F[4,68] = 53.468$, $P = 0.001$), but not their interaction ($F[4,68] = 1.243$, $P = 0.301$). Pre-planned comparison showed higher preference of 2% and 8% alcohol by Bsn^{DAT} males ($P = 0.026$ and 0.028; Figure 4f). Similar to females, no *Bsn*-induced changes in the reinforcing properties of alcohol were observed in males. ANOVA did not reveal effects of the genotype on the time spent in the CC ($F[1,12] = 1.172$, $P = 0.300$; Figure 4g) and alcohol-induced locomotion ($F[1,12] = 0.006$, $P = 0.941$; Figure 4f). Pre-planned comparison showed higher locomotion of Bsn^{DAT} males at the conditioning Day 6 ($P = 0.044$; Figure 4f). Animal number in the drinking study: wt—10 males/7 females; Bsn^{DAT}—9 males/10 females; animal number in the CPP test: wt—7 males/8 females; Bsn^{DAT}—8 males/8 females. Data were analysed by ANOVA for repeated measurements followed by pre-planned comparison using LSD test. DAT, Bsn^{DAT} mice.

3.9 | Bassoon (*Bsn*) deletion in dopaminergic neurons specifically enhances dopamine level in the amygdala of females

In females, *Bsn* knockout in dopaminergic neurons was accompanied with an increase in the tissue DA concentration in the amygdala, but not in the DH or VS (Figure 7a,d,g). Levels of **5-HT** and **NA** remained similar in Bsn^{DAT} and wt *f*emales (Figure 7a–i).

No differences in post-mortem brain levels of all studied neurotransmitters (DA, NA and 5-HT) were found between male Bsn^{DAT} and control mice (Figure 7j–r). Therefore, lack of *Bsn* in dopaminergic neurons results in an amygdala-specific increase in DA level in females, but not males.

4 | DISCUSSION

Impaired synaptic transmission is widely accepted as one of the key mechanisms of psychiatric disorders. Presynaptic scaffolding proteins, such as *Bsn*, which orchestrate numerous functions at the presynaptic active zone (Ivanova et al., 2016; Juranek et al., 2013; Okerlund et al., 2017), are proposed to contribute to the pathogenesis of AUD and affective disorders. In this study, we isolated the dissociating role of *Bsn* in different neuronal types in regulation of affective state and alcohol consumption phenotype. We observed beneficial effects of a *Bsn* deletion in forebrain glutamatergic neurons, including reduced alcohol consumption and preserved affective state in male mice. In females, loss of *Bsn* in glutamatergic neurons resulted in increased anxiety, while depression-like behaviour and alcohol consumption were preserved. Conversely, a *Bsn* knockout in dopaminergic neurons was associated with increased alcohol consumption in males. Bsn^{DAT} males showed reduced depression-like behaviour, but increased anxiety. In contrast, a *Bsn* knockout in dopaminergic neurons did not alter affective or alcohol consumption phenotypes of females. The observed increase in DA concentration in the amygdala of Bsn^{DAT} females likely compensates for effects of Bsn deletion on dopaminergic transmission and prevents behavioural alterations. Although our data do not permit a direct attribution of the observed behavioural phenotypes to Bsn-related alterations in presynaptic activity, Bsn may contribute to the sex-specific manifestation of AUD and negative affective states.

Glutamate plays a crucial role in regulating the development and expression of addictive behaviours, and the NMDA receptors are considered as one of the primary molecular targets for the central effects of alcohol (Gass & Olive, 2008; Kalivas et al., 2009). We have observed a male-specific reduction in alcohol consumption induced by *Bsn* loss in the forebrain glutamatergic neurons. Previous studies showed that inhibition of **NMDA receptor** function by receptor antagonists attenuates ethanol consumption in rodents (Bienkowski et al., 2001; Gass & Olive, 2008; Hölter et al., 2000; Wang et al., 2007), while alcohol promotes synaptic transmission of glutamate in various brain structures (Lominac et al., 2006; Moghaddam & Bolinao, 1994; Szumlinski et al., 2007). Previous data indicate an important role of *Bsn* for neurotransmission: *Bsn* knockout is associated with reduced the rate of vesicle reloading at release sites of cerebellar mossy fibre to granule cell synapses, although the release probability and the number of release sites were unaltered (Hallermann et al., 2010). In turn, in the glutamatergic neurons of the forebrain, lack of *Bsn* was accompanied with increased excitability and baseline synaptic transmission in the medial molecular layer of the dorsal dentate gyrus (Annamneedi et al., 2018). Therefore, potentially altered glutamate synaptic transmission induced by *Bsn* loss in the glutamatergic receptors might determine reduction in alcohol consumption by Bsn^{EMX1} males. Another potential mechanism of the beneficial effects of forebrain *Bsn* knockout on alcohol consumption might involve alterations in autophagy and the **BDNF** system at glutamatergic synapses. Knockout or knockdown of *Bsn* enhances presynaptic autophagy (Okerlund et al., 2017). Accelerated autophagy acts as a protective factor against alcohol-induced toxicity and behavioural impairments (Liu et al., 2019; Pla et al., 2016). On the other hand, *Bsn* knockout leads to a significant, brain region-specific increase in hippocampal BDNF and **TrkB** protein concentrations, as well as altered BDNF/TrkB signalling (Annamneedi et al., 2018; Dieni et al., 2012; Dieni et al., 2015; Heyden et al., 2011). This may, in part, explain the enlarged forebrain volume and enhanced neurogenesis previously observed in Bsn^{Emx1} mice (Annamneedi et al., 2018; Annamneedi et al., 2021). Increased BDNF concentrations are linked to reduced alcohol consumption and reinforcing properties of alcohol in preclinical models (Cui et al., 2015; Jeanblanc et al., 2006; McGough et al., 2004; Pandey, 2016). Considering strong antidepressant effects of enhanced autophagy and BDNF level (Cavaleri et al., 2023; Gassen & Rein, 2019; Tosk et al., 1996; Zhang et al., 2015), these mechanisms might protect male Bsn^{Emx1} mice from development of affective states despite altered glutamatergic transmission. Interestingly, the opposite phenotype observed in Bsn^{Emx1} females, which includes preserved alcohol consumption, but enhanced anxiety-like behaviour, might be determined by the same mechanism. Several studies suggest that increased anxiety is linked to accelerated autophagy (Choe et al., 2024; Wang et al., 2024). However, this hypothesis cannot explain the preserved alcohol consumption in Bsn^{Emx1} females. It should be noted that the discussed literature is mainly focused on the *Bsn*-associated changes in plasticity in the hippocampus. Although the hippocampus is widely shown as one of the key structures contributing to several mechanisms of alcohol dependence (Felipe et al., 2021; Nalberczak-Skóra et al., 2023; Salamian et al., 2025; Stürzenberger et al., 2025), the role of *Bsn* in other forebrain structures is of specific interest.

The dopamine system has been traditionally considered as a crucial mechanism of AUD contributing to reward learning, motivation, drug-seeking behaviour and reinforcement (Dagher & Robbins, 2009; Di Chiara, 1997; Robbins et al., 1989). Alteration of synaptic transmission in dopaminergic neurons, which can be induced by the loss of the crucial presynaptic scaffolding proteins *Bsn*, was associated with enhanced alcohol consumption and anxiety in males. These results are in line with literature data showing the interaction between lack of dopamine and enhanced alcohol consumption (Quarfordt et al., 1991) or anxiety-like behaviour (Selden et al., 1991; Talalaenko et al., 1994).

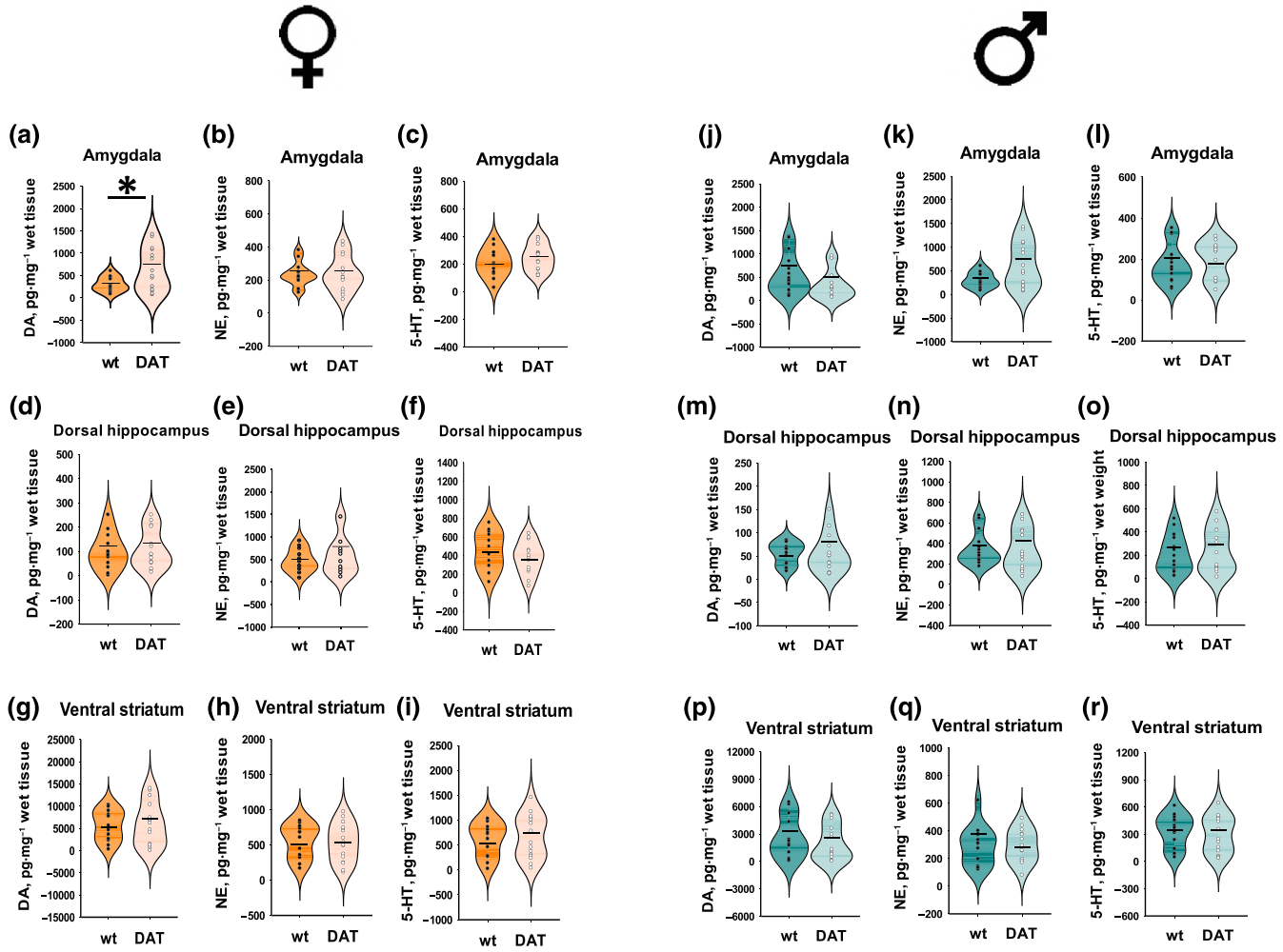

FIGURE 7 Sex-specific effects of Bassoon (*Bsn*) knockout specifically in dopaminergic neurons on brain tissue monoamine levels. In females, loss of *Bsn* specifically in dopaminergic neurons was associated with increased tissue dopamine level in the amygdala ($t = -2.066$, $P = 0.049$; Figure 5a), but not dorsal hippocampus (DH) or ventral striatum (VS) ($P > 0.05$; d–g). Tissue levels of serotonin and noradrenaline in all studied structures did not differ between genotypes ($P > 0.05$; b and c, e and f and h and i). In male Bsn^{DAT} mice, no significant changes in tissue levels of neurotransmitters were observed when compared to wt littermates ($P > 0.05$; j–r). Animal number: wt—16 males/13 females; Bsn^{DAT}—17 males/16 females. Data were analysed by two-tailed *t* test. DAT, Bsn^{DAT} mice.

The observed alcohol consumption phenotype in Bsn^{DAT} males may also result from interactions between *Bsn* and the calcium-binding protein calbindin-D28K (Calb1), which is abundant in dopaminergic neurons. A *Bsn* knockout leads to the loss of this protein in hippocampal mossy fibres (Dieni et al., 2015). Calb1 loss modifies the coupling of Ca^{2+} entry to DA release, limits DA uptake and modulates the effect of DA transporter inhibition on DA release in the nucleus accumbens (Brimblecombe et al., 2019). Alcohol, like other drugs of abuse, promotes DA release (Di Chiara, 1997), and attenuation of this process by the interaction between Calb1 and *Bsn* could contribute to enhanced alcohol drinking in Bsn^{DAT} males. Therefore, the dissociating and even opposite behavioural effects of a *Bsn* knockout in different cell types likely reflect differences in the molecular mechanisms of presynaptic plasticity at dopaminergic and glutamatergic synapses. Interestingly, loss of *Bsn* in the dopaminergic neurons has beneficial effects on depression-like behaviour in males. This is not in line with the expected impairment of dopaminergic transmission. However, the reduction of depression-like behaviour in Bsn^{DAT} males might be explain, as mentioned above, by interactions between *Bsn* and autophagy and BDNF signalling (Annamneedi et al., 2018; Dieni et al., 2015; Heyden et al., 2011; Okerlund et al., 2017; Waites et al., 2013). On the contrary, females are protected against the behavioural effects of a *Bsn* deletion in the dopaminergic neurons. Interestingly, we observed a female-specific increase in DA level in the amygdala of female Bsn^{DAT} mice, which probably serves as a compensatory response to altered synaptic transmission. It might be suggested that enhanced DA level, which plays a crucial role in the pathogenesis of affective disorders and alcohol dependence (Di Chiara, 1997; Opmeer et al., 2010), compensates impaired dopaminergic transmission in females upon *Bsn* deletion in the dopaminergic neurons. This compensation might in turn determine intact affective state and alcohol drinking phenotype of Bsn^{DAT} females.

Our findings reveal a pronounced impact of *Bsn* deletion in dopaminergic neurons on the behavioural phenotype of male mice, whereas the effects in females appear comparatively modest. To our knowledge, no reports have described sex-specific patterns of Bsn expression. Nevertheless, sex-dependent differences have been documented for other presynaptic scaffolding proteins, such as Caskin1 in the striatum and ERC (ELKS/Rab6-interacting/CAST) in the hippocampus of mice (Distler et al., 2020). Consistently, postsynaptic scaffolding proteins including SHANK1 and SHANK3 exhibit sex-dependent expression in the rat hippocampus, which can be further modulated by testosterone treatment (Srancikova et al., 2021). These observations suggest that sex-specific properties of the synaptic cytomatrix may contribute to functional divergences between males and females under both physiological and pathological conditions. Considering that the behavioural alterations observed in *Bsn*-deficient mice may, at least in part, reflect compensatory adaptations within the synaptic machinery, sex-dependent variation in scaffolding protein expression could represent a critical determinant of the observed phenotypic differences. Moreover, a study by Erli et al. (2020) demonstrated an interaction between sex hormones and the presynaptic protein machinery. Treatment with 17β-oestradiol was shown to enrich synaptic proteins at synapses and increase the number of *Bsn*-positive dendritic spines (Erli et al., 2020). While these findings do not directly explain our results, they suggest a sex-specific contribution of *Bsn* to the mechanisms of behavioural control. It should be noted that high sex-specificity as well as wide brain distribution may abolish usage of *Bsn* as a primary target for therapeutic drugs.

The limitations of this study include the absence of data on potential alterations in other presynaptic scaffolding proteins, which may have compensated for the loss of *Bsn* and thereby influenced the observed behavioural outcomes. Given that the presynaptic cytomatrix constitutes a critical structural framework governing the physiological processes of synaptic transmission, developmental perturbations in *Bsn* expression are likely to elicit compensatory adaptations in other synaptic components. Another limitation concerns the assessment of emotional phenotypes, which was based on a battery of behavioural tests. Although appropriate intervals were maintained between the tests, it cannot be excluded that prior testing experiences influenced performance in subsequent tasks and, to some extent, altered monoamine levels within the brain. It should be also noted that only the voluntary alcohol drinking, and not binge-like drinking was investigated in this study. Although consumption of easily available alcohol is one of the key mechanisms of AUD, we did isolate the role of Bsn knockout in glutamatergic and dopaminergic neurons on a model of alcohol abuse.

In conclusion, our findings suggest that *Bsn* is a sex-specific regulator of emotional and alcohol consumption behaviour. *Bsn* exerts dissociating behavioural effects depending on its specific action in different neuronal cell types, such as glutamatergic and dopaminergic neurons. These dissociating effects may be mediated by distinct molecular mechanisms involved in the regulation of neurotransmission and plasticity specific to each neuronal cell type. Further analysis of the molecular mechanisms underlying *Bsn's* contribution to behavioural control is required, as *Bsn* may serve as a male-predominant mechanism in affective disorders and use disorder.

AUTHOR CONTRIBUTIONS

Liubov S. Kalinichenko: Investigation; writing—original draft; methodology; visualization; writing—review and editing; formal analysis; project administration; data curation; supervision. **Mona Krenbauer:** Data curation; investigation; writing—review and editing. **Janosh Isert:** Data curation; investigation; writing—review and editing. **Marlon Kamlah:** Data curation; investigation; writing—review and editing. **Thorben Tanzer:** Data curation; investigation; writing—review and editing. **Hannes Rödel:** Data curation; investigation; writing—review and editing. **Tobias Müller:** Data curation; investigation; writing—review and editing; conceptualization; methodology; funding acquisition; writing—original draft. **Anil Annamneedi:** Investigation; validation; visualization; formal analysis. **Enes Yağız Akdaş:** Data curation; investigation; formal analysis; writing—review and editing. **Volker Eulenburg:** Data curation; investigation; formal analysis; writing—review and editing. **Bernd Lenz:** Conceptualization; funding acquisition; writing—review and editing. **Eckart D. Gundelfinger:** Validation; visualization; investigation; formal analysis. **Johannes Kornhuber:** Conceptualization; funding acquisition; writing—review and editing. **Anna Fejtova:** Conceptualization; validation; investigation; visualization; writing—review and editing. **Christian P. Müller:** Data curation; investigation; writing—review and editing; conceptualization; methodology; funding acquisition; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors claim no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Natural Products Research, Design and Analysis, Immunoblotting and Immunochemistry

and Animal Experimentation and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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REFERENCES

Ackermann, F., Waites, C. L., & Garner, C. C. (2015). Presynaptic active zones in invertebrates and vertebrates. *EMBO Reports*, *16*, 923–938. https://doi.org/10.15252/embr.201540434

Alexander, S. P. H., Fabbro, D., Peach, C. J., Gibb, A. J., Kelly, E., Mathie, A. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Southan, C., Davies, J. A., Beuve, A., Brouckaert, P., Bryant, C., Burnett, J. C., Farndale, R. W., Friebe, A., Garthwaite, J., … Waldman, S. A. (2025). The Concise Guide to PHARMACOLOGY 2025/26: Catalytic receptors. *British Journal of Pharmacology*, *182*-(Suppl 1), S259–S306. https://doi.org/10.1111/bph.70233

Alexander, S. P. H., Gibb, A. J., Kelly, E., Mathie, A. A., Peach, C. J., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Southan, C., Davies, J. A., Amarosi, L., Anderson, C. M. H., Beart, P. M., Broer, S., Dawson, P. A., Gyimesi, G., Hagenbuch, B., Hammond, J. R., … Verri, T. (2025). The Concise Guide to PHARMACOLOGY 2025/26: Transporters. *British Journal of Pharmacology*, *182*(Suppl 1), S404–S496. https://doi.org/10.1111/bph.70235

Alexander, S. P. H., Striessnig, J., Gibb, A. J., Mathie, A. A., Veale, E. L., Kelly, E., Peach, C. J., Armstrong, J. F., Faccenda, E., Harding, S. D., Southan, C., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Beech, D. J., Biel, M., Bill, R. M., … Zhu, M. (2025). The Concise Guide to PHARMACOLOGY 2025/26: Ion channels. *British Journal of Pharmacology*, *182*(Suppl 1), S152–S241. https://doi.org/10.1111/bph.70231

Altrock, W. D., tom Dieck, S., Sokolov, M., Meyer, A. C., Sigler, A., Brakebusch, C., Fässler, R., Richter, K., Boeckers, T. M., Potschka, H., Brandt, C., Löscher, W., Grimberg, D., Dresbach, T., Hempelmann, A., Hassan, H., Balschun, D., Frey, J. U., Brandstätter, J. H., … Gundelfinger, E. D. (2003). Functional inactivation of a fraction of excitatory synapses in mice deficient for the active zone protein bassoon. *Neuron*, *37*, 787–800. https://doi.org/10.1016/s0896-6273(03)00088-6

Annamneedi, A., Caliskan, G., Müller, S., Montag, D., Budinger, E., Angenstein, F., Fejtova, A., Tischmeyer, W., Gundelfinger, E. D., & Stork, O. (2018). Ablation of the presynaptic organizer bassoon in excitatory neurons retards dentate gyrus maturation and enhances learning performance. *Brain Structure & Function*, *223*, 3423–3445. https://doi.org/10.1007/s00429-018-1692-3

Annamneedi, A., del Angel, M., Gundelfinger, E. D., Stork, O., & Çalışkan, G. (2021). The presynaptic scaffold protein bassoon in forebrain excitatory neurons mediates hippocampal circuit maturation: Potential involvement of TrkB signalling. *International Journal of Molecular Sciences*, *22*, 7944. https://doi.org/10.3390/ijms22157944

Bäckman, C. M., Malik, N., Zhang, Y., Shan, L., Grinberg, A., Hoffer, B. J., Westphal, H., & Tomac, A. C. (2006). Characterization of a mouse strain expressing Cre recombinase from the 3′ untranslated region of the dopamine transporter locus. *Genesis*, *44*, 383–390.

Bangasser, D. A., & Cuarenta, A. (2021). Sex differences in anxiety and depression: Circuits and mechanisms. *Nature Reviews. Neuroscience*, *22*, 674–684. https://doi.org/10.1038/s41583-021-00513-0

Benton, R. L. (1989). Planned Comparisons as Better Alternatives to ANOVA Omnibus Tests, 19

Bienkowski, P., Krzascik, P., Koros, E., Kostowski, W., Scinska, A., & Danysz, W. (2001). Effects of a novel uncompetitive NMDA receptor antagonist, MRZ 2/579 on ethanol self-administration and ethanol withdrawal seizures in the rat. *European Journal of Pharmacology*, *413*, 81–89.

Blondiaux, A., Jia, S., Annamneedi, A., Çalışkan, G., Nebel, J., Montenegro-Venegas, C., Wykes, R. C., Fejtova, A., Walker, M. C., Stork, O., Gundelfinger, E. D., Dityatev, A., & Seidenbecher, C. I. (2023). Linking epileptic phenotypes and neural extracellular matrix remodeling signatures in mouse models of epilepsy. *Neurobiology of Disease*, *188*, 106324. https://doi.org/10.1016/j.nbd.2023.106324

Brimblecombe, K. R., Vietti-Michelina, S., Platt, N. J., Kastli, R., Hnieno, A., Gracie, C. J., & Cragg, S. J. (2019). Calbindin-D28K limits dopamine release in ventral but not dorsal striatum by regulating ca 2+ availability and dopamine transporter function. *ACS Chemical Neuroscience*, *10*, 3419–3426. https://doi.org/10.1021/acschemneuro.9b00325

Cavaleri, D., Moretti, F., Bartoccetti, A., Mauro, S., Crocamo, C., Carrà, G., & Bartoli, F. (2023). The role of BDNF in major depressive disorder, related clinical features, and antidepressant treatment: Insight from meta-analyses. *Neuroscience and Biobehavioral Reviews*, *149*, 105159. https://doi.org/10.1016/j.neubiorev.2023.105159

Choe, S., Jeong, H., Choi, J., & Yu, S.-W. (2024). Chronic unpredictable stress induces autophagic death of adult hippocampal neural stem cells. *Molecular Brain*, *17*, 31. https://doi.org/10.1186/s13041-024-01105-6

Costa, K. M., Schenkel, D., & Roeper, J. (2021). Sex-dependent alterations in behavior, drug responses and dopamine transporter expression in heterozygous DAT-Cre mice. *Scientific Reports*, *11*, 3334. https://doi.org/10.1038/s41598-021-82600-x

Curtis, M. J., Alexander, S. P. H., Cortese-Krott, M., Kendall, D. A., Martemyanov, K. A., Mauro, C., Panettieri, R. A. Jr., Papapetropoulos, A., Patel, H. H., Santo, E. E., Schulz, R., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., Wang, X., & Ferdinandy, P. (2025). Guidance on the planning and reporting of experimental design and analysis. *British Journal of Pharmacology*, *182*(7), 1413–1415. https://doi.org/10.1111/bph.17441

Dagher, A., & Robbins, T. W. (2009). Personality, addiction, dopamine: Insights from Parkinson's disease. *Neuron*, *61*, 502–510. https://doi.org/10.1016/j.neuron.2009.01.031

Degenhardt, L., Charlson, F., Ferrari, A., Santomauro, D., Erskine, H., & Mantilla-Herrara, A. (2018). The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990–2016: A systematic analysis for the global burden of disease study 2016. *The Lancet Psychiatry*, *5*, 987–1012. https://doi.org/10.1016/S2215-0366(18)30337-7

Di Chiara, G. (1997). Alcohol and dopamine. *Alcohol Health and Research World*, *21*, 108–114.

Dieni, S., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M. S., Deogracias, R., Gundelfinger, E. D., Kojima, M., Nestel, S., Frotscher, M., & Barde, Y. A. (2012). BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *The Journal of Cell Biology*, *196*, 775–788. https://doi.org/10.1083/jcb.201201038

Dieni, S., Nestel, S., Sibbe, M., Frotscher, M., & Hellwig, S. (2015). Distinct synaptic and neurochemical changes to the granule cell-CA3 projection in bassoon mutant mice. *Frontiers in Synaptic Neuroscience*, *7*, 7. https://doi.org/10.3389/fnsyn.2015.00018

Distler, U., Schumann, S., Kesseler, H.-G., Pielot, R., Smalla, K.-H., Sielaff, M., Schmeisser, M. J., & Tenzer, S. (2020). Proteomic analysis of brain region and sex-specific synaptic protein expression in the adult mouse brain. *Cells*, *9*, 313. https://doi.org/10.3390/cells9020313

Erli, F., Palmos, A. B., Raval, P., Mukherjee, J., Sellers, K. J., Gatford, N. J. F., Moss, S. J., Brandon, N. J., Penzes, P., & Srivastava, D. P. (2020). Estradiol reverses excitatory synapse loss in a cellular model of neuropsychiatric disorders. *Translational Psychiatry*, *10*, 16. https://doi.org/10.1038/s41398-020-0682-4

Felipe, J. M., Palombo, P., Bianchi, P. C., Zaniboni, C. R., Anésio, A., Yokoyama, T. S., Engi, S. A., Carneiro-de-Oliveira, P. E.,

Planeta, C. d. S., Leão, R. M., & Cruz, F. C. (2021). Dorsal hippocampus plays a causal role in context-induced reinstatement of alcohol-seeking in rats. *Behavioural Brain Research*, *398*, 112978. https://doi.org/10.1016/j.bbr.2020.112978

Gass, J. T., & Olive, M. F. (2008). Glutamatergic substrates of drug addiction and alcoholism. *Biochemical Pharmacology*, *75*, 218–265.

Gassen, N. C., & Rein, T. (2019). Is there a role of autophagy in depression and antidepressant action? *Frontiers in Psychiatry*, *10*, 337. https://doi.org/10.3389/fpsyt.2019.00337

Grace, S., Rossetti, M. G., Allen, N., Batalla, A., Bellani, M., Brambilla, P., Chye, Y., Cousijn, J., Goudriaan, A. E., Hester, R., & Hutchison, K. (2021). Sex differences in the neuroanatomy of alcohol dependence: Hippocampus and amygdala subregions in a sample of 966 people from the ENIGMA addiction working group. *Translational Psychiatry*, *11*, 156.

Gundelfinger, E. D., Reissner, C., & Garner, C. C. (2015). Role of bassoon and piccolo in assembly and molecular organization of the active zone. *Frontiers in Synaptic Neuroscience*, *7*, 19. https://doi.org/10.3389/fnsyn.2015.00019

Guzman, S. G., Ruggiero, S. M., Ganesan, S., Ellis, C. A., Harrison, A. G., Sullivan, K. R., Stark, Z., Brown, N. J., Kana, S. L., Tuttle, A., Tenorio, J., Lapunzina, P., Nevado, J., McDonald, M., Jensen, C., Wheeler, P. G., Stange, L., Morrison, J., Keren, B., … Helbig, I. (2025). Variants in BSN, encoding the presynaptic protein bassoon, result in a distinct neurodevelopmental disorder with a broad phenotypic range. *American Journal of Human Genetics*, *112*, 1415–1429. https://doi.org/10.1016/j.ajhg.2025.04.011

Hall, C. S. (1934). Drive and emotionality: factors associated with adjustment in the rat. *Journal of Comparative Psychology*, *17*(1), 89–108. https://doi.org/10.1037/h0073676

Hallermann, S., Fejtova, A., Schmidt, H., Weyhersmüller, A., Silver, R. A., Gundelfinger, E. D., & Eilers, J. (2010). Bassoon speeds vesicle reloading at a central excitatory synapse. *Neuron*, *68*, 710–723. https://doi.org/10.1016/j.neuron.2010.10.026

Heyden, A., Ionescu, M.-C. S., Romorini, S., Kracht, B., Ghiglieri, V., Calabresi, P., Seidenbecher, C., Angenstein, F., & Gundelfinger, E. D. (2011). Hippocampal enlargement in bassoon-mutant mice is associated with enhanced neurogenesis, reduced apoptosis, and abnormal BDNF levels. *Cell and Tissue Research*, *346*, 11–26. https://doi.org/10.1007/s00441-011-1233-3

Hölter, S. M., Danysz, W., & Spanagel, R. (2000). Novel uncompetitive N-methyl-D-aspartate (NMDA)-receptor antagonist MRZ 2/579 suppresses ethanol intake in long-term ethanol-experienced rats and generalizes to ethanol cue in drug discrimination procedure. *The Journal of Pharmacology and Experimental Therapeutics*, *292*, 545–552.

Hyde, J. S., Mezulis, A. H., & Abramson, L. Y. (2008). The ABCs of depression: Integrating affective, biological, and cognitive models to explain the emergence of the gender difference in depression. *Psychological Review*, *115*, 291–313. https://doi.org/10.1037/0033-295X.115.2.291

Ivanova, D., Dirks, A., & Fejtova, A. (2016). Bassoon and piccolo regulate ubiquitination and link presynaptic molecular dynamics with activity-regulated gene expression. *The Journal of Physiology*, *594*, 5441–5448. https://doi.org/10.1113/JP271826

Jeanblanc, J., He, D.-Y., McGough, N. N. H., Logrip, M. L., Phamluong, K., Janak, P. H., & Ron, D. (2006). The dopamine D 3 receptor is part of a homeostatic pathway regulating ethanol consumption. *The Journal of Neuroscience*, *26*, 1457–1464.

Juranek, J. K., Mukherjee, K., Siddiqui, T. J., Kaplan, B. J., Li, J. Y., Ahnert-Hilger, G., Jahn, R., & Calka, J. (2013). Active zone protein expression changes at the key stages of cerebellar cortex neurogenesis in the rat. *Acta Histochemica*, *115*, 616–625. https://doi.org/10.1016/j.acthis.2013.01.003

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. https://doi.org/10.1093/cercor/bhac106

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. https://doi.org/10.1038/s41380-021-01304-w

Kalinichenko, L. S., Zoicas, I., Bienia, A.-M., Bühner, C., Robinson, J., Kütemeyer, J., Labonte, A., Raveendran, T., Warth, L., Smaga, I., Filip, M., Eulenburg, V., Rhein, C., Fejtova, A., Gulbins, E., Kornhuber, J., & Müller, C. P. (2025). Brain acid sphingomyelinase controls addiction-related behaviours in a sex-specific way. *Neurobiology of Disease*, *206*, 106800. https://doi.org/10.1016/j.nbd.2025.106800

Kalivas, P. W., LaLumiere, R. T., Knackstedt, L., & Shen, H. (2009). Glutamate transmission in addiction. *Neuropharmacology*, *56*, 169–173.

Lilley, E., Stanford, S. C., Kendall, D. E., Alexander, S. P. H., Cirino, G., Docherty, J. R., George, C. H., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Sobey, C. G., Stefanska, B., Stephens, G., Teixeira, M., & Ahluwalia, A. (2020). ARRIVE 2.0 and the *British Journal of Pharmacology*: Updated guidance for 2020. *British Journal of Pharmacology*, *177*(16), 3611–3616. https://doi.org/10.1111/bph.15178

Liu, Y., Zhang, Y., Peng, J., Wang, H., Li, X., Li, X., Rong, X., Pan, J., & Peng, Y. (2019). Autophagy alleviates ethanol-induced memory impairment in association with anti-apoptotic and anti-inflammatory pathways. *Brain, Behavior, and Immunity*, *82*, 63–75. https://doi.org/10.1016/j.bbi.2019.07.033

Lominac, K. D., Kapasova, Z., Hannun, R. A., Patterson, C., Middaugh, L. D., & Szumlinski, K. K. (2006). Behavioral and neurochemical interactions between group 1 mGluR antagonists and ethanol: Potential insight into their anti-addictive properties. *Drug and Alcohol Dependence*, *85*, 142–156.

Markou, A. (1998). Neurobiological similarities in depression and drug dependence: A self-medication hypothesis. *Neuropsychopharmacology*, *18*, 135–174. https://doi.org/10.1016/S0893-133X(97)00113-9

McGough, N. N. H., He, D.-Y., Logrip, M. L., Jeanblanc, J., Phamluong, K., Luong, K., Kharazia, V., Janak, P. H., & Ron, D. (2004). RACK1 and brain-derived neurotrophic factor: A homeostatic pathway that regulates alcohol addiction. *The Journal of Neuroscience*, *24*, 10542–10552.

Moghaddam, B., & Bolinao, M. L. (1994). Biphasic effect of ethanol on extracellular accumulation of glutamate in the hippocampus and the nucleus accumbens. *Neuroscience Letters*, *178*, 99–102.

Montenegro-Venegas, C., Fienko, S., Anni, D., Pina-Fernández, E., Frischknecht, R., & Fejtova, A. (2021). Bassoon inhibits proteasome activity via interaction with PSMB4. *Cellular and Molecular Life Sciences*, *78*, 1545–1563. https://doi.org/10.1007/s00018-020-03590-z

Montenegro-Venegas, C., Guhathakurta, D., Pina-Fernandez, E., Andres-Alonso, M., Plattner, F., Gundelfinger, E. D., & Fejtova, A. (2022). Bassoon controls synaptic vesicle release via regulation of presynaptic phosphorylation and <scp>cAMP</scp>. *EMBO Reports*, *23*, e53659. https://doi.org/10.15252/embr.202153659

Müller, C. P., & Schumann, G. (2011). Drugs as instruments: A new framework for non-addictive psychoactive drug use. *The Behavioral and Brain Sciences*, *34*, 293–310. https://doi.org/10.1017/S0140525X11000057

Müller, C. P., Schumann, G., Rehm, J., Kornhuber, J., & Lenz, B. (2023). Self-management with alcohol over lifespan: Psychological mechanisms, neurobiological underpinnings, and risk assessment. *Molecular Psychiatry*, *28*, 2683–2696. https://doi.org/10.1038/s41380-023-02074-3

Nalberczak-Skóra, M., Beroun, A., Skonieczna, E., Cały, A., Ziółkowska, M., Pagano, R., Taheri, P., Kalita, K., Salamian, A., & Radwanska, K. (2023). Impaired synaptic transmission in dorsal dentate gyrus increases

impulsive alcohol seeking. *Neuropsychopharmacology*, *48*, 436–447. https://doi.org/10.1038/s41386-022-01464-5

Okerlund, N. D., Schneider, K., Leal-Ortiz, S., Montenegro-Venegas, C., Kim, S. A., Garner, L. C., Waites, C. L., Gundelfinger, E. D., Reimer, R. J., & Garner, C. C. (2017). Bassoon controls presynaptic autophagy through Atg5. *Neuron*, *93*, 897–913.e7. https://doi.org/10.1016/j.neuron.2017.01.026

Opmeer, E. M., Kortekaas, R., & Aleman, A. (2010). Depression and the role of genes involved in dopamine metabolism and signalling. *Progress in Neurobiology*, *92*, 112–133. https://doi.org/10.1016/j.pneurobio.2010.06.003

Pandey, S. C. (2016). A critical role of brain-derived neurotrophic factor in alcohol consumption. *Biological Psychiatry*, *79*, 427–429. https://doi.org/10.1016/j.biopsych.2015.12.020

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, *18*, e3000410. https://doi.org/10.1371/journal.pbio.3000410

Pla, A., Pascual, M., & Guerri, C. (2016). Autophagy constitutes a protective mechanism against ethanol toxicity in mouse astrocytes and neurons. *PLoS ONE*, *11*, e0153097. https://doi.org/10.1371/journal.pone.0153097

Quarfordt, S. D., Kalmus, G. W., & Myers, R. D. (1991). Ethanol drinking following 6-OHDA lesions of nucleus accumbens and tuberculum olfactorium of the rat. *Alcohol*, *8*, 211–217.

Ramsey, P. (1993). Multiple comparisons of independent means. In L. Edwards (Ed.), *Applied analysis of variance in behavioral science* (pp. 25–61). Dekker, M.

Robbins, T. W., Cador, M., Taylor, J. R., & Everitt, B. J. (1989). Limbic-striatal interactions in reward-related processes. *Neuroscience and Biobehavioral Reviews*, *13*, 155–162.

Salamian, A., Pagano, R., Skonieczna, E., Kalinichenko, L. S., Puchalska, M., Yiğit Ünlü, G., Gajewska-Woźniak, O., Kruashvili, L., Grochowicz, M., Wojtas, B., & Gielniewski, B. (2025). Hippocampal cofilin and *CFL1* gene variants are linked to alcohol use disorder phenotypes. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-025-03226-3

Schattling, B., Engler, J. B., Volkmann, C., Rothammer, N., Woo, M. S., Petersen, M., Winkler, I., Kaufmann, M., Rosenkranz, S. C., Fejtova, A., Thomas, U., Bose, A., Bauer, S., Träger, S., Miller, K. K., Brück, W., Duncan, K. E., Salinas, G., Soba, P., … Friese, M. A. (2019). Bassoon proteinopathy drives neurodegeneration in multiple sclerosis. *Nature Neuroscience*, *22*, 887–896. https://doi.org/10.1038/s41593-019-0385-4

Selden, N. R. W., Everitt, B. J., Jarrard, L. E., & Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience*, *42*, 335–350.

Seney, M. L., Huo, Z., Cahill, K., French, L., Puralewski, R., Zhang, J., Logan, R. W., Tseng, G., Lewis, D. A., & Sibille, E. (2018). Opposite molecular signatures of depression in men and women. *Biological Psychiatry*, *84*, 18–27. https://doi.org/10.1016/j.biopsych.2018.01.017

Srancikova, A., Mihalj, D., Bacova, Z., & Bakos, J. (2021). The effects of testosterone on gene expression of cell-adhesion molecules and scaffolding proteins: The role of sex in early development. *Andrologia*, *53*, 53. https://doi.org/10.1111/and.14153

Stürzenberger, S., Bülow, N., Kalinichenko, L. S., Licha, R., Eulenburg, V., Dahlmanns, M., Müller, C. P., Zheng, F., & Alzheimer, C. (2025). Heavy adolescent drinking makes the adult brain more vulnerable to ethanol by permanently altering the age-dependent interplay between alcohol, GIRK channels and activin. *Molecular Psychiatry*, *31*(2), 1027–1040. https://doi.org/10.1038/s41380-025-03210-x

Szumlinski, K. K., Diab, M. E., Friedman, R., Henze, L. M., Lominac, K. D., & Bowers, M. S. (2007). Accumbens neurochemical adaptations produced by binge-like alcohol consumption. *Psychopharmacology*, *190*, 415–431.

Talalaenko, A. N., Abramets, I. A., Stakhovskii, Y. V., Shekhovtsov, A. A., Chernikov, A. V., & Shevchenko, S. L. (1994). The role of dopaminergic mechanisms of the brain in various models of anxious states. *Neuroscience and Behavioral Physiology*, *24*, 284–288.

Toivainen, S., Xu, L., Gobbo, F., Della Valle, A., Coppola, A., Heilig, M., & Domi, E. (2024). Different mechanisms underlie compulsive alcohol self-administration in male and female rats. *Biology of Sex Differences*, *15*, 17. https://doi.org/10.1186/s13293-024-00592-5

Tosk, J. M., Farag, M., Ho, J. Y., Lee, C. C., Maximos, B. B., & Yung, H.-H. (1996). The effects of nerve growth factor and ganglioside GM1 on the anti-proliferative activity of cocaine in PC 12 cells. *Life Sciences*, *59*, 1731–1737.

Waites, C. L., Leal-Ortiz, S. A., Okerlund, N., Dalke, H., Fejtova, A., Altrock, W. D., Gundelfinger, E. D., & Garner, C. C. (2013). Bassoon and piccolo maintain synapse integrity by regulating protein ubiquitination and degradation. *The EMBO Journal*, *32*, 954–969. https://doi.org/10.1038/emboj.2013.27

Wakita, M., Yaguchi, H., Otuski, M., Tanikawa, S., Miki, Y., Aiba, I., Yoshida, M., Nomura, T., Uwatoko, H., Mito, Y., Sinpo, K., Ikeuchi, T., Tanaka, S., Wakabayashi, K., & Yabe, I. (2025). Pathological study of progressive supranuclear palsy the cases with mutations in bassoon. *Neuropathology*, *45*, 140–152. https://doi.org/10.1111/neup.13009

Wang, J., Carnicella, S., Phamluong, K., Jeanblanc, J., Ronesi, J. A., Chaudhri, N., Janak, P. H., Lovinger, D. M., & Ron, D. (2007). Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: Implications for alcohol drinking behavior. *The Journal of Neuroscience*, *27*, 3593–3602.

Wang, W., Zhu, Q., Zhou, S., Fang, S., Cai, F., Zhang, X., Li, W., Huang, Z., & Yu, K. (2024). The downregulation of autophagy in amygdala is sufficient to alleviate anxiety-like behaviors in post-traumatic stress disorder model mice. *Translational Psychiatry*, *15*(1), 394.

Wilsnack, R. W., Vogeltanz, N. D., Wilsnack, S. C., & Harris, T. R. (2000). Gender differences in alcohol consumption and adverse drinking consequences: Cross-cultural patterns. *Addiction*, *95*, 251–265. https://doi.org/10.1046/j.1360-0443.2000.95225112.x

Zhang, Y., Liu, C., Zhao, Y., Zhang, X., Li, B., & Cui, R. (2015). The effects of calorie restriction in depression and potential mechanisms. *Current Neuropharmacology*, *13*, 536–542.

SUPPORTING INFORMATION

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

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